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Poster

836. Proliferation: Cell Cycle and Molecular Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:836.01/A1

Topic: A.02. Neurogenesis and Gliogenesis

Title: A novel role of Nonsense-mediated mRNA decay in neurodevelopment

Authors: M. HARRISON, Y. ZHOU, C. HUANG, M. LI, *Y. MAO
Biol., PSU, State College, PA

Abstract: Nonsense-mediated mRNA decay (NMD) is a well-known RNA surveillance mechanism that ensures the speedy degradation of mRNAs carrying premature termination codons (PTCs). This mechanism relies on several key exon junction complex (EJC) factors to distinguish PTC from the normal stop codon. But how this mechanism involves in brain development is not clear. Strikingly, mutations in EJC factor like Upf3b have been reported to cause X-linked mental retardation and autism. This strongly indicates that NMD may be a novel pathway that involves in mental retardation. We identify that a key EJC factor is required for neural progenitor proliferation. It binds to several genes involved in the Wnt pathway, and activity dependent genes that are important for neuroplasticity. In this study, we use mouse models to examine NMD function in neurodevelopment, and how it may affect behavior. Studying these mechanisms will provide insights into the causes of mental retardation and eventually lead to new therapeutic strategies for neurodevelopmental illnesses.

Disclosures: M. Harrison: None. **Y. Zhou:** None. **C. Huang:** None. **M. Li:** None. **Y. Mao:** None.

Poster

836. Proliferation: Cell Cycle and Molecular Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:836.02/A2

Topic: A.02. Neurogenesis and Gliogenesis

Support: Vision Science Research Program

NSERC

University of Toronto-School of Graduate Studies

Fighting for Blindness

Title: Dmbx1 is required for cell cycle progression of retinal and tectal progenitors during neurogenesis in zebrafish

Authors: *L. WONG, V. TROPEPE
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Abstract: Neurogenesis is a process by which progenitor cells proliferate and subsequently undergo terminal differentiation to become various subtypes of neurons and glia. Cell cycle duration of these progenitor cells is crucial for regulating the growth (through proliferation) and cell specification (timing of cell cycle exit) in order to generate regional diversity in developing neural tissue. A key regulator of cell cycle progression of progenitor cells in the zebrafish retina and brain is a transcription factor called diencephalon/mesencephalon homeobox 1 (*dmbx1*). Loss of function of *dmbx1* using antisense morpholino oligonucleotides showed that progenitor cells in the retina and the optic tectum were progressing through the cell cycle at least 5 times slower in the absence of *dmbx1*. As a result, most of these progenitors were still cycling and unable to differentiate on time. In the present study we determined that the expression of *cyclinD1*, which is an important factor that promotes G1 to S transition and guides the cell to re-enter the cell cycle, was sustained in progenitor cells with reduced *dmbx1* levels. This finding suggested that Dmbx1 might repress *cycinD1* expression and allow cells to become post-mitotic and undergo differentiation. By knocking down *cyclinD1* and *dmbx1* simultaneously, we showed that cells were now able to exit the cell cycle and differentiate. We are testing the hypothesis that Dmbx1 is a direct transcriptional repressor of *cyclinD1*. Together, our findings highlight an important relationship between homeobox related transcription factors and the cell cycle machinery in the regulation of the timing of neurogenesis.

Disclosures: L. Wong: None. **V. Tropepe:** None.

Poster

836. Proliferation: Cell Cycle and Molecular Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:836.03/A3

Topic: A.02. Neurogenesis and Gliogenesis

Support: National Nature Science Foundation of China 81000485, 30525017, 30770696, 81070904

The Ministry of Science and Technology of China 2007CB512303

Title: FoxM1 is involved in interkinetic nuclear migration by controlling the length of cell cycle in the developing telencephalon

Authors: *X. WU, X. GU, Y. JIANG, X. HAN, Y. YUAN, Y. WANG, C. ZHAO
Neurodevelopment Lab., Inst. of Life Sci., Jiangsu, China

Abstract: During telencephalic development, interkinetic nuclear migration (INM) is a remarkable feature of neuroepithelium which is designed to make full use of germinal zone and maximize its output. By this way, the neuroepithelium can generate such a great number of new born neurons that make up the entire cerebral cortex. The migration of nuclear of apical progenitors (APs) is reported to be highly related with cell cycle, however the detailed mechanism between INM and cell cycle regulators has not been revealed. Here we first report that conditional disruption of the key cell cycle regulator FoxM1 in dorsal telencephalon results in abnormal INM and affect the transition from apical to basal progenitors by controlling the cell cycle length.

In this study, we first examined the expression of FoxM1 by in situ hybridization and found that FoxM1 was specifically expressed at the germinal zone of the telencephalon during embryonic development. Conditional disruption of FoxM1 in developing dorsal telencephalon by crossing FoxM1fl/fl mice with Emx1-Cre results in decreased proliferation of APs. Meanwhile the basal-to-apical INM is observed to be slowed-down, indicating the S or G2 phase may be affected. We further measured the length of S phase by 2h IdU and 0.5h BrdU double labeling, and found that in control the length of S phase was about 5.5 hour long, whereas lengthened to 8 hours in FoxM1 cko. More cells are observed to be remained in the cell cycle when a 14 hours-BrdU labeling was preformed at E14.5, which is consistent with the slowed INM. Moreover, among PH+ M phase cells, the percentage of PH3+ Tbr2+ basal progenitors is increased in FoxM1 cko when compared to that of control. These results indicate that ablation of FoxM1 slows INM by lengthening cell cycle and may play an important role in the transition from APs to BPs. In brief, FoxM1 is required for INM of APs in the developing telencephalon and involved in the neuron differentiation. Further studies are needed to elucidate the molecular mechanisms.

Disclosures: X. Wu: None. **X. Gu:** None. **Y. Jiang:** None. **X. Han:** None. **Y. Yuan:** None. **Y. Wang:** None. **C. Zhao:** None.

Poster

836. Proliferation: Cell Cycle and Molecular Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:836.04/A4

Topic: A.02. Neurogenesis and Gliogenesis

Support: UPM RUGS 04-01-11-1163RU

MOHE ERGS/1/11/SKK/UPM/03/1

NHMRC Grants 219176, 257501 and 257529

Title: Molecular and functional characterisation of a novel long noncoding RNA at Sox4 gene locus in the developing mouse brain

Authors: *K.-H. LING[1,3,4], P. J. BRAUTIGAN[3], S. MOORE[3], R. FRASER[3], P.-S. CHEAH[2,5], J. M. RAISON[6], M. STANKOVIC[3], Y.-K. LEE[3], T. DAISH[5], D. M. MATTISKE[7], J. R. MANN[7], D. L. ADELSON[5], P. Q. THOMAS[5], C. N. HAHN[3], H. S. SCOTT[3]
[1]Clin. Genet. Unit, Dept. of Obstectrics & Gynaecology, [2]Dept. of Human Anat., Universiti Putra Malaysia, UPM Serdang, Malaysia; [3]Dept. of Mol. Pathology, The Inst. of Med. and Vet. Sci. and The Hanson Inst., Adelaide, Australia; [4]Sch. of Med., [5]Sch. of Mol. and Biomed. Sci., [6]eResearchSA, Univ. of Adelaide, Adelaide, Australia; [7]Theme of Lab. and Community Genet., Murdoch Childrens Res. Institute, Royal Children's Hosp., Parkville, Australia

Abstract: The role of long noncoding RNAs (lncRNAs) in cellular development and regulatory processes are remained under-characterised. To date, only a handful of functionally characterised lncRNAs, such as *Xist*, *Evf-2*, *Air*, *HOTAIR* and *Kcnq1ot1*, were reported to have significant regulatory properties during development. In this study, we demonstrated the expression of multiple overlapping sense and natural antisense transcripts (NATs) at the *Sox4* gene locus in the *Mus musculus* C57BL/6 (mouse) brain. These NATs were spatiotemporally regulated throughout cerebral corticogenesis and formed dsRNA aggregates in the cytoplasm of brain cells with the *Sox4* mRNAs. The dsRNAs formation, however, did not alter the level of *Sox4* sense expression at RNA or protein level. Instead, the dsRNA served as a template to produce a novel endogenous siRNA (endo-siRNA), whose biogenesis is Dicer1-dependent. The endo-siRNA is expressed in the marginal and germinative zones of the developing and postnatal mouse brains suggesting a specific role in neurogenesis. To our knowledge, this is the first demonstration in the mammalian system that sense-NATs serve as Dicer1-dependent templates in the cytoplasm to produce an endo-siRNA. The finding will change the way we interpret these emerging class of noncoding RNAs and may explain the large proportion of uncharacterised biogenesis of various nontypical noncoding small RNAs in the mammalian system especially in neural plasticity.

Disclosures: **K. Ling:** Employment; Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia.. Research Grant; Universiti Putra Malaysia RUGS 04-01-11-1163RU, Ministry of Higher Education, Malaysia ERGS/1/11/SKK/UPM/03/1. **P.J. Brautigan:** None. **S. Moore:** None. **R. Fraser:** None. **P. Cheah:** Employment; Universiti Putra Malaysia. Research Grant; Universiti Putra Malaysia RUGS 04-01-11-1163RU, Ministry of Higher Education ERGS ERGS/1/11/SKK/UPM/03/1. **J.M. Raison:** None. **M. Stankovic:** None. **Y. Lee:** None. **T. Daish:** None. **D.M. Mattiske:** None. **J.R. Mann:** None. **D.L. Adelson:** None. **P.Q. Thomas:** Research Grant; Pfizer Australia. **C.N. Hahn:** None. **H.S. Scott:** Research Grant; NHMRC Grants 219176, 257501 and 257529.

Poster

836. Proliferation: Cell Cycle and Molecular Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:836.05/A5

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH/NIAAA AA016654

NIH/NHLBI HL64917

Title: Nox4-generated superoxide drives angiotensin II-induced neural stem cell self-renewal

Authors: *W. ZAWADA[1], E. TOPCHIY[3], E. PANZHINSKIY[3], S. W. BARGER[1,4], M. DAS[2]
[1]Geriatrics - The Reynolds Inst. on Aging, [2]Med., Univ. of Arkansas For Med. Sci., Little Rock, AR; [3]Univ. of Wyoming, Laramie, WY; [4]Grecc, Central Arkansas Veterans Healthcare Syst., Little Rock, AR

Abstract: Although reactive oxygen species (ROS) play an important role in neural development and stem cell responses in the adult CNS, the mechanism by which ROS affect neural stem cell (NSC) self-renewal is poorly understood. Because angiotensin II (Ang II) has been reported to increase numbers of neuronal precursor cells, we have hypothesized that Ang II drives NSC self-renewal by stimulating production of non-toxic levels of superoxide that serve as pro-proliferative signals. To address our hypothesis, we have modeled self-renewal in a murine neural stem cell line (C17.2). Addition of Ang II (100nM) to these NSC cultures stimulated superoxide production as detected by an increase in dihydroethidine (DHE) fluorescence. Significant elevation in superoxide was first observed one hour following stimulation with Ang II and returned to baseline by 24 hours. By 48 hours, a single treatment with Ang II doubled the number of NSCs in the culture and an antioxidant N-acetyl-cysteine (NAC) blocked this Ang II-induced response, a finding consistent with superoxide being responsible for the observed

increase in proliferation. Confocal microscopy using DHE and a mitochondrial marker (MitoTracker) revealed that C17.2 cells have not one, but rather two major sources of ROS with distinct sub-cellular localizations. These ROS sources were found to be either mitochondrial or extramitochondrial; indicating that in addition to superoxide production in the mitochondria, membrane bound NADPH oxidase might be responsible for the extramitochondrial generation of ROS. Although we have identified NADPH oxidase catalytic subunits Nox3 and Nox4 to be constitutively expressed in these cells, Nox4 mRNA was more abundant. Angiotensin II increased synthesis of Nox4 protein, whereas siRNA targeting Nox4 reduced Nox4 protein levels back to baseline and abolished Ang II-induced ROS production and the proliferative response. Our findings suggest that Ang II-induced proliferation of NSCs requires Nox4-generated superoxide. This mechanism might be important for neurogenesis during brain development as well as during disease states affecting the nervous system.

Disclosures: W. Zawada: None. **E. Topchiy:** None. **E. Panzhinskiy:** None. **S.W. Barger:** None. **M. Das:** None.

Poster

836. Proliferation: Cell Cycle and Molecular Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:836.06/A6

Topic: A.02. Neurogenesis and Gliogenesis

Title: mTOR signaling mediates GSK-3 regulation of neural progenitor proliferation in the developing brain

Authors: *M. KA, R. TELFER, W.-Y. KIM
Developmental Neuroscience, Munroe-Meyer Inst., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Recent studies have shown that glycogen synthase kinase-3 (GSK-3) is a master molecule in the regulation of neural progenitor proliferation and neurogenesis during nervous system development. However, it remains unclear what downstream mechanisms mediate the activities of GSK-3 in neural progenitors. Here, we show that the mammalian target of rapamycin (mTOR) signaling plays an important role as a downstream of GSK-3 in cortical progenitor regulation. First, we discovered that GSK-3 controls mTOR activity in neural progenitors. Deletion of GSK-3 increases phosphorylations of 4EBP1 and S6 proteins, markers for mTOR activity, while overexpression of GSK-3 decreases the phosphorylations. Then, we generated and characterized a conditional mutant mouse in which mTOR is deleted in neural progenitors in the developing nervous system. Genetic ablation of mTOR leads to abnormal

progenitor development. There are decreases in progenitor proliferation and neurogenesis in the developing brain. Finally, we found that knockdown of mTOR activity suppresses the hyperproliferation of neural progenitors induced by GSK-3 deletion. Together, these findings reveal that GSK-3 functionally interacts with mTOR signaling and this interaction is important in neural progenitor regulation during brain development.

Disclosures: M. Ka: None. **R. Telfer:** None. **W. Kim:** None.

Poster

836. Proliferation: Cell Cycle and Molecular Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:836.07/A7

Topic: A.02. Neurogenesis and Gliogenesis

Support: ANSEF biochem-2757

ANSEF biochem-2381

Title: Role of DPD in the metabolism of embryonic human brain derived cells

Authors: *K. DANIELYAN[1], O. MANUKYAN[2], T. MANUCHARYAN[2], R. ABRAHAMYAN[3], A. GALOYAN[1]
[1]H Buniatian Inst. of Biochem., H. Buniatian Inst. of Biochem., Yerevan, Armenia; [2]Biochem., Yerevan State Univ., Yerevan, Armenia; [3]Republican Maternity Hosp. , Inst. of Perinatology, Obstetrics and Gynaecology, Yerevan, Armenia

Abstract: Background. Previously by the utility of Allopurinol, classical inhibitor of Xanthine Oxidoreductase activity (EC 1.17.3.2), one of the last enzyme in the cycle of purines' catabolism, we have delineated the role of the last one in the processes of human brain E90 derived cells' proliferation and development (1). Dihydropyrimidine dehydrogenase (DPD; EC 1.3.1.2) catalyzes the initial and rate-limiting step in the catabolism of the pyrimidine bases - uracil and thymine. In our current work by the treatment of primarily human embryonic brain cell culture with the DPD inhibitor - Captopril we have estimated the metabolic activity of cultured cells by means of measurement of deposited amino acids pool.
Methods. We have used described by M.P. Mattson and B. Rychlik (2), method with some modifications. There was used the media with the d-Biotin, Catalase, L-Carnitine HCl, Corticosterone, Insulin, Progesterone, Putrescine, Superoxide Dismutase, DL Alpha-Tocopherol etc. Brain of the embryos was obtained from abortion material of the E90 fetuses. All procedures with the utility of biomaterials were carried out in accordance with the Declaration of Helsinki.

The statistics of the obtained results were calculated by the utility of t-student test and were considered significant if $p< 0.05$ (presented data are introduced as SEM). The amino acid pools was estimated by the classical Ninhydrine reaction method. Cells were grown over the 7 days with/without treatment of Captopril.
Results. In contrast to the control group the amino acid pool in the cells treated over 7 days with the Captopril was higher in statistically significant way (10.67 ± 0.02 vs 15.13 ± 2.43, $p<0.05$). Further addition of NADH, coenzyme of DPD didn't initiate the increase of amino acids quantity not in the control groups of the cells neither in the Captopril treated cells whereas addition of the Captopril into the final reaction mixture increases the quantity of amino acids in the control group in contrast to the Captopril treated group of the cells (12.22 ± 0.39; 13.86 ± 4.93; $p< 0.05$ after comparison with their above mentioned controls.
Conclusion. Treatment of the primerely human brain derived cells culture with the DPD inhibitor Captopril over 7 days initiates the increase in the amino acids pool.
Refernces. Danielyan KE, Kevorkian GA: Xanthine oxidase activity regulates human embryonic brain cells growth. Biopolym. Cell. 2011;27(5):350-353. Mattson MP, Ruchlik B: Cell culture of cryopreserved human fetal cerebral cortical and hippocampal neurons: neuronal development and resposes to trophic factors. Brain Research. 1990;552:2004-212.

Disclosures: K. Danielyan: None. **O. Manukyan:** None. **T. Manucharyan:** None. **R. Abrahamyan:** None. **A. Galoyan:** None.

Poster

836. Proliferation: Cell Cycle and Molecular Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:836.08/A8

Topic: A.03. Postnatal Neurogenesis

Support: NSERC Discovery Grant

UVIC start up funds

Title: Pannexin 1 regulation of neurogenesis

Authors: L. E. WICKI-STORDEUR, A. D. DZUGALO, R. PRAGER, R. M. SWANSBURG, *L. SWAYNE
Med. Sci., Univ. of Victoria, Victoria, BC, Canada

Abstract: The ventricular zone of the postnatal brain contains unspecialized neural stem cells. Through a complex developmental process initiating with these unspecialized cells, new neurons

are produced. This series of dynamic changes in cellular behaviours, collectively termed neurogenesis, is essential for the development of social memories and behaviours and also plays an important role in brain repair following injury. Ion channels are emerging as key regulators of this process. We recently detected pannexin 1 (Panx1) ion and metabolite-permeable channels in neural stem and progenitor cells (Wicki-Stordeur et al. Neural Development Mar 29;7(1):11). By manipulating Panx1 expression and activity we uncovered its important impact on cell proliferation. Panx1 mediates the release of adenosine triphosphate (ATP), which in turn, activates purinergic (P2) receptors to stimulate cell proliferation. To build on this foundational work, and expand our understanding of Panx1 regulation of neural stem and progenitor cell proliferation, we then identified protein interaction partners using an unbiased proteomics approach. Here we explore how these data have provided further insight into the role of Panx1 in neural stem and progenitor cells. As these special cells are highly responsive to neurological injury and disease, and are gaining attention as putative targets for brain repair, understanding the fundamental role of Panx1 channels in neural stem cells is of critical importance for brain health and disease.

Disclosures: L.E. Wicki-Stordeur: None. **A.D. Dzugalo:** None. **R. Prager:** None. **R.M. Swansburg:** None. **L. Swayne:** None.

Poster

836. Proliferation: Cell Cycle and Molecular Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:836.09/A9

Topic: A.03. Postnatal Neurogenesis

Title: From neonate to adult: Restricted phenotypic potential of newly born striatal neurons.

Authors: *J. WRIGHT, L. H. THOMPSON
Neurodegeneration, Florey Neurosci. Inst., Melbourne, Australia

Abstract: Substantial advances have been made in the last decade on our understating of the basic physiology underlying neurogenesis in the postnatal mammalian brain. The bulk of the work in this area has been based on analysis of the adult brain. Relatively less is known about the capacity for neurogenesis in specific structures within the neonatal brain. Here we demonstrate that the production of medium spiny striatal projection neurons (MSNs) in the striatum extends into the early neonatal period under normal physiological conditions in the rat brain, with low but stable contributions of striatal interneurons during this time-period. Using retroviral labeling, we further show how MSNs born neonatally, correctly acquire their axonal targets in the globus

pallidus and midbrain. These data provide evidence of latent MSN development in the neonatal striatum post-embryogenesis.
It has been recently shown that upon damage to the striatum as seen in stroke and Huntington's disease models, migratory neuroblasts born from the sub-ventricular zone (SVZ) lining the lateral ventricles are redirected into the lesioned striatum and mature into neurons. Whether adult neurogenic niches in the SVZ maintain the phenotypic plasticity as seen in the early neonatal brain and hence are able to contribute new MSN neurons to the damaged striatum remains controversial. This repair mechanism provides minimal replacement of lost neurons due to poor long-term survival. Therefore, in an excitotoxic forebrain injury model in adult rats, I have utilized two neurotrophic factors GDNF and the newly defined neurotrophic factor 'Meteorin' in an attempt to increase cell survival and migration of newly-born cells into the damaged striatum. In line with previous studies, GDNF lead to an increase in mature neurons 6 weeks post-injury, whilst Meteorin increase neuroblast migration into the damaged striatum. However, in all circumstances including lesion alone, no newly-born MSNs were found in the striatum only Calretinin+ interneurons, illustrating that phenotypic plasticity present in early neonatal SVZ neurogenic precursors is lost in the adult.

Disclosures: J. Wright: None. **L.H. Thompson:** None.

Poster

837. Visual System I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:837.01/A10

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NIMH grant MH084080

Title: Superior temporal sulcus activity to unexpected goal-directed motion decreases longitudinally in neurotypical children

Authors: A. AHMED, *B. C. VANDER WYK
Child Study Ctr., Yale Univ., New Haven, CT

Abstract: Objectives: To investigate longitudinal neurodevelopmental trends in social cognition using functional magnetic resonance imaging (fMRI).
Methods: Seventeen typically developing children (7 males, 10 females) participated in two fMRI sessions approximately 1 year apart. These longitudinal subjects had a mean age of 11.69 years at their first observation and 12.94 years at their second observation. At each visit, children were shown a 6'16'' fMRI paradigm consisting of a human actor who displays positive affect

(smile) or negative affect (frown) towards one of two cups on the screen. The actor then reaches and grabs one of the two cups in either a congruent or incongruent fashion. Congruent trials were defined as those that matched the displayed intention; the actor reached for the same cup if she displayed positive affect or the opposite cup if she displayed negative affect. Action did not match intention in the incongruent trials; the actor reached for the same cup if she displayed negative affect, or the opposite cup if she displayed positive affect. One full run consisted of 32 trials.
Regions-of-interest in the right and left superior temporal sulcus (STS) and the right dorsolateral prefrontal cortex were created using an independent sample of adults and children who viewed the same imaging paradigm. These functionally-defined regions represent significant clusters of activation that significantly differentiated incongruent and congruent conditions. Waveforms from these regions were extracted within the longitudinal sample and the peak response was defined as activation at 10 seconds after stimulus onset relative to initial activation.
Results: We investigated how the difference between incongruent and congruent conditions varied across observations. Peak brain responses in the left STS showed a significant difference between observations ($t = 2.325$, $p = 0.027$; mean at time 1 = +0.297, time 2 = -0.076). The right STS showed a similar decreasing trend but did not reach statistical significance ($t = 1.333$, $p = 0.192$; mean at time 1 = +0.216, time 2 = -0.012). Furthermore, the aggregate response to motion (the average of peak responses in both conditions) did not significantly differ between observations.
Conclusions: Preferential activation to incongruent stimuli in the left STS showed a drop-off leading to preferential activation to congruent stimuli between the initial viewing of the stimulus and a second viewing approximately one year later. This suggests that the left STS may be more sensitive to developmental changes than the right STS, and that these changes result in diminished peak brain responses to incongruent goal-directed motion.

Disclosures: A. Ahmed: None. **B.C. Vander Wyk:** None.

Poster

837. Visual System I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:837.02/A11

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: MRC Grant G0801242

MRC Grant G0600107

MRC Grant G1100162

Title: Functional imaging in the zebrafish optic tectum reveals emergent properties associated with visual motion processing

Authors: *P. HUNTER, A. S. LOWE, I. D. THOMPSON, M. P. MEYER
MRC Ctr. for Developmental Neurobio., King's Col. London, LONDON, United Kingdom

Abstract: In zebrafish the optic tectum is the principal target of retinal ganglion cells (RGCs) and is required for visually driven behaviours such as prey capture and predator avoidance. However, we know little about the specific computations that the tectum performs in order to elicit these behaviours. This is due, in large part, to the fact that little is known about the type of visual information that is delivered to the tectum by RGCs. We have previously addressed this by using *in vivo* functional imaging and a drifting bar stimulus to categorise orientation- and direction-selectivity in the population of RGC inputs to the tectum. Here we begin to use this functional description of retinotectal inputs as a basis for asking how these inputs are processed in the postsynaptic tectal neurons.
Using bulk loading of tectal neurons with the calcium indicator, Oregon Green BAPTA 488-AM, we apply the same drifting bar stimulus and unbiased, voxel-wise analysis strategy to characterise the population of tectal cells. Individual cell somas were automatically delineated by clustering ‘like’ voxels: neighbouring voxels that exhibit comparable descriptive statistics. The normalised vector sum and 1-circular variance defined cells as direction- and orientation-selective cells respectively. Cumulative statistics across multiple fish identified two populations of orientation-selective tectal cells that match those present in the population of RGC inputs. However, we identify a population of direction-selective tectal neurons that are tuned to head-to-tail motion - a response type not present in the RGC input population. To the best of our knowledge this is the first emergent property associated with tectal circuits in zebrafish.
By co-registering data from multiple larvae we can generate composite, functional parametric maps to reveal the spatial organisation of direction- and orientation-selective tectal cells. Registering these maps with similar parametric maps of the RGC inputs will show whether the retinotectal biases evident in the input populations are mirrored in the postsynaptic tectal cells. The behavioural significance of these emergent properties and functional architecture is something that can be addressed in the future using zebrafish as a model system.

Disclosures: P. Hunter: None. **A.S. Lowe:** None. **I.D. Thompson:** None. **M.P. Meyer:** None.

Poster

837. Visual System I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:837.03/A12

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: MRC PhD Programme

Title: Investigating the functional presynaptic variability of single cells in the larval zebrafish

Authors: *A. WALKER, M. P. MEYER
MRC Ctr. for Developmental Neurobio., King's Col. London, London, United Kingdom

Abstract: A major determinant of functional synaptic strength is the probability of Ca2+-induced neurotransmitter release from a presynaptic terminal. Release probabilities at individual presynaptic terminals can be diverse, even when they belong to the same axon. It is important to understand the underlying causes of synaptic heterogeneity as ultimately these determine the reliability, flexibility and diversity of information transfer in the brain. Here we use expression of a presynaptically targeted, genetically encoded calcium indicator (SyGCaMP3) in single zebrafish retinal ganglion cells (RGCs) to study presynaptic functional heterogeneity across an axonal arbour in vivo. Because the visual system of zebrafish is functional well before the structural development of visual circuits is complete we can use visual stimulation and functional imaging to characterise synaptic properties as RGC axonal arbours develop. Our strategy provides an opportunity to study, in parallel, the development of cell-wide tuning properties of single, identified, RGCs in vivo. By expressing SyGCaMP3 in individual RGCs we are able to target direction- and orientation-selective cells and record visual stimulus-induced calcium responses in discriminated presynaptic boutons. Thus, we can record the magnitude of responses of all presynaptic sites belonging to a single neuron, and assess the spatial organisation by synaptic weight across the axonal arbour. By imaging the same cell throughout development we can not only determine how direction- and orientation-selectivity emerges, but also how synapse functional heterogeneity changes as a function of developmental stage and axon arbour complexity.

Disclosures: A. Walker: None. **M.P. Meyer:** None.

Poster

837. Visual System I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:837.04/A13

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NSERC 7660 (DM)

NSERC (KD)

CIHR (DM, KD)

CPR Network; James S macDonnell Foundation

Title: The rapid recovery of visual acuity promoted by short periods of darkness in amblyopic kittens can be accompanied by the passive acquisition of stereopsis.

Authors: *D. E. MITCHELL[1], K. DUFFY[1], K. HOLMAN[1], K. MACNEILL[2]
[1]Dept Psychology, Dalhousie Univ., Halifax, NS, Canada; [2]Orthoptics, IWK Hosp., Halifax, NS, Canada

Abstract: Earlier we demonstrated (O'Leary et al, *Frontiers Syst. Neurosci* 6: 22, 2012) that short periods of darkness reduce labeling for neurofilament, a cytosketon protein that forms a stable intracellular scaffold and supports neuron structure. In some respects, the change in neurofilament mimics a prior developmental stage. Could this alteration in cytoskeleton protein signal an increased level of plasticity and a greater capacity for recovery from amblyopia?
To investigate this issue, 9 kittens were rendered amblyopic by monocular deprivation from either P7 or P30 until P37. Following introduction of binocular vision at P37, kittens were placed in darkness for 10 days (a dark pulse) either immediately after MD (ID Group; N=4) or 5-8 weeks later (DD Group; N=5). Acuity measurements for gratings were made both binocularly and monocularly by means of a jumping stand. Once acuity had stabilized for at least 2 months, at which time no animal evinced any amblyopia, monocular and binocular measurements were made of depth discrimination thresholds with stimuli that presented sparse monocular depth cues (Mitchell et al, *Perception* 8: 389-396, 1979).
Kittens from the ID group (dark pulse immediately after MD) initially appeared blind in both eyes. Form vision returned in 1- 3 days after which the visual acuity of each eye improved gradually in tandem to normal levels in 2 months. *Amblyopia never developed.* The severe amblyopia that developed in animals from the DD group disappeared rapidly in 5-7 days following the dark pulse. By comparison to animals from the ID group, the dark pulse had no effect on the acuity of the nondeprived eye indicating a short critical period for the effects of a dark pulse on vision (<70 days). For 5 animals, binocular thresholds were an order of magnitude or more better than monocular thresholds; the large binocular superiority makes it likely that they possessed at least local stereopsis. As the binocular depth thresholds of the 4 remaining animals were no better than monocular thresholds, it is likely that they were stereoblind. There was no indication that animals from the ID group, who never developed amblyopia, were more likely to develop stereopsis than those in which the dark pulse was delayed.
Although it is tempting to attribute the rapid changes in acuity and the acquisition of stereopsis to cytoskeletal modification, darkness likely exerts other effects including changes in the excitatory-inhibitory balance in the visual cortex. The dramatic beneficial improvement in visual

acuity and stereoscopic vision suggests that complete elimination of light to both eyes for 10 days may facilitate current behavioral therapies for amblyopia.

Disclosures: D.E. Mitchell: Research Grant; NSERC, CIHR, James S MacDonnell Foundation. **K. Duffy:** Research Grant; NSERC, CIHR. **K. Holman:** None. **K. MacNeill:** None.

Poster

837. Visual System I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:837.05/A14

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Title: Peeking through eggshells - How does light induce visual asymmetries in pigeons before hatch?

Authors: *F. STROECKENS, O. GUNTURKUN
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Abstract: Pigeon embryos take an asymmetrical prehatch position with the right eye pointing towards the semi-translucent shell, resulting in a higher level of visual stimulation of the right eye. This asymmetric light stimulation leads to the development of visual pathway asymmetries and lateralized visual behavior. However, at this stage of development retinal photoreceptors are still not functional, meaning light stimuli must be perceived by a different receptor. It is known that beside the classic rod/cone photoreceptor system a second instance of photoreceptive pigments exists inside retinal ganglion cells (RGC's). Those cells, localized in the ganglion cell layer mature early during embryonic development and establish their first contacts to the optical tectum before the critical phase of asymmetry induction occurs. Therefore, those photoreceptive RGC's are very likely the photoreceptive structure inducing lateralization. Studies in mammals and avian species show, that Cryptochrome 1b (Cry1b), thus far known to be responsible for entrainment of the “inner clock” during circadian rhythm but without any image giving function is expressed in RGC’s. We assume that Cry1b-containing RGC's are also responsible for inducing asymmetry formation during embryonic development in pigeons. To verify this assumption, we used an avian specific Cry1b antibody to label possible expressions sides in retinae of adult, post hatch and embryonic pigeons to test; 1) if Cry1b is in general expressed in pigeons; 2) if Cry1b is expressed during the critical time of asymmetry induction during and shortly after embryonic development. We found, that Cry1b is expressed in a subset of RGC’s and few displaced ganglion cells in adult, post hatch and embryonic pigeons. However,

expression in post hatch and embryonic pigeons was weaker in comparison to adults. Our results show that Cry1b is expressed in the pigeon's retina and is present during the critical period of asymmetry induction. Therefore Cry1b is a feasible candidate for the induction of visual asymmetries in pigeons. We currently plan to double label Cry1b positive cells in light and dark incubated embryonic pigeons with activity markers to show an activation of these cells towards light as well as tracing study to investigate projection targets of Cry1b containing RGC's. In combination with behavioral asymmetry testing of animals which were raised under wavelength only activating Cry1b, this might finally deliver the mechanism initializing visual asymmetries in birds.

Disclosures: F. Stroeckens: None. **O. Gunturkun:** None.

Poster

837. Visual System I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:837.06/A15

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: Children's Glaucoma Foundation

Sharon Stewart Aniridia Research Trust

Title: Evaluation of bmp4 signaling in early eye development in mouse embryo culture system

Authors: *V. K. KALASKAR[1], J. D. LAUDERDALE[2]
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Abstract: Embryos overexpressing (18 copies) 'pairedless' Pax6 (Pax6ΔPD), an isoform of Pax6 gene, exhibit lens degeneration with concomitant upregulation of Bmp4 in the optic cup and PSMAD -1/5/8 expression in the lens tissue. We used a mouse embryo culture system to evaluate the role of BMP4 signaling in these tissue interactions. Wild-type embryos were CD1-C57BL/6J. Embryos with 8 or 18 copies of Pax6ΔPD express green fluorescent protein (GFP) under the control of Pax6 promoter. Embryos at E10.5 were cultured in a standardized serum free stem cell culture medium in an oxygenated rolling culture system. BMP4 or BSA treated affi-gel beads were implanted adjacent to the optic cup. Wild-type E10.5 mouse embryos cultured for 24 to 36 hours showed morphological development and tissue specific gene expression in the ocular tissue consistent with early eye development. BMP4 bead implantation dorsal to the optic cup resulted in upregulation of PSMAD-1/5/8 expression in the surrounding mesenchymal cells and in the dorsal optic cup. BMP4 resulted in expansion of the dorsal region

and under-development of the ventral region of the optic cup and affected the expression tissue specific genes like Pax6. Implantation at 29 - 30 somite stage (E10) inhibited the initiation of pigmentation, while at 34 - 35 somite stage (E10.5) resulted in decreased pigmentation in the retinal pigmented epithelium (RPE). Our results indicate that, BMP4 signaling regulates pigmentation in the RPE in a stage-dependent manner and affects the dorsal-ventral patterning of the optic cup. We believe this mouse embryo culture system will be useful for laboratories needing to utilize whole embryos to study signaling interactions important in early eye development.

Disclosures: V.K. Kalaskar: None. **J.D. Lauderdale:** None.

Poster

837. Visual System I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:837.07/A16

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: EY011261 to HTC

Title: Inter-tectal connections mediate visual avoidance behavior in Xenopus

Authors: *A. C. GAMBRILL, W. SHEN, H. T. CLINE
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Abstract: Interhemispheric connections play an important role in mediating activity in multiple experimental models. The existence of axons crossing the midline from one lobe of the optic tectum to the other in Xenopus has been anecdotally documented; however the function of these inputs is still unknown. While retinal inputs to the optic tectum are almost exclusively contralateral, we find that neurons in the optic tectum send axon outputs to contralateral tectum and that disruption of the rostral-posterior portion of these commissures abolishes visually-guided avoidance behavior, indicating a critical role for these connections in processing visual information. The optic tectum, the equivalent of the mammalian superior colliculus, receives and integrates multimodal sensory inputs. To determine the role of inter-tectal connections in the development of the neural networks which shape visual behavior, and in the integration of information from multiple modalities, we have undertaken an anatomical and electrophysiological characterization of inter-tectal connections. We show that these processes are distributed throughout the rostrocaudal axis of the optic tectum, and that a significant fraction of neurons in the tectum receive either direct or indirect input from the contralateral lobe. Stimulation of ipsilateral tectum using a bipolar electrode can evoke synaptic responses in the

contralateral lobe of the optic tectum. These synaptic responses are comprised of both excitatory currents, which are sensitive to blockade with AMPAR- and NMDAR- selective antagonists (57.7 +/- 7.5% blockade when recorded at +40mV in the presence of 2uM NBQX and 50uM APV), as well as inhibitory currents, which are sensitive to blockade with GABA-A-antagonists (61.6 +/- 5.2% blockade when recorded at 0mV with 40uM picrotoxin). We have characterized these responses from stage 44 to stage 48, a developmental window critical for synaptic and network maturation in Xenopus. We conclude that through a combination of excitatory and inhibitory input, inter-tectal fibers play a critical role in mediating visual behavior.

Disclosures: A.C. Gambrill: None. **W. Shen:** None. **H.T. Cline:** None.

Poster

837. Visual System I

Location: Hall F-J

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Program#/Poster#:837.08/A17

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NSF (MB)

SBE 0542013 to the Temporal Dynamics of Learning Center

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IBRO Return Home Grant (YL)

Title: Developmental changes in inter-subject correlation of cortical activity.

Authors: *Y. LERNER[1], S. SCHERF[2], M. KATKOV[3], M. BEHRMANN[4], U. HASSON[5]
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Abstract: In typical adults, a surprising amount of shared responses across different individuals is found during free viewing of real life movies (Hasson et al., 2004). These findings indicate that a large extent of human adult cortex is stereotypically responsive to naturalistic audiovisual stimuli and lead to natural questions about how these shared responses emerge developmentally. To investigate this issue, we used functional magnetic resonance imaging (fMRI) to measure the inter-subject correlation of neural responses evoked by an 11-minute child appropriate movie

('Escape to Witch Mountain'), in children (ages 9-14), adolescents (ages 15-19) and adults (ages 20-32). The resulting fMRI time course acquired from each brain area in each individual was used to compute within-age group correlations (children-children, adolescents- adolescents, adults-adults) and between-age group correlations (children-adults, adolescents-adults). The analysis was performed separately in each area defined by the cluster analysis of local responses, without the need to hypothesize a priori about the differences in the response patterns across age groups. The within-age group correlations assess whether each brain area responds reliably to the movie within a given age group, where the comparison to the adult responses assesses whether each brain area responds in a stereotypical “adult-like” pattern across age groups. Our results showed that the responses in primary sensory areas in children and adolescents are very similar to the responses observed in the adults, suggesting that these areas functionally mature at early stages of the development. However, some regions exhibited higher within-age correlation in the children and/or adolescents than within the adults. We plot the brain responses across whole cortex and identify the different patterns of maturation through which these areas evolve. These findings reflect quantitative changes in the widespread cortical networks that support emerging stereotypical responses to naturalistic stimuli and will have important implications for future studies of cortical development.

Disclosures: Y. Lerner: None. **S. Scherf:** None. **M. Katkov:** None. **M. Behrmann:** None. **U. Hasson:** None.

Poster

837. Visual System I

Location: Hall F-J

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Program#/Poster#:837.09/A18

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NSC-100-2311-B-002-010

NSC 100-2321-B-002-001

Title: Presynaptic SNAP-25b regulates the firing size of retinal waves during development

Authors: *Y.-T. HSIAO[1], P.-C. HUANG[1], Y.-C. CHEN[1], S.-Y. KAO[1], C.-T. WANG[1,2,3,4]
[1]Inst. of Mol. and Cell. Biol., [2]Dept. of Life Sci., [3]Neurobio. and Cognitive Sci. Ctr., [4]Genome and Systems Biol. Program, Natl. Taiwan Univ., Taipei, Taiwan

Abstract: SNARE complex plays an important role in neurotransmitter release. SNAP-25 (SN25), one of three SNARE proteins, can be phosphorylated by PKA and PKC, providing the

versatility in the mechanisms regulating neurotransmitter release. Our previous studies in PC12 cells suggested that SN25b phosphorylation by PKA and PKC differentially regulates fusion pore kinetics during Ca^{2+}-dependent exocytosis. However, it is unclear whether SN25b in presynaptic neurons could further influence the large-scale network activity. Retinal waves, patterned spontaneous activity in the developing binocular retina, are initiated by neurotransmitter release and thus serve as a useful model for the study of synaptic transmission on a large scale. In this study, we determined whether SN25b could modulate the characteristics of retinal waves. Wild-type SN25b (wt-SN25b) was specifically overexpressed in presynaptic starburst amacrine cells (SACs) of neonatal rat retinas. By combining molecular perturbation and live calcium imaging, we examined the changes of wave properties in the postsynaptic retinal ganglion cells (RGCs). Wave frequency remained the same by overexpressing wt-SN25b in SACs compared to control, suggesting that the expression level of SN25b in presynaptic SACs may not involve in regulating wave initiation. In contrast, both duration and intensity of single waves were significantly increased by overexpressing wt-SN25b compared to control, suggesting that SN25b overexpression in presynaptic SACs enhances wave firing size. Given that retinal waves are augmented by cAMP-PKA signaling, our results thus suggest that the expression level of SN25b in presynaptic SACs may up-regulate the firing size of retinal waves, possibly through phosphorylation by cAMP-PKA signaling.

Disclosures: Y. Hsiao: None. **P. Huang:** None. **Y. Chen:** None. **S. Kao:** None. **C. Wang:** None.

Poster

837. Visual System I

Location: Hall F-J

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Program#/Poster#:837.10/A19

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: FAPESP (2009/53595-6)

CNPq (303822/2010-4)

CNPq (400837/2010-2)

CNPq (478755/2011-2)

Title: Cartesian and non-Cartesian contrast sensitivities are changed in acute and early malnourished children

Authors: *C. C. ALENCAR[1], N. A. SANTOS[2], L. R. G. BRITTO[1]
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Abstract: Acute malnourished (AM) children present current malnutrition, resulting in normal height according to age but underweight according to height. Early malnourished (EM) children have experienced inadequate nutrition during the critical period of nervous system development, followed by nutritional recovery, resulting in a short stature according to age but normal weight according to the short stature. We measured Cartesian and non-Cartesian contrast sensitivities (CS) in sixty children of both sexes, aged 7 to 11 years-old (M=9.2, SD=.99). Twenty children were eutrophic (E) - without malnutrition, twenty were AM and twenty were EM, based on state healthcare records and Waterlow's anthropometric parameters. CS to four spatial frequencies (.25, 1.0, 2.0, and 8.0 cycles per degree [cpd]) was measured using a temporal two-alternative forced-choice psychophysical method, with mean luminance of 40.1 cd/m2. The Repeated Measure Analysis of Variance applied to the Cartesian CS showed significant differences between the groups [$F(2,57)=48.5, p<.05; \alpha=1$]. The Fisher LSD post hoc test showed that the AM children were 1.10 and 1.11 times less sensitive than the E group to the frequencies of 1.0 and 2.0 cpd, respectively. The EM group was 1.34 and 1.24 times less sensitive than the E and AM groups, respectively, to the frequency of .25 cpd; 1.60 and 1.45 to the frequency of 1.0 cpd; 1.53 and 1.37 to the frequency of 2.0 cpd. The EM children were 1.27 times more sensitive than the E group to the frequency of 8.0 cpd. The Repeated Measure Analysis of Variance applied to the non-Cartesian CS showed significant differences between the groups [$F(2,57)=13.3, p<.05; \alpha=1$]. The Fisher LSD post hoc test showed that the EM children were 1.48 and 1.35 times less sensitive than the E and AM groups, respectively, to the frequency of 8.0 cpd. The AM group was 1.12 and 1.09 times less sensitive than the E and EM groups, respectively, to the frequency of 1.0 cpd; 1.12 and 1.08 to the frequency of 2.0 cpd. These results suggest that acute malnutrition impairs CS to medium-spatial-frequencies Cartesian and non-Cartesian gratings, whereas early malnutrition impairs CS to low-to-medium-spatial-frequencies Cartesian gratings and high-spatial-frequencies non-Cartesian gratings in children. The assessment of Cartesian and non-Cartesian CS may help establish a more nuanced characterization of the effects of malnutrition on the developing visual system and aid in diagnosis, prognosis, and follow-up treatment in children who experienced malnutrition.

Disclosures: C.C. Alencar: None. **N.A. Santos:** None. **L.R.G. Britto:** None.

Poster

837. Visual System I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:837.11/A20

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: EY015387

EY11369

EY03991

Title: Spontaneous retinal activity is critical for establishing the anatomical and functional organization of the lateral geniculate nucleus in the absence of eye-specific segregation

Authors: *S. W. FAILOR, B. CHAPMAN
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Abstract: The lateral geniculate nucleus (LGN) in ferrets undergoes a series of developmental periods involving the formation of eye-specific laminae, sublaminae that receive input from ON-center and OFF-center retinal ganglion cells, and the refinement of synaptic inputs to individual geniculate cells. These developmental processes result in highly stereotyped laminar patterning (Speer et al. 2010), receptive field structures (Tavazoie et al., 2000), and retinotopy (Zahs et al., 1985). It has been well established that spontaneous retinal activity plays a role in eye-specific segregation (Penn et al., 1998; Stellwagen et al., 2002), and additionally retinotopic refinement in mice (Grubb et al., 2003); here we show that in the ferret spontaneous retinal activity plays a role in the establishment of correct retinogeniculate lamination, as well as both receptive field structure and retinotopy, when eye-specific segregation is absent.

Ferrets at postnatal day zero (P0) were monocularly enucleated to eliminate the retinogeniculate afferents from one eye. Starting at P0 the remaining eye was injected with either epibatidine (epi) or saline intravitreally every 48 hours until P10 to disrupt spontaneous retinal activity (Penn, et al. 1998; Sun et al. 2008). In epi treated enucleates the shape of the ipsilateral (ipsi) lamina failed to expand, as it normally does due to enucleation, and its borders were unrefined, whereas the contralateral (contra) lamina lacked afferents in a caudal medial region. When enucleates were allowed to recover to P25 following epi injections, abnormal lamination was again observed for both the ipsi and contra projection. In these animals the ipsi lamina was fragmented and both ipsi and contra lamina lacked the defined ON/OFF sublaminae seen in control enucleates. Adult ferrets (P60+) were anesthetized and single-unit electrophysiology recordings were carried out to characterize the functional organization of the LGN following early enucleation and epi treatment. Areas of the LGN in epi treated animals contained cells that developed abnormal receptive field size and structure, with retinotopy also being disrupted.

Our results align with previous work in supporting a role for activity in retinotopic refinement; additionally we demonstrate that activity drives the establishment of retinogeniculate lamination even in the absence of eye-specific segregation. These results suggest that activity-dependent mechanisms besides Hebbian-like axonal competition are at play during development, such as

the readout of axon guidance molecules or the competition-independent refinement and/or elaboration of retinogeniculate axons.

Disclosures: S.W. Failor: None. **B. Chapman:** None.

Poster

837. Visual System I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:837.12/A21

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NIH grant EY013588

Title: Developmental refinement of LGN receptive fields contributes to the maturation of orientation selectivity in the cortex

Authors: *C. W. KILEY, W. M. USREY
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Abstract: In contrast to the growing number of studies providing support for the Hubel and Wiesel model of the organization of geniculocortical connections that underlie the receptive field properties of simple cells, we know relatively little about the mechanisms that guide the specificity and refinement of these connections during development. To test the hypothesis that correlation-based mechanisms may play a role, we investigated the relationship between the maturation of LGN receptive fields and the refinement of correlated activity patterns between neighboring LGN neurons. Single-unit recordings were made from small ensembles of LGN neurons in the kitten at various stages of development to compare the spatiotemporal receptive field properties of individual neurons with the patterns of the cells' correlated activity evoked by stimuli drifting at various orientations. Our results demonstrate a tight correspondence between the size and location of LGN receptive fields and the orientation tuning of their correlated activity. In particular, as LGN receptive fields mature and decrease in size, the number of neurons co-excited by a common stimulus decreases and the orientation tuning of their correlated activity sharpens. These results support the idea that the refinement of receptive fields in the LGN contributes to the development of orientation tuning in the cortex.

Disclosures: C.W. Kiley: None. **W.M. Usrey:** None.

Poster

837. Visual System I

Location: Hall F-J

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Program#/Poster#:837.13/A22

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NIH Grant EY019578-02

Title: Information processing in the optic tectum mediates collision avoidance behavior in the Xenopus laevis tadpole

Authors: *A. S. KHAKHALIN, D. KOREN, J. GU, C. D. AIZENMAN
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Abstract: The optic tectum of anuran tadpoles is a main integrative sensory center in these animals, mediating orienting behaviors, such as collision avoidance, schooling, and visually-driven escape responses. While a lot is known about the development and cellular organization of the optic tectal circuitry, it is still unclear how information coming from the environment is encoded in the activity of tectal cells, and how the computations are performed.
Here we describe a collision avoidance behavior in Xenopus laevis tadpoles, and through a series of sequentially reduced preparations we illustrate the mechanisms underlying this behavior. In the first set of experiments we presented freely swimming animals with computer-generated two-dimensional objects, approaching them at either collision or non-collision trajectories. By tracking the animals, and by reconstructing the geometry of approaches, and the projections computer-generated objects would have on the retinas, we identified temporal and spatial properties of those stimuli that successfully triggered the collision avoidance response. Then, in the second set of experiments, we presented partially immobilized animals with computer generated stimuli that were projected on a microfiber screen placed near one of the eyes. The stimuli were specifically designed so that their retinal projections mimicked our reconstructions from the first set of experiments. A high speed camera was used to track the movements of the tail, and we show that different stimuli induced different sub-variants of the avoidance motor program, with different latencies, and probabilities. Finally, we presented the same set of stimuli to fully immobilized animals, recording either the spiking activity, or the inhibitory and excitatory postsynaptic currents from individual cells in the optic tectum. We show that the spatial and temporal computations that are required for the identification of behaviorally relevant stimuli start at the level of the ganglion cells in the retina, and continue in the optic tectum, where they rely on the temporal interaction between excitatory and inhibitory drive to the tectal cells.

Disclosures: A.S. Khakhalin: None. **D. Koren:** None. **J. Gu:** None. **C.D. Aizenman:** None.

Poster

837. Visual System I

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Program#/Poster#:837.14/A23

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: DFG (Deutsche Forschung Gemeinschaft

Title: Role of SPAR3/Sipa1L3 in lens and brain development

Authors: *N. KANWAL[1], J. BOCKMANN[1], M. KÜHL[2], S. J. KÜHL[2], T. M. BOECKERS[1]
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Abstract: Spine associated Rap-GAP 3 is a novel, hitherto uncharacterized component of the post synaptic density (PSD). SPAR3 belongs to the SPAR family of small GTPase activators. It is well conserved among species (Rattus norvegius, Mus musculus, Xenopus laevis), and consists of 20 exons encoding 1776 amino acids in mouse.
We generated SPAR3 knockout mice to uncover the functional properties of this protein in vivo. SPAR3 mutant mice display a severe phenotype of juvenile cataract. Already during fetal development an abnormal protrusion of the lens, an altered structure of the optical cup surrounding the developing lens can be observed. Moreover, further investigation revealed an abnormal arrangement, shape, structure and packing of lens fiber cells. Interestingly, most of the lens fiber cells retain their nuclei. Known interaction partners of SPAR3 (LAPSER1, beta-catenin) and neuronal marker proteins (ßIII-Tubulin, MAP2, Synaptophysin, PSD95) are up-regulated in lenses of mutant mice, but not in the respective brain regions. Furthermore, other members of the SPAR family (SIPA, SPAR1, and SPAR2) are highly expressed in brain, but not in lenses thus implicating possible compensatory effects of these family members in brain. Taken together SPAR3 seems to have a crucial role during early lens development and that lens fiber cells in SPAR3 knockouts may lose their early differentiation path. Further investigations of our SPAR3 mutants will help to elucidate the functional aspects of this novel protein during the development of the ocular system.

Disclosures: N. Kanwal: None. **J. Bockmann:** None. **M. Kühl:** None. **S.J. Kühl:** None. **T.M. Boeckers:** None.

Poster

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Program#/Poster#:837.15/A24

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NSC-100-2311-B-002-010

NSC 100-2321-B-002-001

Title: Synaptotagmin III regulates the firing rate of retinal waves in the developing rat retina

Authors: *S.-Y. KAO[1], Y.-C. CHEN[1], C.-T. WANG[1,2,3,4]
[1]Inst. of Mol. and Cell. Biol., [2]Dept. of Life Sci., [3]Neurobio. and Cognitive Sci. Ctr., [4]Genome and Systems Biol. Program, Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Binocular visual circuit refinement requires a robust patterned spontaneous activity in the developing retina (i.e. retinal waves). In postnatal rats (P0-P9), retinal waves are initiated via a subset of starburst amacrine cells (SACs) releasing acetylcholine (ACh) onto postsynaptic retinal ganglion cells (RGCs). Synaptotagmin (Syt) family serves as a calcium sensor during Ca^{2+}-regulated exocytosis to mediate neurotransmitter release. Although more than seventeen Syt isoforms have been discovered, the Syt isoform responsible for Ca^{2+}-regulated exocytosis in the initiation of stage-II waves remains obscure. Our previous findings suggested that Syt III is abundantly expressed in the RGC layer and inner plexiform layer (IPL) during the first week of postnatal rats, consistent with the developmental time course of stage-II waves. In this study, we used molecular perturbation and subsequent live Ca^{2+} imaging to determine the role of Syt III in regulating retinal waves. Whole-mount developing rat retinas (P0-P2) were transfected by the DNA vectors carrying Syt III or antisense-Syt III. Control was the vector carrying the transfection marker EGFP only. After 70 hours post transfection, spontaneous and correlated Ca^{2+} transients in the developing RGCs were imaged by the Ca^{2+} indicator fura-2 at 1 s-interval for 10 min. By calculating the interval of calcium transients associated with retinal waves, we found that inter-wave interval was significantly decreased in Syt III-overexpressing retinas compared to control. In contrast, inter-wave interval was not changed in antisense-Syt III-overexpressing retinas compared to control. Overexpressing Syt III or antisense-Syt III did not alter the duration and intensity of single waves, indicating that the firing size of retinal waves remains the same. Thus, our results suggest that Syt III regulates the firing rate of stage-II waves in the developing rat retina.

Disclosures: S. Kao: None. **Y. Chen:** None. **C. Wang:** None.

Poster

837. Visual System I

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Program#/Poster#:837.16/A25

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: Swiss National Foundation Grant 3100A0_122527/1 and 31-138676

Title: Nogo-A influences the eye-specific segregation of retinogeniculate projections

Authors: *A. M. GUZIK-KORNACKA, V. E. PERNET, M. E. SCHWAB
Brain Res. Institute, Univ. of Zurich/ETH, Zurich, Switzerland

Abstract: The retinogeniculate pathway of the mouse is an excellent model to study the refinement of connections and stabilisation of axonal arbours and synapses. Before eye opening, retinal axons from the two eyes in the dorsal lateral geniculate nucleus (dLGN) undergo extensive pruning and segregate into non-overlapping territories. After eye opening, retinogeniculate synapses are further strengthened and stabilized, or pruned, respectively. Nogo-A, a potent neurite growth inhibitory protein, is highly expressed in early postnatal retinal ganglion cells (RGCs), whereas in the adult visual system it is mainly present in myelin. The developmental switch in localization of Nogo-A coincides with the maturation of retinogeniculate system. There is a growing body of evidence that Nogo-A restricts synaptic plasticity; e.g. Nogo-A/B knock out (KO) or Nogo receptor (NgR1) KO mice have been shown to possess high visual cortex plasticity in adult life, far beyond the end of the so-called critical period. Here we compared the segregation of retinogeniculate axonal terminals from both eyes between the wild type and Nogo-A KO mice. We visualised retinal axons from the two eyes by two coloured fluorescent cholera toxin subunit B tracing and analysed the segregation of eye-specific domains by two independent methods. Strikingly, in adult Nogo-A deficient mice we observed a significantly lower degree of eye-specific terminal segregation, i.e. a marked overlap of left- and right eye termination zones. Further experiments will help to answer whether the observed phenotype is due to developmental defects or to increased plasticity causing sprouting and instability of axonal arbours in the absence of the growth inhibitory cue Nogo-A.

Disclosures: A.M. Guzik-Kornacka: None. **V.E. Pernet:** None. **M.E. Schwab:** None.

Poster

837. Visual System I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:837.17/A26

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NIH Grant EY019049

Kirchgessner Foundation

Title: Broadening of cortical inhibition mediates developmental sharpening of orientation selectivity

Authors: *Y. LI[1,2], L. IBRAHIM[2], W.-P. MA[2], L. I. ZHANG[2], H. W. TAO[2]
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Abstract: Orientation selectivity (OS) of visual cortical neurons is progressively sharpened during development. However, synaptic circuit mechanisms underlying the OS sharpening remain unclear. In the current study, in vivo whole-cell voltage-clamp recordings from layer 4 excitatory neurons in the developing mouse primary visual cortex revealed changes of orientation tuning profiles of their excitatory and inhibitory inputs during a post-eye-opening period when OS of their spiking responses becomes sharpened. In addition to a parallel strengthening of excitation and inhibition during this developmental period, the orientation tuning of excitatory inputs keeps relatively constant, whereas the tuning of inhibitory inputs is broadened, and becomes significantly broader than that of excitatory inputs. Neuron modeling and dynamic-clamp recording demonstrated that this developmental broadening of the inhibitory tuning is sufficient for sharpening OS. Depriving visual experience by dark rearing impedes the normal developmental strengthening of excitation, but a similar broadening of inhibitory tuning, likely caused by a nonselective strengthening of inhibitory connections, results in the apparently normal OS sharpening in excitatory neurons. Our results thus provide the first demonstration that an inhibitory synaptic mechanism can primarily mediate the functional refinement of cortical neurons.

Disclosures: Y. Li: None. **L. Ibrahim:** None. **W. Ma:** None. **L.I. Zhang:** None. **H.W. Tao:** None.

Poster

837. Visual System I

Location: Hall F-J

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Program#/Poster#:837.18/A27

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NIH Grant EY019968

Title: Alternative splicing of the LIM-homeodomain transcription factor Isl1 in retinal ganglion cells

Authors: *I. E. WHITNEY[1,2], B. REESE[3,2]
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Abstract: The LIM-homeodomain (LIM-HD) transcription factor Isl1 plays a role in the differentiation of multiple cell-types in the retina. An alternative isoform of Isl1, Isl1β, has been identified in pancreatic cells; alignment to the canonical isoform, Isl1α, reveals the absence of 23 amino acids corresponding to a protein-binding domain. This study seeks to determine the role of *Isl1* alternative splicing in the differentiation of neuronal cell-types in the mouse retina.
The pattern of *Isl1* alternative splicing across multiple tissues was determined using RT-PCR with one primer pair capable of amplifying both isoforms. Quantitative real time RT-PCR with isoform-specific primers was used to characterize expression changes in the developing retina. The translation of *Isl1* isoforms in the retina was assessed by western blotting using a commercially available monoclonal mouse antibody that recognizes both isoforms. This pan-Isl1 antibody, in conjunction with a custom-made rabbit polyclonal antibody specific to the alpha isoform, was also used with standard immunofluorescence techniques.
We found both *Isl1* isoforms to be expressed in the developing and adult mouse retina, with *Isl1α* in greater abundance. This pattern of expression was also found in other *Isl1* positive tissues, including brain and spinal cord. Quantification of *Isl1* transcript abundance revealed a progressive increase in expression differences across retinal development with *Isl1α* having a 2 (E12), 4 (E16), 5 (P1), 6 (P10) and 13 (adult) fold greater expression than *Isl1β.* We also identified two peaks in both *Isl1α* and *Isl1β* expression at E16 and P10, coincident with the genesis of *Isl1* expressing cells. The pan-Isl1 antibody recognizes two protein products differing in size by the expected 23 amino acids absent in Isl1b, confirming the presence of both isoforms in the retina at the protein level. Certain bipolar, amacrine, and ganglion cells are labeled with the pan-Isl1 antibody; while every pan-Isl1 bipolar and amacrine cell was co-localized with the Isl1-alpha antibody, a subset of pan-Isl1 RGCs were not, revealing the existence of a beta-isoform only population of RGCs. We subsequently analyzed the soma size of these pan+/alpha+ and pan+/alpha- RGCs and found the beta-only population to be restricted to RGCs of small and medium soma sizes (50-175 μm^2) and never found to be RGCs with large somas (176-300 μm^2). These results indicate possible functional differences in the Isl1 isoforms leading to the differentiation of distinct RGC types, supporting alternative splicing as a mechanism for generating neuronal diversity in the CNS.

Disclosures: I.E. Whitney: None. **B. Reese:** None.

Poster

837. Visual System I

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Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NIH Grant EY020108

NIH Grant EY017137

David and Lucile Packard Foundation

Title: Melanopsin ganglion cells are critical for the proper development of the visual system

Authors: *J. M. RENNA[1], K. CHEW[2], G. LOEVINSOHN[1], S. WENG[1], S. HATTAR[2], D. M. BERSON[1]
[1]Neurosci., Brown Univ., Providence, RI; [2]Biol., Johns Hopkins Univ., Baltimore, MD

Abstract: Waves of correlated activity sweep across the early postnatal retina and contribute to segregation and refinement of retinofugal projections. The waves have been viewed as light-independent, as they appear before rods and cones can affect the inner retina. Amacrine cells have been thought to drive the waves, and ganglion cells to be passive recipients. However, we recently showed that, during this period, light alters both retinal waves and maturation of retinofugal projections by exciting melanopsin ganglion cells (mRGCs; Renna et al., 2011). At this age, mRGCs are excited not only by light, but by the waves themselves. Since mRGCs alter waves when excited by light, we wondered whether they might also do so in the dark, by feeding back wave-generated signals to the wave-generating network. We therefore tested whether, in the absence of light, genetic ablation of mRGCs would affect the retinal waves and the development of retinofugal projections.
We made multi-electrode array recordings in darkness from retinas of postnatal day 6 (P6) mice lacking melanopsin ganglion cells (*Opn4*$^{dta/dta}$) and matched controls (*Opn4*$^{+/+}$). Relative to controls, single ganglion cells in *Opn4*$^{dta/dta}$ mice exhibited prolonged bursting during waves (10.8% increase; $p<0.05$) and more spikes per burst (40% increase; $p<0.05$;n= 3 retinas/54 cells *Opn4*$^{dta/dta}$, and n= 3 retinas/64 cells *Opn4*$^{+/+}$). At distances greater than 600μm, pairs of cells exhibited more correlated firing in *Opn4*$^{dta/dta}$ mice relative to controls ($p<0.05$). To assess the effects of the loss of mRGCs on the ocular segregation of retinofugal projections, *Opn4*$^{dta/dta}$ and *Opn4*$^{+/+}$ mice were raised in continuous darkness. At P7, we made ocular injections of contrasting fluorescent anterograde tracers (cholera toxin B [CTB]-Alexa-488 and CTB-Alexa-594) and harvested the tissue at P8. Data were processed and analyzed as described by Renna et. al. (2011). *Opn4*$^{dta/dta}$ mice lacked a well-defined ipsilateral sector within the dLGN and

exhibited a 20% increase in the number of pixels with substantial label from both eyes ($p < 0.0001$; n= 4 *Opn4*$^{+/+}$, 5 *Opn4*$^{dta/dta}$, 10 sections/mouse/dLGN).
Retinal wave activity drives the development of the image forming visual system. mRGCs, the main conduits of input to non-image forming visual centers, are excited by retinal waves and alter retinal wave activity when depolarized (Renna et. al., 2011). Here we have shown that when mRGCs are removed from the circuit, wave activity is altered and the projections to the dLGN are less segregated. These results suggest that, even in the absence of light, mRGCs contribute to the proper development of the image-forming visual system.

Disclosures: J.M. Renna: None. **K. Chew:** None. **G. Loevinsohn:** None. **S. Weng:** None. **S. Hattar:** None. **D.M. Berson:** None.

Poster

837. Visual System I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:837.20/A29

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Title: Preliminary study: Frontal gamma dynamics of infants

Authors: *M. BALLAN, J. H. GILMORE
UNC Chapel Hill, Chapel Hill, NC

Abstract: In the proposed study, the frontal asymmetries of infants from normal control families are discussed. The age groups of participants are 2 weeks old, one year old and two years old. In order to understand the temporal dynamics of brain activities, high array electroencephalogram (EEG) recording technique has used while the infant quietly plays in the caregiver's lap. A short video has shown to engage 1-year old and 2-year old toddlers during the recording whereas the 2-week old infants are kept awake (eye open). First, a bandpass filter is applied to remove low frequency and high frequency components of EEG signals with a low cutoff of 1.5 Hz and high cutoff of 57 Hz. Later, the power spectrum of signals between 30-50 Hz is calculated in order to identify the frontal and occipital gamma activities. Our preliminary results showed that the frontal gamma activity is a biomarker for the early developmental differences. We are currently recruiting infants with family history of Schizophrenia. Therefore, we will be able to compare these two groups.

Disclosures: M. Ballan: Employment; UNC Chapel Hill. **J.H. Gilmore:** None.

Poster

838. Visual System II

Location: Hall F-J

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Program#/Poster#:838.01/A30

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: San Paolo 2008.1267

Title: Early depolarizing GABA controls critical period plasticity in the rat visual cortex

Authors: M. ALLEGRA[1], G. DEIDDA[2], C. CERRI[3], G. BONY[2], M. CALEO[3], *L. CANCEDDA[2]
[1]Scuola Normale Superiore, Pisa, Italy; [2]NBT, Italian Ins. Technol. (IIT), Genova, Italy; [3]Inst. di Neuroscienze, Consiglio Nazionale delle Ricerche, CNR, Pisa, Italy

Abstract: GABA, the main inhibitory neurotransmitter in the adult mammalian brain, plays a fundamental role for development and plasticity of the visual system. Conversely, GABA exerts depolarizing and mostly excitatory actions during early development due to high expression of the Na+/K+/Cl- cotransporter NKCC1. Interestingly, nothing is known about depolarizing GABA control of plasticity mechanisms in the visual system. Here, we interfered with depolarizing GABA action by treating rat pups with the NKCC1 inhibitor bumetanide or vehicle from P3 to P8 and examined young adult animals at P35. We found that basic physiological parameters (visual acuity, contrast threshold and binocularity) developed normally. However, differently from in control animals, the critical period for ocular dominance plasticity was still open in bumetanide-treated animals, as shown by a persistent sensitivity to the effects of a brief monocular deprivation. Moreover, visual cortical slices from bumetanide-treated rats showed reliable LTP in response to theta-burst stimulation of the white matter. Similar results were obtained with another inhibitor of NKCC1 at perinatal ages, furosemide. As we found no difference in controls and bumetanide-treated animals in experiments of monocular deprivation and LTP at P20 (the peak of the critical period) and in adult rats at P75, we concluded that the critical period was prolonged in bumetanide-treated animals. To explain the higher level of plasticity in bumetanide-treated rats at P35, we analysed basal GABAergic transmission and the extracellular matrix, which normally limit experience-dependent plasticity in the adult. We found a reduction of both the inhibitory tone and the density of perineuronal net-surrounded cells. As Brain Derived Neurotrophic Factor (BDNF) regulates the time course of critical period plasticity in the visual system by modulating maturation of inhibitory GABAergic circuits, we analysed BDNF levels in the visual cortex. We found a significant lower expression of BDNF in bumetanide-treated animals, in agreement with the delayed closure of the critical period. These

results demonstrate that early depolarizing GABA exerts a long-lasting modulation of the level of plasticity in adulthood.

Disclosures: M. Allegra: None. **G. Deidda:** None. **C. Cerri:** None. **G. Bony:** None. **M. Caleo:** None. **L. Cancedda:** None.

Poster

838. Visual System II

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Program#/Poster#:838.02/A31

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: HFSP Career Development Award

Title: Heterogeneous developmental time courses of higher visual areas in mice

Authors: I. T. SMITH, L. B. TOWNSEND, C. M. MAZZONE, *S. L. SMITH
UNC Sch. of Med., Chapel Hill, NC

Abstract: Specific visual processing deficits are observed in many developmental cognitive disorders (Grinter et al 2010; Simmons et al. 2009). For example, thresholds for global motion detection are often elevated in young children with autism spectrum disorders, despite normal performance on other visual tasks. This specificity suggests that visual processing circuitry is unequally affected. In neocortex, visual pathways diverge downstream of primary visual cortex (V1) via parallel cortico-cortical projections into multiple higher visual areas (HVA). HVAs exhibit a diverse range of selectivities to visual stimuli (Marshel et al. 2011; Andermann et al. 2011), suggesting that they may be specialized for processing different aspects of visual information. Thus, if a subset of HVAs exhibit greater sensitivities to genetic and/or environmental influences in development, this could underlie the specificity of visual defects in some developmental cognitive disorders. To investigate how HVAs are affected in developmental disorders, it is crucial to first understand how these circuitries are formed during normal development. To study the development of HVAs, we turned to the mouse visual system model. Mouse primary visual cortex (V1) is surrounded by at least nine HVAs in a compact area less than 3 mm across (Wang and Burkhalter 2007). In this study, we examined the functional development of V1 and HVAs in mice during the week following eye opening (P14 - P20). Using intrinsic signal optical imaging, we mapped cortical activation in response to a battery of visual stimuli. To locate HVAs, we mapped retinotopy using individual drifting bars. To drive activity in HVAs, gratings and random dot kinetograms were used. We found that HVAs developed heterogeneously. Lateral HVAs (areas AL and LM) developed early, exhibiting

functional responses at eye opening that strengthened over days in concert with responses in V1. By contrast, medial HVAs (areas AM and PM) developed more slowly. Responses in medial HVAs were very weak to undetectable for the first two days after eye opening, and then progressively strengthened. Due to these temporally segregated time courses, the development of medial HVAs are potentially more sensitive to visual experience than that of lateral HVAs.

Disclosures: I.T. Smith: None. **L.B. Townsend:** None. **C.M. Mazzone:** None. **S.L. Smith:** None.

Poster

838. Visual System II

Location: Hall F-J

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Program#/Poster#:838.03/A32

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NIH Grant P20RR016816

Title: Global hyper-synchronous spontaneous activity in the developing optic tectum

Authors: *K. IMAIZUMI[1], J. Y. SHIH[2], H. E. FARRIS[3]
[1]LSU Sch. of Vet. Med., Baton Rouge, LA; [2]Univ. of California, San Francisco, San Francisco, CA; [3]LSUHSC, New Orleans, LA

Abstract: Correlated synchronous spontaneous activity in the developing brain carries a blueprint of neuronal circuits underlying topographic organization. To understand the development of topographic organization, thus, requires knowledge of spatio-temporal patterns of spontaneous activity. We addressed this question in the developing optic tectum of albino tadpoles, Xenopus laevis, by employing an in vivo two-photon calcium imaging technique combined with a bulk-loading cell-permeant Oregon Green BAPTA-1 AM. This technique allowed us to separately monitor spontaneous activity in the input (the neuropil consisting of ascending axons and tectal dendrites) and output (tectal cell bodies) layers of the tectum. To understand the influence of visual experience, tadpoles were separately raised in normal (visual experience) and dark (no visual experience) conditions. We found that spontaneous events in the input layer depend on the developmental stage but not visual experience, while those in the output layer depend on both developmental stage and visual experience. In the output layer of the tectum, neurons are tightly correlated with average maximum correlation coefficient of ~0.5 despite the spontaneous activity, which we refer to as hyper-synchronous spontaneous activity. Furthermore, all identified neurons and the neuropil are significantly correlated with structured temporal patterns, suggesting global synchronization. In terms of global synchronization, a large

number of neuronal pairs (83 %) show the maximum correlation coefficients at 0 s time lag, suggesting that tectal neurons receive input from a common source to generate spontaneous activity (e.g., self-generation). To understand the source of this spontaneous activity, we directly manipulated sensory input to the tectum. The optic tectum receives input from visual and mechanosensory (e.g., lateral line, somatosensory, and auditory) systems through retino-tectal and hindbrain-tectal projections, respectively. The manipulation of each sensory input alone has a small or no effect on spontaneous events. However, the manipulation of both visual and mechanosensory inputs significantly alters frequency, magnitude, and temporal patterns of spontaneous activity, while preserving weak activity. The results suggest that spontaneous activity in tectal neurons is self-generated and enhanced by a non-linear combination of visual and mechanosensory inputs. This is the first study to show global hyper-synchronous spontaneous activity enhanced by a non-linear combination of visual and mechanosensory inputs in the developing brain.

Disclosures: K. Imaizumi: None. **J.Y. Shih:** None. **H.E. Farris:** None.

Poster

838. Visual System II

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Program#/Poster#:838.04/A33

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: Natural sciences and engineering research council of Canada

Title: Changes in white matter connectivity in the visual system of people with one eye

Authors: *K. R. KELLY[1], L. MCKETTON[2], K. A. SCHNEIDER[2], J. K. E. STEEVES[1]
[1]Psychology, [2]Biol., York Univ., Toronto, ON, Canada

Abstract: Background. The present study investigated the development of white matter connectivity in the visual pathway of adults who have had one eye surgically removed (enucleated) during infancy. Previous research in our lab has revealed decreases in cortical thickness and surface area in visual cortices of this population, as well as a decrease in lateral geniculate nucleus (LGN) volume. More interestingly, these decreases were less severe in the hemisphere ipsilateral to the remaining eye, suggesting aberrant connections are made between the remaining eye and cells associated with the removed eye. This is consistent with monkey models of enucleation showing recruitment of LGN cells contralateral to the remaining eye. It is evident that grey matter development of the visual system is atypical in this population, however the effect of enucleation on white matter connectivity in this system is unclear. Methods. A

group of adults whose eye was enucleated early in life due to retinoblastoma (cancer of the retina) were compared to binocularly intact controls. All participants were scanned using a 3T Siemens Trio MRI scanner and 32-channel head coil. Anatomical regions of interest (ROIs) for the optic nerve, optic chiasm, and LGN were created by manually tracing these areas from high-resolution T1 weighted (nerve, chiasm) and proton density weighted (LGN) images. All ROI masks were registered to diffusion tensor imaging (DTI) space to determine fractional anisotropy and mean diffusivity values, and the voxel number of optic radiations (OR) per hemisphere for each participant. Probabilistic tractography was performed using DTI information to visualize and quantify tracts throughout the visual pathway. Tractography was also performed using a separate diffusion spectrum imaging (DSI) scan which allows for visualization of crossing fibers. Results. Changes in white matter connectivity were found within the visual pathway of the monocular enucleation group compared to binocularly intact controls. Specifically, we found an increase in fibers projecting to the contralateral LGN from the remaining eye. Further, an increase in the number of ORs projecting to V1 in the hemisphere contralateral to the remaining eye was also found. Conclusions. Our data are consistent with hemisphere asymmetries in visual system grey matter following monocular enucleation. The complete disruption of half of the visual inputs from enucleation appears to promote reorganization within the visual pathway via cell recruitment by the remaining eye.

Disclosures: K.R. Kelly: None. **L. McKetton:** None. **K.A. Schneider:** None. **J.K.E. Steeves:** None.

Poster

838. Visual System II

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Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NSERC

AHFMR

CIHR

Title: Maturation of visual attention for hand advance and somatosensory attention for hand withdrawal of the reach-to-eat movement in human infants aged 6 to 12 months

Authors: *L.-A. R. SACREY, J. M. KARL, I. Q. WHISHAW
Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: The reach-to-eat movement is a natural act in which an object or food item is grasped and transported to the mouth and it is one of the earliest forelimb behaviors displayed by human infants. In adults, there is a tight coupling between visual attention and the advance phase of the reach-to-eat movement and somatosensory attention and the withdrawal phase of the reach-to-eat movement. The present study examined when and how this relationship between sensory attention and movement develops in infancy. In a longitudinal study, eight infants, aged six months to twelve months, and twenty adults reached for familiar inanimate objects and food items. Hand and eye movements and accuracy were measured using manual, frame-by-frame kinematic analysis. The results show that there was a gradual increase in sensory coupling of vision to hand advance and somatosensation to hand withdrawal, such that by twelve months of age, infants approximated the adult pattern. A large variability in infant performance was likely the result of the concomitant maturation of the arm, hand, and rotatory movements, the development of precision grasping, and the improving targeting accuracy both for grasping and placing the target into the mouth. The results are discussed in relation to the idea that online sensory attention and motor control for reaching develops in parallel.

Disclosures: L.R. Sacrey: None. **J.M. Karl:** None. **I.Q. Whishaw:** None.

Poster

838. Visual System II

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Program#/Poster#:838.06/A35

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: 5R01EY007060-22

Title: The role of Progranulin-a during the development of the zebrafish retina

Authors: *C. WALSH[1], L. KAKUK-ATKINS[2], P. HITCHCOCK[2]
[1]Ann Arbor, MI; [2]Univ. of Michigan, Ann Arbor, MI

Abstract: Pgrn is a secreted growth factor implicated in several processes, including embryogenesis, tumorigenesis and wound repair. In humans, autosomal dominant point mutations in the progranulin gene (PGRN) result in truncated progranulin protein and haploinsuficiency. Such mutations occur in 5-10% of individuals with Frontotemperal Lobar Degeneration (FTLD), the second most common cause of early onset dementia. Our lab recently demonstrated Pgrna expression is strongly upregulated in retinal microglia in adult zebrafish after the selective death and during stem-cell based regeneration of photoreceptors. However, the specific functions of Pgrna during developmental and regenerative neurogenesis remain

unknown.
The work presented here used expression studies and reverse genetics approaches to determine the cellular expression and function of progranulin-a (Pgrna) during embryonic retinal development in zebrafish. Pgrna protein synthesis was diminished by injecting morpholino oligonucleotides (MOs), targeting the 5'UTR or third splice site (SS). Gross eye morphology and cell specific antibody markers revealed that at 72 hours post fertilization (hpf) Pgnra knock down results in the reduction of cellular differentiation in the retina compared to control or uninjected embryos. EdU labeling of dividing cells showed that in the absence of cellular differentiation, the retina persisted as a proliferative neuroepithelium. Co-injection of pgrna morpholinos and hGRN mRNA rescued the morpholino-induced phenotype. These data suggest that for retinal progenitors Pgrna functions to govern the transition from proliferation to a differentiation, and that in the vertebrate retina microglia-derived growth factors regulate aspects of developmental neurogenesis.

Disclosures: C. Walsh: None. **L. Kakuk-Atkins:** None. **P. Hitchcock:** None.

Poster

838. Visual System II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:838.07/A36

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Title: Maturation of intrinsic properties in the optic tectum of the Xenopus tadpole

Authors: *A. S. HAMODI, K. G. PRATT
Univ. of Wyoming, Laramie, WY

Abstract: The caudal edge of the optic tectum of the *Xenopus* tadpole consists of a proliferative zone where neural progenitor cells give rise to neurons. These immature neurons then migrate rostrally into the tectum proper where they are incorporated into the retinotectal circuit (retinal ganglion cell axons projecting directly onto tectal neurons of the optic tectum). Ongoing incorporation of immature neurons from the caudal proliferative zone into the functional retinotectal circuit creates a spatial developmental gradient in which the most immature neurons are situated in the caudal portion of the optic tectum and the most mature in the rostral portion. Many aspects of neuronal maturation have been described along this rostrocaudal (RC) axis including increasing dendritic arbor complexity, increasing frequency and amplitudes of spontaneous synaptic events, increasing AMPA:NMDA ratios, and decreasing levels of calcium-permeable AMPA receptors. Much less has been reported about how the intrinsic properties may

be changing along this RC axis. Since the way a neural circuit functions is determined by both a neuron's synaptic and intrinsic properties, and since there is much evidence that these two properties are functionally coordinated, we wondered how intrinsic properties may also be changing along the same axis. Therefore, using stage 48 tadpoles, we recorded in whole cell voltage clamp configuration from neurons residing in different parts of the tectum. So far, we identified a gradual and significant increase in both peak sodium and potassium currents along the RC axis. No significant difference in cell capacitance or input resistance was observed. Furthermore, we noticed that this location-dependent increase in intrinsic currents is correlated with an increase in the amount of synaptic input received by individual neurons. This suggests that the development of intrinsic currents could be an activity-dependent process. We begin to test this possibility by altering the amount or pattern of synaptic activity received by the tectal neurons. Preliminary data suggest that chronic visual deprivation may disrupt the normal maturational gradient of intrinsic properties.

Disclosures: A.S. Hamodi: None. **K.G. Pratt:** None.

Poster

838. Visual System II

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Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NIH P30 EY000785

NIH R01 EY015788

NIH T32 NS007224

Title: Spontaneous retinal waves propagate throughout the developing visual system *in vivo*

Authors: *J. B. ACKMAN, T. J. BURBRIDGE, M. C. CRAIR
Neurobiol, Yale Univ., NEW HAVEN, CT

Abstract: The display of patterned spontaneous activity is an emergent property of the immature nervous system that is thought to mediate synaptic competition and instruct self-organization in many developing neural circuits. In the visual system, isolated preparations of developing retina exhibit propagating waves of electrical activity among neighboring retinal ganglion cells, termed 'retinal waves'. Since retinal ganglion cells relay visual information to higher order structures in the central nervous system, retinal waves are thought to play a key role in activity-dependent

refinement of topographic neural maps in the superior colliculus, lateral geniculate nucleus, and visual cortex. However, the role of retinal waves in neural circuit development remains controversial, in part because their existence has never been demonstrated in vivo. Here we use optical methods to demonstrate in live neonatal mice that waves of spontaneous retinal activity are present and propagate throughout the entire visual system before eye opening. This patterned activity encompassed the visual field, preferentially initiated in the binocular retina, and exhibited spatiotemporal correlations between the two hemispheres. Retinal waves were the primary source of activity in the midbrain and primary visual cortex and modulated ongoing activity in secondary visual areas. Thus, patterned activation of immature visual circuits by spontaneous retinal activity is likely to play an instructive role for intra- and inter- hemispheric circuit refinement across multiple visual areas.

Disclosures: J.B. Ackman: None. **T.J. Burbridge:** None. **M.C. Crair:** None.

Poster

838. Visual System II

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Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: PRESTO, JST

The 21st COE Program

The Global COE Program

MEXT

HFSP

JSPS

Title: FoxP2 is a parvocellular-specific transcription factor in the visual thalamus of monkeys and ferrets

Authors: *L. IWAI[1,2], Y. OHASHI[3], D. VAN DER LIST[4], W. M. USREY[4,5], Y. MIYASHITA[3], H. KAWASAKI[2]
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Abstract: Although the parallel visual pathways are a fundamental basis of visual processing, our knowledge of their molecular properties is still limited. Here we uncovered the first parvocellular-specific molecule using the dorsal lateral geniculate nucleus (dLGN) of higher mammals. We found that FoxP2 transcription factor was specifically expressed in X cells of the adult ferret dLGN. Interestingly, FoxP2 was also specifically expressed in parvocellular layers 3-6 of the adult monkey dLGN, providing new evidence for a homology between X cells in the ferret dLGN and parvocellular cells in the monkey dLGN. Furthermore, this expression pattern was established as early as gestation day 140 in the embryonic monkey dLGN, suggesting that parvocellular specification has already occurred when the cytoarchitectonic LGN layers are formed. Our results should help in gaining a fundamental understanding of the development, evolution and function of the parallel visual pathways, which are especially prominent in higher mammals.

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Poster

838. Visual System II

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:838.10/A39

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: MIUR grant PRIN to LM

Title: Acceleration of visual acuity induced by EE is precociously primed by early increase of IGF-1 levels in the visual cortex

Authors: L. BARONCELLI[1], *M. CENNI[1], S. LANDI[2], G. DEIDDA[3], R. NARDUCCI[4], L. CANCEDDA[3], N. BERARDI[4,1], L. MAFFEI[1,2]
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Abstract: Continuous exposure to enriched environment (EE) from birth to post-weaning determines a marked acceleration of visual system development both at retinal and cortical levels. Insulin-like growth factor-1 (IGF-1) is crucial in mediating EE effects on visual acuity development. Indeed, in the visual cortex, IGF-1 levels are increased by EE at P18, and post-

weaning administration of IGF-1 mimics, while post-weaning block of IGF-1 action blocks, EE effects. Interestingly, we have recently found that also limited exposure to EE in a pre-weaning period (up to P12), accelerates the much later occurring process of visual acuity development. This suggests that factors affected by EE during the first 10 days of life may be sufficient to trigger visual cortical maturation. As IGF-1 is a crucial mediator of pre-weaning EE effects on retinal development, here we explored the possible involvement of IGF-1 in mediating the effects of pre-weaning EE on visual cortical maturation.
We found that IGF-1 levels increased in the visual cortex of EE rats as early as P6. Then, we investigated whether this precocious expression of IGF-1 was a first crucial event to set in motion the process leading to a faster development of visual acuity. For this purpose, we assessed whether cortical infusions of IGF-1 by intraventricular (i.v.) injections in non EE rats from P1 to P7, was sufficient to mimic EE effects on visual acuity development, and whether blocking intracortical IGF-1 action in EE rats by means of i.v. injections of IGF-1 antagonist JB1 blocked EE effects. We found that visual acuity of non EE rats treated with IGF-1 was higher than in control rats and similar to that of rats exposed to pre-weaning EE; conversely, EE rats treated with JB1 showed visual acuity comparable to that of non EE rats.
Altogether, these results suggest that the acceleration of visual-acuity development induced by pre-weaning EE is mediated by early increase of IGF-1 levels in the visual cortex.
Moreover, to understand the mechanisms of action of this priming effect of early IGF-1 cortical levels on visual acuity development, we are currently assessing the developmental expression of chloride transporters in the visual cortex of IGF-1-treated rats. Preliminary results indicate that IGF-1 treatment accelerates the developmental decrease of NKCC1.

Disclosures: L. Baroncelli: None. **M. Cenni:** None. **S. Landi:** None. **G. Deidda:** None. **R. Narducci:** None. **L. Cancedda:** None. **N. Berardi:** None. **L. Maffei:** None.

Poster

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Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NIH P30 EY000785

NIH R01 EY015788

NIH T32 NS007224

Title: *In vivo* examination of retinal waves and visual map development in β2 nicotinic acetylcholine receptor whole animal and conditional deletion knockout mice

Authors: *T. J. BURBRIDGE, H. XU, J. B. ACKMAN, M. C. CRAIR
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Abstract: The topographic and eye-specific organization of the mouse visual system, long a model for developmental circuit refinement, is largely organized before eye-opening and true sensory experience, but it is also known to be dependent on activity. Retinal waves, a spontaneous, patterned bursting activity studied ex vivo in dissected retinas, have provided the best guess as to the source of visual circuit-informing activity for the past twenty years, but both their in vivo existence and relative importance have been questioned. One source of this confusion stems from conflicting reports of the most widely utilized model of disrupted waves, deletion or mutation of the β2 subunit of the nicotinic acetylcholine receptor (β2-nAChR). While some in vitro reports have demonstrated a complete lack of correlated bursting retinal activity in these knockouts, others have found either more frequent retinal waves or no waves and sparse bursting in small subsets of retinal ganglion cells. We therefore developed a technique to examine and characterize spontaneous retinal activity before eye opening in awake animals in vivo through pre- and postsynaptic calcium imaging in the superior colliculus. From these experiments, we find that retinal waves do exist in vivo in β2-nAChR knockout mice, but their frequency, speed, amplitude, and direction of propagation are dramatically disrupted. Moreover, the effects of β2-nAChR knockout on retinal waves are greatest when retinofugal circuit refinement is most pronounced. Using Cre/loxP conditional knockouts, we further observed that deletion of β2-nAChRs in the retina alone was sufficient to alter spontaneous retinal activity and visual map development, while deletion of β2-nAChRs from the midbrain had no effect on retinal waves or visual circuit anatomy. These experiments thus verify the existence of altered waves in whole animal β2-nAChR knockout mice, demonstrate a pronounced developmental effect of β2-nAChR deletion on retinal wave properties, and confirm through within-animal controls in conditional mutants the hypothesis that retinal wave disruption leads directly to altered visual circuit development.

Disclosures: T.J. Burbridge: None. **H. Xu:** None. **J.B. Ackman:** None. **M.C. Crair:** None.

Poster

838. Visual System II

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Program#/Poster#:838.12/A41

Topic: A.08. Development of Motor, Sensory and Limbic Systems

Title: Molecular basis for the development of ocular dominance patterns

Authors: *Y. WEI, A. KOULAKOV
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Abstract: Neurons in the primary visual cortex of the cat and monkey form regions that are dominated by either the right or left eye. The layout of these regions is referred to as the ocular dominance pattern (ODP). The mechanism of the formation of these patterns is not fully understood. Here we propose a mechanism for the simultaneous formation of ODPs and topographic maps that is based on the interplay between the gradients of molecular labels and activity-dependent
synaptic plasticity. We propose that the segregation of geniculate inputs dominated by either eye is controlled by two competing factors. Activity-dependent synaptic plasticity tends to segregate inputs dominated by different eyes due to weaker interocular than intraocular correlations in the patterned electric activity. Molecular gradients of topographic labels, such as Eph receptors and ephrins, on the contrary, restrict the degree of such segregation. To test this mechanism, we study a computational model in which activity-dependent and activity-independent factors are combined. We make several observations about the model that make the proposed mechanism plausible. First, we show that the periodicity of ODP can vary in a wide range in this model, even within the same map. Steeper molecular gradients yield narrower ocular dominance columns, while stronger effects of correlated activity within the critical period produce wider columns. The periodicity of ODP can therefore be determined genetically, through the distribution of molecular labels in the cortex. Second, we show that monocular eye occlusion can lead to the decrease in the number of neurons dominated by one of the eyes. Third, we show that an increase/decrease in the intraocular correlations leads to the increase/decrease in the number of binocular neurons. Fourth, overall strengthening of the effects of correlated activity can lead to pattern's desegregation. Fifth, occlusion of one of the eyes may lead to the induction of ODP in the case when the ODP is not formed. Sixth, in the model, the topographic maps experience discontinuities on the interfaces between two columns of observed magnitude. Finally, we demonstrate how the direction of the molecular gradients in 2D can affect local orientation of ODP. The latter observation suggests that molecular labels may play an instructive role in the formation of ocular dominance maps.

Disclosures: Y. Wei: None. **A. Koulakov:** None.

Poster

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Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: Polish Ministry of Science and Education Grant N N301 654140 to M. Zapasnik

Polish Ministry of Science and Education Grant N N401 557640 to K. Burnat

Title: Zif268 expression in adult visual cortex uncovers perceptual learning potential in cats regardless of early visual experience

Authors: *M. ZAPASNIK[1], K. LASKOWSKA - MACIOS[1,2], T.-T. HU[2], M. KOSSUT[1], L. ARCKENS[2], K. BURNAT[1]
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Abstract: Visual input during early life is crucial to establish correct vision. We analyzed the impact of early binocular pattern deprivation (BD) on motion perception. BD reduces retinal illumination and interferes with the OFF pathway development (Burnat et al., 2012). At the end of a 2-year period of behavioral motion perception training, interaction of the ON and OFF pathways was tested using a global motion task designed by Edwards and Badcock (1994). Afterwards, to test whether different behavioral outcomes of early BD are reflected in distinct cortical circuit activity, the expression profile of the activity reporter gene zif268 in the visual cortical areas 17, 19, OFF-driven PMLS and multimodal area 7 was measured. Adult BD cats deprived from eye opening for the first 2 or 4 months of life (2BD and 4BD), or for 2 months after 2 months of normal vision (2N2BD) were tested. Normal cats were raised in a normal visual environment. After completing the training cats were exposed to overnight darkness prior to 1-hour light stimulation and then sacrificed. In situ hybridization was applied using an oligonucleotide probe complementary to the nucleotides encoding amino acids 2-16 of the rat zif268 gene. Analysis of visual areas present on sections from Horsley-Clark levels A.5-A.7 was performed. Extraction of the OFF-driven global motion signal was only impaired in the 2N2BD group. As compared to the normal group, the 2BD and 4BD cats performed equally well. Nevertheless, the zif268 signal was elevated in areas PMLS, 17 and 19 in the 2BD and 4BD groups as compared to normal cats, whereas the poorly performing 2N2BD cats exhibited normal zif268 levels. We therefore suggest that the increased zif268 levels in the 2BD and 4BD cats reflect a compensation mechanism allowing the use of a different cortical circuit to perform in the global motion task. No differences in the zif268 signal between any of the BD and normal groups were found in multimodal area 7. As expected, BD altered cortical activity in motion-sensitive OFF-driven PMLS area - the 2BD and 4BD groups had significantly higher zif268 levels in the PMLS than normal cats.

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Poster

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Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: CNPq

FAPERJ

PROAP-UFF

CAPES

Title: Interleukin-4 intravitreous injection disrupts retinotectal topography in rodents

Authors: *P. CAMPELLO-COSTA, V. G. GOULART, G. D. MENEZES, A. C. FARIA-MELIBEU, C. A. SERFATY
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Abstract: The superior colliculus (SC) is a major subcortical visual center in mammals, receiving afferents from retinal ganglion cells, which establish synaptic contacts in an orderly fashion on target. The visual system undergoes an extensive structural and functional adaptation by environment modification. The main plasticity in the SC occurs during the critical period of development which corresponds to the first two/three post-natal weeks. Interleukin-4 is an anti-inflammatory cytokine that plays a role in immune and nervous systems. In the nervous system, it has been related to hippocampal neuronal survival and differentiation and retinal cells proliferation as well. In retina, IL-4 receptor (IL-4R) was identified both in photoreceptors and Müller cells process. The aim of this study was to observe the in vivo effect of IL-4 in the retinofugal pathway. Furthermore, we are looking for the IL-4R- related signaling pathways. Lister Hooded rats were injected with IL-4 or PBS (control) in the right eye at post natal day 10 (PND 10). In PND 14 the animals were injected with HRP, a neuronal marker, in the left eye. Afterwards, animals were perfused and processed to HRP histochemistry to reveal the distribution pattern of retinal axons in the SC. Alternatively, animals were processed for immunofluorescence or western blotting analysis to verified p-ERK and p-AKT content in the retina. Quantitative analysis was performed using Scion Image and Prism software. All animal use procedures were approved by the local animal care committee (CEPA-UFF) under protocol number 129/09. Our results show that animals treated with intraocular injection of PBS show a normal distribution pattern of retinotectal afferent, characterized by the presence of fibers and

terminal fields defined in clusters in the ventral and anterior region of SC. On the other hand, animals submitted to IL-4 in vivo treatment at PND10 presents an increase in retinotectal arbors, with many fibers emerging to anomalous regions of the superior colliculus. Moreover, IL-4 treatment leads to an increase in pERK content in the retina compared to PBS, control group. There was no significant difference in the content of pAKT between groups. Our results show that intravitreous treatment with IL-4 induces an increase in pERK expression in the retina and a disruption in the uncrossed retinotectal pathway of the opposite eye. Altogether, this data suggest that interleukin-4 induces a change in intra-retinal electrical activity and the consequent weakening of the contralateral retinal projections which may be contributing to the sprouting of ipsilateral retinal connections of the intact eye.

Disclosures: P. Campello-Costa: None. **V.G. Goulart:** None. **G.D. Menezes:** None. **A.C. Faria-Melibeu:** None. **C.A. Serfaty:** None.

Poster

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Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NIH Grant EY018605

Title: Intracellular mechanisms of DSCAM and DSCAML1 during retinal development

Authors: *A. M. GARRETT[1], A. L. D. TADENEV[1], P. G. FUERST[1,2], R. W. BURGESS[1]
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Abstract: Understanding how precise neural networks form is a major goal of developmental neurobiology and will provide important insights into human disease. Retinal circuits are relatively well understood with well-defined members and organization; thus the retina is a valuable model for investigating the processes and molecules that underlie neural network formation. The retina contains five major cell types, each of which can be further divided into subtypes. The cells of each subtype are spaced non-randomly to form intermingled mosaics such that each subtype is represented across the entire retina. The Ig superfamily members Down syndrome cell adhesion molecule (DSCAM) and Dscam-Like1 (DSCAML1) are expressed in non-overlapping subtypes. In mice mutant for either of these molecules, dendrites fail to form orderly, overlapping arbors, but rather fasciculate, pulling the cell bodies of other cells of the same subtype (homotypic) into clumps. Fasciculation occurs specifically among homotypic cells, not between different subtypes, and prevention of fasciculation requires homophilic DSCAM

interaction. Our working hypothesis is that DSCAM and DSCAML1 mask a cell subtype specific adhesive code. In addition to the fascicles and clumps, synapses in the mouse mutant retinas fail to mature properly. Presumably, both functions of self-avoidance to mask adhesion and contributing to synaptic structure involve interactions with the intracellular domain of DSCAM. While DSCAMs are clearly important for normal neural development, little is known about the intracellular mechanisms downstream of DSCAM. The C-termini of DSCAM and DSCAML1 are both comprised of PDZ interacting motifs, which bind specifically to the PDZ domain proteins MAGI-2 and -3 (membrane-associated guanylate kinase with inverted domain structure). DSCAM also interacts directly with the serine-threonine kinase Pak1 (p21-activated kinase 1), activating the kinase. The functional significance of these interactions in retinal development is unknown.
We generated knock in mice in which the PDZ-interacting domains of DSCAM and DSCAML1 were replaced with epitope tags. We are using these mice to ask which aspects of DSCAM and DSCAML1 function depend on their interaction with MAGI proteins and other PDZ-domain containing molecules. Using primary retinal ganglion cells, we have found that neurons mutant for DSCAM display increased self-crossings *in vitro*. We are using this system to investigate the intracellular mechanisms, including PAK1 and PDZ-domain proteins, to DSCAM-mediated self-avoidance. Together, our results will further our understanding of the mechanism of DSCAM function.

Disclosures: A.M. Garrett: None. **A.L.D. Tadenev:** None. **P.G. Fuerst:** None. **R.W. Burgess:** None.

Poster

838. Visual System II

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Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NIH Grant EY019968

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Title: Identification of three genomic loci that control AII amacrine cell number in the mouse retina

Authors: *P. W. KEELEY[1,2], I. E. WHITNEY[1,2], S. A. LEONG[3], B. E. REESE[3,2]
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Abstract: The establishment of neuronal populations requires specific patterns of gene expression that control different aspects of development including cellular production, differentiation, and survival. Natural variations in cell number exist across individuals, suggesting the presence of polymorphisms in the genes that control these processes. By identifying such polymorphic genes, we may gain insight into the molecular mechanisms underlying these developmental events.
In this study, we utilized recombinant inbred (RI) mice to determine the genomic loci that determine the size of the AII amacrine cell (AC) population in the mouse retina. A total of 129 mice were analyzed, with a minimum of 3 mice per strain, including the A/J (A) and C57BL/6J (B6) parental strains, and the 26 RI strains derived from them (AxB/BxA RI strain set). Each wholemounted retina was immunostained using antibodies to Prox1, and the total number of AII ACs was estimated by sampling Prox1+ cells in four quadrants across the amacrine cell layer. QTL mapping was performed using GeneNetwork (www.genenetwork.org).
While there was only a slight difference in the total number of AII ACs between the parental strains (A: 64,777 cells, B6: 69,223 cells), there was conspicuous and graded variability between the lowest and highest RI strains (BxA26: 57,141 cells, BxA2: 86,557 cells). Despite this large variability in total number, there was only slight variation within any strain as the average CoV was 0.05 for all strains, while the heritability (h^2) for this trait was estimated to be 0.74, indicating nearly three-quarters of the variation can be accounted for by an effect of strain. Because the number of AII ACs for each parental strain fell within middle of this range for the RI strains, multiple polymorphic genes must exist where the variants in a given genotype work in opposing directions. This was confirmed, as we mapped two suggestive QTLs, one on the proximal end of Chromosome (Chr) 11, where B6 alleles increased trait values by 13,252 cells, and another on the proximal end of Chr 19, where A alleles increased trait values by 13,696 cells. Composite interval mapping was conducted to control for each of these primary QTLs, which identified a third QTL on Chr 9, where A alleles increased trait values by 11,140 cells. Using chromosome substitution mice in which the A haplotype for one chromosome has been introgressed upon an otherwise B6 background, we have validated the QTLs on Chr 9 and 19, showing significant increases in the number of AII ACs (increases of 8,728 and 9,862 cells, respectively). Future work will interrogate these loci for candidate genes that contribute to the establishment of AII AC number.

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Poster

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Topic: A.08. Development of Motor, Sensory and Limbic Systems

Support: NIH Grant EY10894

NIH Grant T32 EY022312

Title: Optical imaging of spontaneous retinal waves with genetically encoded calcium and glutamate indicators

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Abstract: Spontaneous retinal waves play an important role in visual system development. While the mechanism of acetylcholine-mediated retinal waves (stage II waves) has been extensively studied, the mechanism of glutamatergic retinal waves (stage III waves) remains unclear. Here we report the use of a combination of two-photon imaging of genetically encoded fluorescent proteins and electrophysiological recordings to investigate the properties of stage III wave propagation in the postnatal mouse retina. The fluorescent Ca2+ indicator GCaMP3 and the glutamate sensing protein GluSnFR were expressed in specific types of retinal cells, including On and Off bipolar cells (BCs), On and Off starburst amacrine cells (SACs), Muller cells, and ganglion cells (RGCs), following in vivo electroporation and/or viral infection of respective DNAs. Under two-photon imaging, rhythmic Ca2+ activities, with cell type-specific spatiotemporal characteristics, were recorded from BCs, SACs and RGCs during spontaneous retinal waves. BCs displayed rhythmic Ca2+ activities in the axon terminals, but not in the somata, whereas SACs and RGCs showed correlated Ca activities in both dendrites and somata. Patch-clamp recordings also detected rhythmic current inputs to BCs, SACs, RGCs, and Muller cells in the wholemount retina and revealed different kinetic properties in different cell types. Pharmacological blockade of ionotropic GABA and glycine receptors enhanced excitatory current inputs to these cells, which could, in turn, be blocked by ionotropic glutamate receptor antagonists CNQX and CPP, demonstrating a glutamate input to these cells during stage III waves. The rhythmic release of glutamate during stage III waves was further characterized by imaging the spatiotemporal properties of extracellular glutamate with GluSnFR expressed in various cell types and retinal layers. Our results show that fluorescent imaging of genetically encoded Ca2+ and glutamate sensors in specific retinal cell types, together with targeted patch-clamp recording, provides an effective approach to understanding the mechanism of stage III retinal waves.

Disclosures: M. Ye: None. **B.G. Borghuis:** None. **R. Duvoisin:** None. **L.L. Looger:** None. **Z.J. Zhou:** None.

Poster

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Topic: A.01. Brain Patterning

Support: Consolider-Ingenio CDS2007-023

Title: Epha/ephrina signaling and retinal spontaneous activity control sequential refinement steps during the formation of topographic maps

Authors: *I. M. BENJUMEDA
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Abstract: Prior to the onset of sensory experience, both molecular guidance factors and spontaneous activity have been shown to play fundamental roles in the development of neural circuits and topographic maps. The relative contribution of these two mechanisms, however, is still a matter of significant debate. In particular, whether or not the presence of patterned spontaneous activity is required to activate the repulsive response of retinal axons to caudal collicular areas remains uncertain. By manipulating retinal EphA levels and blocking spontaneous activity in mouse retinal ganglion cells, we demonstrate that neural activity is not necessary to activate the EphA/ephrinA-mediated repulsive response of retinal axons during topographic map formation in the visual targets. Together, these results demonstrate that, in vivo EphA/ephrinA signaling determines the location of the axonal termination zone independently of the presence of retinal spontaneous activity and that activity is subsequently needed for proper terminal arborization.

Disclosures: I.M. Benjumeda: None.

Poster

839. PNS Regeneration II

Location: Hall F-J

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Topic: A.09. Transplantation and Regeneration

Support: USAMRMC Grant W81XWH-11-2-0047

Title: Optimization of nanofiber configuration in nerve guidance conduits

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Abstract: There are over 200,000 peripheral nerve repair surgeries performed each year in the United States, and the current gold standard for bridging nerve gaps is the autologous nerve grafts. Synthetic nerve guidance conduits avoid the donor site morbidity of the autologous graft, yet have limited efficacy, especially over longer gaps of 15 mm or more. Incorporating aligned electrospun nanofibers into the conduits has been shown to improve nerve regeneration in a rat sciatic nerve model. Many properties of electrospun nanofibers have been shown to significantly influence axonal outgrowth and migration of Schwann cells. However, the optimal fiber properties have yet to be determined to maximize regeneration outcomes. This study aims to identify ideal configurations through in vitro analysis of cell response to polycaprolactone (PCL)-gelatin nanofibers. Fiber properties analyzed include the nanofiber alignment (random versus aligned), average diameter (300 nm to 1.6 μm), fiber composition and degradation rate (PCL/gelatin ratio ranging from 0 to 100% PCL), fiber density, and fiber-conduit configuration. Primary rat Schwann cells and dorsal root ganglion explants are cultured on nanofibers both in 2D coverslips and 3D conduits to monitor cell adhesion, proliferation, migration rate, and maturation state. The cell response to the nanofibers is analyzed via fluorescence imaging and live-cell imaging. The Schwann cell maturation state is monitored by PCR and co-culture myelination assay. This study helps describe the ideal nanofiber properties to be incorporated into nerve guidance conduits to maximize functional regeneration.

Disclosures: R. Martin: None. **R. Mi:** None. **A. Mullen:** None. **B. Ginn:** None. **A. Hoke:** None. **H. Mao:** None.

Poster

839. PNS Regeneration II

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Topic: A.09. Transplantation and Regeneration

Support: Grant-in-Aid for Scientific Research JSPS 23390461

Title: Effects of antibody to BDNF on nerve regeneration following transection of inferior alveolar nerve

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Abstract: [Introduction] Peripheral nerve injury sometimes induces neuroma formation at the injury site.Inferior alveolar nerve (IAN) is often damaged
by dental treatments, resulting in painful symptoms. Antagonizing neurotrophin has been reported toreduce neuroma formation and neuropathic pain without damaging the transecting neurons following peripheral nerve injury, indicating the possibility that inhibition of BDNF at the injury site can repress neuroma formation. The present study aimed to examine the effects of a local application of antibody to BDNF (anti-BDNF) at the injury site on nerve regeneration and neuroma formation in a rat experimental model with IAN transection.
[Materials and Methods] A total of 22 S-D rats were used. Under general anesthesia, mandible canal at the right side was opened, and the IAN was transected. For observation of histopathological change and nerve regeneration, the animals were sacrificed at appointed time depending
on timing after the transection of IAN; postoperative (PO) 1 week (n= 2), PO 2 weeks (n=4), and PO 3 weeks (n= 8). Furthermore, the IAN transection sites (n=5) were applied with anti-BDNF (1 μg/μl) when the
IAN was cut**.** The animals with physiological saline were treated as a control (n=3). These tissue sections were processed for either AZAN staining or immunohistochemistry for protein gene product 9.5 (PGP 9.5).
[Results] An apparent morphological change was found on the proximal nerve bundles at PO 1 week; the injury site showed enlarge with a remarkable high amount of collagen fibers, indicating the neuroma formation. This neuroma became more enlarge, reached a higher level at PO 2 weeks, and remained in the almost the same volume thereafter.In experimental animals with local application of anti-BDNF, injured nerve bundles with PGP9.5 immunoreaction showed continuity with a little shrinkage. No accumulation of collagen fibers existed at the injury site in the animals treated with anti-BDNF. On the other hand, the control animals exhibited the disrupted and discontinuous IAN.
[Conclusion] IAN transection induced the formation of neuroma containing an accumulation of collagen fibers at injury site, which was inhibited by a local administration of anti-BDNF, suggesting that a local application of anti-BDNF is effective for prevention of neuroma formation in a case of peripheral nerve injury.

Disclosures: Y.M. Valverde Guevara: None. **H. Yoshikawa:** Research Grant; Aid for Scientific Research JSPS. **K. Seo:** Research Grant; Aid for Scientific Research JSPS. **T. Maeda:** Research Grant; Aid for Scientific Research JSPS. **I. Saito:** None.

Poster

839. PNS Regeneration II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:839.03/A50

Topic: A.09. Transplantation and Regeneration

Support: Alberta Innovates Health Solutions

Canadian Institutes of Health Research

Canadian Foundation for Innovation

Multiple Sclerosis of Canada

Hotchkiss Brain Institute

Title: AlphaB-crystallin knockout mice display decreased functional recovery following sciatic nerve injury.

Authors: *E.-M. F. LIM[1], S. T. NAKANISHI[2], R. KUMAR[2], P. WHELAN[3], J. BIERNASKIE[3], S. S. OUSMAN[4]
[1]Neurosci., Univ. of Calgary, Calgary, Canada; [2]Neurosci., [3]Comparative Biol. and Exptl. Med., [4]Clin. Neurosciences, Univ. of Calgary, Calgary, AB, Canada

Abstract: Partial or incomplete regeneration of peripheral nerves and the associated poor functional recovery in humans is attributed in part to an inability of Schwann cells (SCs) to maintain a supportive environment for regrowth. SCs play an important role in regeneration: they promote macrophage infiltration to clear debris, serve as scaffolds for regenerating axons, create a permissive environment by secreting neurotrophic factors, and ensheath and remyelinate regenerating axons. AlphaB-crystallin (αBC) is a small heat shock protein expressed in both mature myelinating and non-myelinating SCs. αBC has been shown to have chaperone properties, promote cell survival, prevent neurotoxicity, and suppress inflammation. We investigated whether αBC promoted SC proliferation, survival, myelination and/or neurotrophic factor secretion during sciatic nerve regeneration. The right sciatic nerve of 8-12 week old female 129S6 wild-type (WT) and αBC null mice (αBC-/-) was crushed at mid-thigh and left to recover for 28 days post-surgery. To assess for functional recovery, the DigiGait system (Mouse,

Inc.) was used to measure multiple gait parameters including swing duration, braking duration, propulsion duration, stance width, stance width variation, stance duration and paw area in naïve animals and at 28 days after injury. Motor nerve conduction velocities were also measured. No significant difference for all gait parameters was observed between WT and αBC-/- mice before injury. However, αBC-/- mice displayed gait abnormalities in all measured gait parameters that were indicative of pain sensitivity, abnormal mobility, decrease in strength and decrease in stability. In addition, there was no significant difference in the motor nerve conduction velocity between WT and αBC-/- mice before injury. Of interest however, the conduction velocity in αBC-/- mice was significantly slower compared to WT mice at 28 days post-crush. Altogether, these results demonstrate impaired functional recovery following peripheral nerve injury in αBC-/- mice following sciatic nerve crush. We are currently investigating the molecular mechanisms underlying the motor and behavioural deficits in αBC-/- mice. These findings aim to develop a greater understanding of the molecular signals that assist in SC biology and have implications for understanding disease mechanisms in inherited and acquired neuropathies, as well as provide potential ideas for therapeutic approaches to enhance peripheral nerve regeneration.

Disclosures: E.F. Lim: None. **S.T. Nakanishi:** None. **R. Kumar:** None. **P. Whelan:** None. **J. Biernaskie:** None. **S.S. Ousman:** None.

Poster

839. PNS Regeneration II

Location: Hall F-J

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Program#/Poster#:839.04/A51

Topic: A.09. Transplantation and Regeneration

Support: NIH Grant T32EB003383: Neuroengineering Training Grant

USAMRMC Grant W81XWH-11-2-0047: Electrospun Fiber Nerve Guide for Peripheral Nerve Repair and Regeneration

Title: Neurotrophic factor gradient generation for directional peripheral nerve growth guidance and regeneration

Authors: *K. D. KRICK[1,4], A. KHADEMHOSSEINI[5], A. HOKE[6], H.-Q. MAO[4,2,3]
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Abstract: Traumatic injury to the peripheral nervous system causing large localized nerve lesions can result in significant loss of nerve function without medical intervention. We are focusing on recapitulating the extracellular environment of naturally-regenerating nerves using hydrogel-based methods to provide localized and controlled presentation of neurotrophic cues to enhance peripheral nerve regeneration across large lesion gaps. This study aims to elucidate the importance of various neurotrophic factor (NF) gradient characteristics and presentation modalities (bound vs. diffusible) to directed axonal regrowth and Schwann cell migration using a highly-controllable hydrogel-based gradient generation method in combination with in vitro live-cell imaging platforms. By using a surface tension-driven microfluidics platform, we have generated multi-centimeter NF gradients with controlled gradient steepness, length, and concentration range. Through incorporation of immobilized heparin, we can also provide variable NF release kinetics and tunable NF presentation. The gradient hydrogels can then be incorporated into a live-cell imaging system to track and analyze the migration and outgrowth of dorsal root ganglion cells and Schwann cells; analysis using this system is ongoing. Combining a controllable NF gradient generation platform with a programmable analysis system will create a powerful tool for optimizing NF gradients for nerve regeneration applications and will further our understanding of the mechanisms underlying NF gradient-based nerve regeneration.

Disclosures: K.D. Krick: None. **A. Khademhosseini:** None. **A. Hoke:** None. **H. Mao:** None.

Poster

839. PNS Regeneration II

Location: Hall F-J

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Program#/Poster#:839.05/A52

Topic: A.09. Transplantation and Regeneration

Support: NIH R01 NS034484-12

Institute of Nanobiotechnology

Title: A two-compartment organotypic model of mammalian peripheral nerve repair

Authors: *R. SIDDIQUE[1], A. VYAS[2], N. V. THAKOR[1], T. M. BRUSHART[2]
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Abstract: Several growth factors are upregulated by Schwann cells soon after they are denervated. The role played by these factors in promoting the regeneration of adult motor neurons is poorly understood, however, because of the difficulties encountered in modeling this complex system. An experimental model is thus needed that both reproduces the complex three-

dimensional environment of peripheral nerve and facilitates control of the regeneration environment. As a first step in addressing this need, our lab developed an *in vitro* organotypic co-culture model of adult mammalian nerve repair (Vyas et al., 2009). Spinal cord sections from mice that express YFP in their motor neurons were co-cultured with segments of peripheral nerve. Once the nerve was populated with fluorescent motor axons, it was transected and joined to a nerve graft that it could then reinnervate. This system accurately models nerve repair, but cannot isolate the repair environment from that of parent motor neurons. We now describe a two-compartment model that provides this isolation. A PDMS base is divided into two fluidically isolated compartments that are separated by a central bridge. A Transwell™ membrane that rests on the bridge is transected between the compartments, and a peripheral nerve segment conveys axons from the motor neuron compartment to the experimental compartment. We have confirmed fluidic isolation of the compartments for 3 weeks, excellent axonal growth across the bridge, and robust reinnervation of nerve grafts in the experimental compartment. With this system, the regeneration environment can be manipulated by enhancing or reducing the concentration of specific growth factors without influencing parent motoneurons. The growth of regenerating axons can be monitored in real time, allowing a new level of precision in studying axon regeneration.

Disclosures: R. Siddique: None. **A. Vyas:** None. **N.V. Thakor:** None. **T.M. Brushart:** None.

Poster

839. PNS Regeneration II

Location: Hall F-J

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Program#/Poster#:839.06/A53

Topic: A.09. Transplantation and Regeneration

Support: NIH Grant 5P20RR020173

NIH Grant NS30255

Title: RNA-binding protein ZBP1 associates with beta-actin and GAP-43 mRNAs with different affinities for axonal localization

Authors: *H. KIM[1], P. KIM[1], N. I. PERRONE-BIZZOZERO[2], J. L. TWISS[3], S. YOO[1]
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Abstract: Proper mRNA transport and local protein synthesis are crucial not only during the development of the nervous system, but also for regeneration after injury. Numerous studies

have shown that targeting mRNAs to the distal processes of neurons is driven by localization signal(s), most often in the 3' untranslated regions (UTRs) of the mRNAs, and RNA-binding proteins (RBPs) that are important for RNA localization and translational regulation. 40 nucleotides in GAP-43 mRNA's 3'UTR (including the HuD binding site) are both necessary and sufficient for its axonal transport. GAP-43 and β-actin mRNAs compete for binding to ZBP1 for axonal localization (Donnelly et al., 2011). Interestingly, ZBP1 and HuD were previously shown to interact within a ribonucleoprotein (RNP) complex associated with tau mRNA (Atlas et al., 2004). Based on these observations, we asked how these HuD and ZBP1 interact to form a RNP complex containing cognate mRNAs. We transiently transfected F11 cells, a hybridoma of embryonic rat DRG neurons and mouse neuroblastoma, with Flag-tagged ZBP1 and Myc-tagged HuD. RNA-immunoprecipitation (RIP) experiments using the specific anti-Flag or -Myc antibodies demonstrate that HuD and ZBP1 co-immunoprecipitate in an RNA- dependent fashion. Both β-actin and GAP-43 mRNAs are present in the immunoprecipitates. We then determined the relative binding affinity of β-actin and GAP-43 mRNAs for binding to ZBP1 using quantitative RT-PCR analysis. Results show that although ZBP1 interacts specifically with both β-actin and GAP-43 mRNAs; this RNA-binding protein shows a higher affinity for β-actin than for GAP-43 mRNA. *In vitro* binding assays with recombinant ZBP1 and HuD proteins and qPCR analyses were used to determine if ZBP1 and HuD interact directly with the mRNAs or require additional protein binding partners. These data confirmed that ZBP1 binds to both β-actin and GAP-43 mRNAs but shows different affinities for these transcripts.

Disclosures: H. Kim: None. **P. Kim:** None. **N.I. Perrone-Bizzozero:** None. **J.L. Twiss:** None. **S. Yoo:** None.

Poster

839. PNS Regeneration II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:839.07/A54

Topic: A.09. Transplantation and Regeneration

Support: Axonia Medical

Title: Tissue engineered nerve constructs for repair of peripheral nerve injury in swine.

Authors: M. I. EZRA, J. A. WOLF, K. D. BROWNE, *D. H. SMITH
Div. Neurosurg., Univ. of Pennsylvania/Penn Ctr. For Brain Injury and Repair, PHILADELPHIA, PA

Abstract: Peripheral nerve injuries present a serious medical concern, constituting approximately 2.8 % of all trauma cases in the US, and approximately 100,000 neurosurgical procedures in the US and Europe annually. Although peripheral nerves have the ability to regenerate on their own, current techniques used to repair peripheral nerve damage do not sufficiently restore sensory and motor function, as axons are slow to extend across the injury gap and make reconnections on the distal end. The resulting limited functional recovery is often associated with inadequate muscle strength, allodynia, and an overall lower quality of life for the patient. The use of a living axonal graft to bridge a nerve injury site provides axon-mediated axon guidance to regenerating axons, increasing the rate of regeneration and therefore the likelihood of functional recovery following repair.
Previously, our group has demonstrated the ability to engineer living axonal constructs *in vitro* by applying continued mechanical stretch to axons spanning two populations of embryonic rat dorsal root ganglia (DRG) neurons to repair damaged nerves in a rat model. In the current study, we aim to scale up the living nerve constructs to a larger, more clinically relevant model by using dissociated adult porcine DRGs. Here, axons are rapidly stretch grown ex vivo to a length necessary to span the nerve damage in a pig model of peripheral nerve injury. This novel method attempts to emulate neuronal growth mechanisms observed during normal development in large animals, such as the blue whale and the neck of the giraffe. For the current work, we plate dissociated DRGs from an adult pig onto 2 separate plastic membranes: one population on the stationary side and the other on the “towing” side. First, axons are allowed to grow and synapse with neurons on the other membrane for 5 days. Then, the population on the towing membrane is slowly pulled away over several days, aided by a precise computer-controlled motor system, creating long tracts of living axons. We have successfully optimized a protocol to isolate porcine DRGs, dissociate and purify the cells, and culture them under various conditions *in vitro* for greater than 7 weeks. Additionally, we have begun to culture the dissociated DRGs in our elongation systems, and experimented with stretching the axons at various rates in order to optimize overall length and health of the constructs. In the future, we aim to construct porcine axonal constructs in vitro up to 10 cm in length for use to repair defects in the porcine peroneal nerve. This approach may be used to establish or promote functional connections needed for nervous system repair that have not been achieved by other methods.

Disclosures: M.I. Ezra: None. **J.A. Wolf:** None. **K.D. Browne:** None. **D.H. Smith:** Ownership Interest; Douglas Smith.

Poster

839. PNS Regeneration II

Location: Hall F-J

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Program#/Poster#:839.08/A55

Topic: A.09. Transplantation and Regeneration

Support: NIH-NINDS Grant R01 NS047175 (PGP)

NIH Grant R01 HD057521 (VPL)

Title: Glucocorticoids and stress enhance regenerative axon growth in adult sensory neurons

Authors: *J. K. LERCH[1,2], J. K. ALEXANDER[5], A. ZHA[1], D. MOTTI[2], J. C. GENSEL[1], J. I. WEBSTER-MARKETON[1], V. P. LEMMON[3], J. L. BIXBY[4], P. G. POPOVICH[1]
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Abstract: Recently, we showed that stress and glucocorticoids (GCs) exacerbate neuropathic pain caused by injuring the peripheral branches of axons from dorsal root ganglion (DRG) neurons. Stress and GCs are known to affect neuroplasticity in the hippocampus; however, it is not known if similar effects occur in DRG neurons. Western blots revealed greater expression of glucocorticoid receptors (GR) in DRG neurons relative to other stress-responsive regions of the central nervous system, including the hippocampus. DRG neurons isolated from mice subjected to acute (60 min) restraint stress exhibited robust structural plasticity in vitro; both the length and complexity of axon growth was increased compared to neurons isolated from non-stressed mice. These effects were GC- and mTOR-dependent, as pre-treatment of mice with a glucocorticoid receptor (GR) antagonist or mTOR inhibitor (rapamycin) prevented the axon growth-promoting effects of stress on DRG neurons. To explore molecular mechanisms of GR-mediated sensory axon plasticity, regeneration-associated genes (RAGs) that potentially interact with GR were identified by *in silico* gene networking analysis, and interactions were tested using luciferase reporter assays. A constitutively active form of GR significantly increased transcription of several RAGs identified in the network analysis. A transcription factor binding site prediction program identified GR-responsive elements in the promoter regions of these same RAG genes. To test RAG upregulation in vivo, mice were subjected to acute stress followed by crush of the sciatic nerve. PCR analysis of mRNA isolated from DRG neurons confirmed that stress induced RAG expression in vivo. Overall, our experiments reveal the sensitivity of DRG neurons to stress and GCs, which may result in profound anatomical and functional consequences after injury.

Disclosures: J.K. Lerch: None. **J.K. Alexander:** None. **A. Zha:** None. **D. Motti:** None. **J.C. Gensel:** None. **J.I. Webster-Marketon:** None. **V.P. Lemmon:** None. **J.L. Bixby:** None. **P.G. Popovich:** None.

Poster

839. PNS Regeneration II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:839.09/A56

Topic: A.09. Transplantation and Regeneration

Support: Crafoord foundation

Swedish research council

nanometer consortium in Lund

Title: Sensory neurite outgrowth and guidance by electrospun fibres and microtubes *in vitro*

Authors: M. KANJE[1], A. GHASEMI AZAR[1], *F. JOHANSSON[2]
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Abstract: We have previously shown that it is possible to orient nerve fibres by nano- and µ-sized structure alteration of surfaces[1]. Others have demonstrated that neurites can grow along electrospun polymer fibres[2].
In the present study we investigated neurite outgrowth from mouse dorsal root ganglia on electrospun µm-thick fibres made of poly L-lactic acid (PLLA) and assemblies of tubes of polystyrene with an average inner diameter in a range of 20-100 µm. The electrospun fibres were collected on coverslips. The tubes were produced by a tailor made dry spinning system. Individual tubes were assembled into bundles and the space between the tubes filled with polymethylsiloxane (PDMS). Suitably sized (a few mm long)) cross sections were then cut from the tube assemblies. In cross section, only the tube openings were thus left for possible in-growth of neurites. Excised dorsal root ganglia from adult mice were subsequently mounted in Matrigel® on top of the cut tubes or the electrospun fibres. The entire preparation was incubated in a tissue culture dish containing serum-free medium supplemented with nerve growth factor. After a few days culturing the preparations were washed, fixed and stained with antibodies to neurofilaments in order to visualize regenerating neurites.
The neurites grew and oriented along the electrospun fibres.
In the tube experiments, neurites occasionally grew into and down the holes in the tubes. However, most neurites were found on the surface of the tube assembly.

We conclude that either fibres or tubes can act as guides for neurites. The present models for neurite outgrowth are suitable for studies of neurite guidance effects of both tropic, trophic factors.

[1] Kanje M, Johansson F. Nanomodified surfaces and neurite outgrowth. Prog Brain Res 2011;194:253-62.
[2] Bockelmann J, Klinkhammer K, von Holst A, Seiler N, Faissner A, Brook GA, et al. Functionalization of Electrospun Poly(epsilon-Caprolactone) Fibers with the Extracellular Matrix-Derived Peptide GRGDS Improves Guidance of Schwann Cell Migration and Axonal Growth. Tissue Eng Pt A 2011;17:475-86.

Disclosures: M. Kanje: None. **A. Ghasemi Azar:** None. **F. Johansson:** None.

Poster

839. PNS Regeneration II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:839.10/A57

Topic: A.09. Transplantation and Regeneration

Title: Characterization of the Octopus arm morphology and its regenerative capability: Role of Acetylcholinesterase during morphological modification.

Authors: *S. FOSSATI[1], F. CARELLA[2], G. DE VICO[2], F. BENFENATI[1], L. ZULLO[1]
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[2]2Federico II Univ. of Naples, Dept. of Biol. science, Naples, Italy

Abstract: The ability of regenerate whole-body structures has been long studied and still has a peculiar interest in stem cell biology for its therapeutic potential. Regeneration of arms in Cephalopods and in particular in Octopods has been the subject of several studies. The first study on the regeneration of the Octopus arm demonstrated that all regenerated tissues, with the exception of the dermal connective tissue, are produced by the pre-existing tissues of the same kind. However, the details of the regenerative process in the Octopus arm remain to be elucidated. Using Octopus vulgaris as model of regeneration, we are investigating the role of different cell types and neurotransmitters in the regenerative process. Since Octopus has a typical cholinergic innervation at the level of the arm neuromuscular system, we investigated the involvement of acetylcholinesterase (AChE) in the Octopus arm regeneration. AChE has been demonstrated to have non-cholinergic functions in various cell types and to be involved in the regulation of cell proliferation, differentiation and apoptosis. Our hypothesis is based on previous studies, which showed that in lower invertebrates and amphibians different expression levels of

AChE are associated with the regeneration phases. In order to follow cell replacement in the Octopus arm, we first assessed the expression of specific markers involved in cellular proliferation (AgNOR and PCNA). Our results showed that the activity of the enzyme AChE is related to the proliferation stage of the arm regenerative process. Interestingly AgNOR and AChE assay showed a similar trend in particular during the stages when the morphogenesis was mostly dependent upon cell proliferation. We suggest that AChE protein may have an important influence in the process of regeneration and that it could be considered as a potential target to promote or regulate the regenerative process. In order to follow the expression of the AChE gene during regeneration we are now completing the cloning of the entire gene of AChE. AChE is a molecule that evolved very early in evolution and is well conserved across species. So far we obtained a fragment of 1044 bp, corresponding to a protein of 348 aa, which has high homology with the AChEs of other animal species. Our next step is to produce a probe for in-situ experiments to detect different levels of expression of AChE in samples at various stages of regeneration. This will allow us understanding some latent basic pathways to unlock to promote regeneration.

Disclosures: S. Fossati: None. **F. Carella:** None. **G. De Vico:** None. **F. Benfenati:** None. **L. Zullo:** None.

Poster

839. PNS Regeneration II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:839.11/A58

Topic: A.09. Transplantation and Regeneration

Support: DARPA

Title: Carbon nanotubes enhance sensitivity and safety of regenerative multi electrode interfaces

Authors: A. KANNEGANTI[1], S. ANAND[1], R. GRANJA[1], E. KEEFER[2], *M. I. ROMERO-ORTEGA[1]
[1]Bioengineering Deparment, Univ. of Texas at Arlington, Arlington, TX; [2]Plexon Inc, Dallas, TX

Abstract:
Advanced robotic limbs are multi-fingered lightweight devices capable of up to 22 degrees of freedom. However, providing users with natural control and feel of such robotic limbs remains a formidable challenge. Peripheral nerves in the residual limbs of amputees, offers a readily accessible portal to the bidirectional flow of information between the nervous system of the user

(motor), and smart robotic prosthetic devices (sensory). To access such portal, several types of peripheral nerve interfaces (PNI) have been developed along a spectrum of invasiveness and sensitivity. We have reported enhanced sensitivity in single spike recordings using carbon nano tube (CNT) coated electrodes for acute brain implantation. Despite the significant advantages of increased surface area and decreased impedance by CNTs, the long-term reliability in the recording of such electrodes has not been investigated. Here, we show the long term recording of the peripheral neural activity with CNT-coated multi-electrode
arrays in rats during bipedal locomotion. We implanted adult Lewis rats with electrodeposited CNTs on 18-electrode Pt/Ir arrays placed in the regenerative path of the transected sciatic nerve, using 7 mm collagen-filled polyurethane tubes. Neural activity was recorded over time and the changes in the signal quality were quantified. Persistent single spike
activity was observed in chronic nerve implants 30 days post-implantation, and the regenerated tissue was harvested and processed for the visualization of various molecular markers, targeting the molecules associated with the tissue response to the implant. Preliminary results show comparable immune response to uncoated multi electrode implants. This study shows for the first time that CNT-coated REMIs can provide enhanced signal to noise ratio and sensitive recordings of single spike activity in regenerative peripheral neurointerfaces after long term implantation. Together, this data shows that CNT-REMI electrodes provide a sensitive interface for the chronic recording of single spike activity and the safe stimulation of regenerated axons.

Disclosures: A. Kanneganti: None. **S. Anand:** None. **R. Granja:** None. **E. Keefer:** None. **M.I. Romero-Ortega:** None.

Poster

839. PNS Regeneration II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:839.12/A59

Topic: A.09. Transplantation and Regeneration

Title: Adhesion molecule L1-mediated axonal myelination correlates with L1's proteolytic cleavage

Authors: *D. GUSEVA[1,2], M. SCHACHNER[2,3,4]
[1]Cell. Neurophysiol., Hannover Med. Sch., Hannover, Germany; [2]Zentrum für Molekulare Neurobiologie, Univ. Hamburg, Hamburg, Germany; [3]Keck Ctr. for Collaborative Neuroscience, Rutgers Univ., Piscataway, NJ, NJ; [4]Ctr. for Neuroscience, Shantou Univ. Med. Col., Shantou, China

Abstract: Adhesion molecules, which are involved in neurogenesis, synapse formation and myelination, are proteolytically cleaved to achieve functional activity. This posttranslational modification has become of particular interest in morphogenetic events during development of the nervous system. The adhesion molecule L1 shapes development of the nervous system and is beneficial for repair processes in the adult central nervous system of vertebrates by promoting axonal regrowth and neuronal survival. In the peripheral nervous system, L1 is up-regulated by myelination-competent Schwann cells and regenerating axons after nerve damage. L1 is a 200 to 220 kD type I transmembrane protein and consists of six immunoglobulin (Ig)-like domains followed by five fibronectin type III-like repeats and a single transmembrane region that is linked to a short cytoplasmic domain that is highly conserved among members of the L1 family. In the mouse brain, L1 protein is detectable in four distinct molecular forms with sizes of 200, 180, 140, and 80 kD. Previously it was shown that L1 is processed by ADAM10 to yield 85 and 32 kD fragments. The proprotein convertase PC5A also plays an essential role in L1 processing leading to the proteolytic 180 and 140 kD fragments. Here we show that transgenic mice overexpressing L1 under the control of the neuronally active Thy-1 promoter are not different in functional recovery compared with wild-type littermates 12 weeks after femoral nerve injury. However, remyelination in the distal stump of the injured nerve was considerably more improved in transgenic mice than in wild-type littermates. Interestingly, in L1-overexpressing mice the levels of the transmembrane 70 kD fragment of L1 are decreased in the femoral nerve after injury and in the lesion-corresponding spinal cord segments at 5 days after injury, when compared with wild-type littermates. We interpret these findings by assuming that the 70 kD fragment attenuates remyelination by allowing preferential full-length homophilic L1 trans-interactions between Schwann cells and axons, coincident with blocking sites for cleavage, thus allowing full-length L1 to signal transduce in the two interacting cell types of the peripheral nervous system. Our data suggest that L1-mediated axonal myelination requires proteolytic processing of L1 during remyelination after peripheral nerve injury.

Disclosures: D. Guseva: None. **M. Schachner:** None.

Poster

839. PNS Regeneration II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:839.13/A60

Topic: A.09. Transplantation and Regeneration

Title: RNA-Seq and microRNA expression profiling reveal networks of RNA interactions in regenerating dorsal root ganglion neurons

Authors: *D. MOTTI[1], J. H. GANS[1], F. KUO[1], J. K. LERCH[1], R. MORRIS[1,2], J. L. BIXBY[1,2,3], V. P. LEMMON[1,4]
[1]Miami Project To Cure Paralysis, [2]The Hussman Inst. for Human Genomics, [3]Dept. of Cell. and Mol. Pharmacol., [4]Dept. of Neurolog. Surgery, Univ. of Miami, Miami, FL

Abstract: Robust axonal regeneration in the central nervous system remains elusive, while peripheral neurons are known to regenerate their axons after injury. Changes in gene expression, including changes in specific mRNA isoforms (PLoS One, 7(1): e30417) comprise one mechanism controlling the regeneration process. We took advantage of Next Generation Sequencing of the Transcriptome (RNA-Seq) to identify mRNA isoforms differentially expressed in the neurons of the dorsal root ganglion (DRG) after sciatic nerve crush, an injury that is known to initialize the axonal regeneration program in these neurons. The expression of different RNA species is influenced by and influences the activity of small regulatory RNAs (e.g. microRNAs). Therefore we also utilized Quantitative Real-Time PCR to explore differential expression of microRNAs in the same experimental paradigm, to reveal events regulating transcription of individual isoforms. We identified more than 2000 mRNA isoforms and more than 40 microRNAs that were differentially expressed in DRG neurons 7 days after axotomy. Among these changes were a number that have been previously described, but our approach also revealed, for the first time, differential expression on an isoform specific basis. Among the microRNAs, we observed downregulation of mir-9, a neuronal specific microRNA whose inhibition increases axon length in cortical cells (Nat Neurosci., 15: 697-699). We are currently using a bioinformatic approach to predict the interactions among these mRNAs and microRNAs, and thus to describe the regulatory network behind the transcriptional profile of regenerating DRG neurons. A High Content Analysis approach will be used to confirm in vitro the functional role of predicted network nodes in axon regeneration of peripheral and central nervous system neurons. Overall these experiments will enable us to elucidate new mechanisms behind gene expression regulation, by revealing changes in regulatory RNAs and how they regulate specific RNA isoform expression, thus furthering our understanding of the genetic mechanisms underlying the process of axon regeneration.

Disclosures: D. Motti: None. **J.H. Gans:** None. **F. Kuo:** None. **J.K. Lerch:** None. **R. Morris:** None. **J.L. Bixby:** None. **V.P. Lemmon:** None.

Poster

840. Acetylcholine and Acetylcholinesterase

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:840.01/A61

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Federal funds from the Biomedical Advanced Research and Development Authority, Office of the Assistant Secretary for Preparedness and Response, Office of the Secretary, DHHS, under contract HHSO100201100030C with Countervail Corp.

Title: Dose dependency of the effectiveness of galantamine to counter the neurotoxicity of the organophosphorus poison VX

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Abstract: In recent years galantamine, a drug currently approved for treatment of Alzheimer's disease, emerged as a potential medical countermeasure against the acute and delayed toxicity induced by organophosphorus compounds (OPs), including the nerve agents soman, sarin, and VX, and a number of pesticides (PNAS103:13220, 2006; Neurotoxicology 32:785, 2011). The present study was designed to assess the immediate and delayed toxicity of the nerve agent VX and the effectiveness of a delayed post-treatment with galantamine to counter the effects of VX. The guinea pig was the animal model of choice because the sensitivity of humans and non-human primates more closely resembles that of guinea pigs than of other rodents. Here, adult male guinea pigs were injected subcutaneously with saline or VX (0.6x or 1.0xLD50). Within 1 h after the challenge with 1.0xLD50 VX, guinea pigs started to present with signs of acute intoxication, including facial twitches, chewing, and head tremors. Within 3 h, more severe signs of acute intoxication, including convulsions and respiratory distress, became readily pronounced in approximately 50% of the animals. Animals were euthanized, according to the IACUC-approved protocols, whenever signs of acute intoxication became life-threatening. By 24 h following the initial injection of 1.0xLD50 VX, 45% to 50% of the animals remained alive in the different experimental groups. At this time, galantamine (2-10 mg/kg) or saline (0.5 ml/kg) was injected intramuscularly to the animals in all test groups. Neither galantamine nor saline altered the survival outcome of the animals that had been injected 24 h earlier with VX (0.6x or 1.0xLD50) or saline. Three months later, locomotor and exploratory activities of the guinea pigs were assessed in a large open field arena. The overall locomotor activity of the VX-challenged guinea pigs was comparable to that of control (saline/saline-injected) animals. However, VX-exposed guinea pigs presented with anxiety-like behavior; the number of entries to the center zone of the arena as well as the distance traveled and the time spent in this zone were significantly lower among VX-challenged animals than among control animals. The effect of VX was dose dependent, being more pronounced at 1.0xLD50 than 0.6xLD50. Galantamine, dose dependently, countered the anxiety-like behavior triggered by exposure of the guinea pigs to VX; the most effective dose of galantamine was found to be 4 mg/kg. These results support the

contention that post-treatment with galantamine can effectively counter delayed behavioral alterations triggered by an acute exposure to the nerve agent VX.

Disclosures: E. Dowlati: None. **C. Hawkins:** None. **Y. Aracava:** None. **G.W. Basinger:** Employment; Countervail Corp. **E.F.R. Pereira:** None. **J. Mamczarz:** None. **E.X. Albuquerque:** None.

Poster

840. Acetylcholine and Acetylcholinesterase

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:840.02/A62

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant ES019282

Title: Effect of prenatal exposure to chlorpyrifos on cognitive performance of guinea pigs

Authors: L. GAVRUSHENKO, J. D. PESCRILLE, *E. F. PEREIRA, E. X. ALBUQUERQUE, J. MAMCZARZ
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Abstract: An ever growing number of epidemiological studies have provided evidence of organophosphorus (OP) pesticide toxicity to the developing human brain. The present study was designed to test the hypothesis that changes in cognitive and non-cognitive behavior become apparent in offspring born to mothers exposed to low doses of OP pesticides, including chlorpyrifos. To this end, we used guinea pigs, a precocious rodent species that is considered the best non-primate model of human OP intoxication. Pregnant guinea pigs received one daily injection of chlorpyrifos (25 mg/kg s.c.) or peanut oil for 10 days starting on gestation day (GD) 50-53. Offspring were born on GD 65-67, weaned from mothers on postnatal day (PND) 20, and housed according to their sexes. Around PND 30 evaluation of locomotor and exploratory activity started in open field and novel object exploration tests. The overall locomotor activity of the animals was very similar across sexes and treatments. However, in the presence of a novel object, males maintained higher locomotion than females, and males that were exposed in utero to chlopyrifos spent more time close to the object than sex-matched animals that had been exposed to peanut oil. For evaluation of cognitive functions, animals were first trained to find a hidden escape platform in a fixed position in the Morris water maze (MWM) (5 days x 4 trials). After a two-day break, animals were subjected to two days of probe tests, consisting of complete removal of the escape platform, and then two days of platform relocation (4 trials with 1 hour

inter-trial-interval). In the reference memory training, males performed better than females. In addition, males and females that had been exposed in utero to chlorpyrifos did not perform as well as sex-matched guinea pigs that had been exposed to peanut oil. In the first probe test, chlorpyrifos-exposed males showed no spatial bias, whereas peanut oil-exposed males swam preferentially in the area where the escape platform used to be located. On the second probe test neither sex showed spatial bias. When subjected to training to find the escape platform in new locations, male and female guinea pigs that had been exposed in utero to chlorpyrifos were significantly impaired compared to sex-matched animals that had been exposed to peanut oil. These results demonstrate that following in utero exposure to chlorpyrifos young adult guinea pigs present with significant cognitive impairment.

Disclosures: L. Gavrushenko: None. **J.D. Pescrille:** None. **E.F. Pereira:** None. **E.X. Albuquerque:** None. **J. Mamczarz:** None.

Poster

840. Acetylcholine and Acetylcholinesterase

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant ES019282

Title: *In utero* chlorpyrifos exposure leads to augmented CA1 pyramidal GABAergic transmission in the adult male guinea pig

Authors: *R. D. BURKE, E. X. ALBUQUERQUE, E. F. R. PEREIRA
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Abstract: The organophosphorus (OP) pesticide chlorpyrifos (CPF) is among the most commonly used pesticides in the US. Accounting for roughly 50% of the millions of pounds of OP pesticides used yearly, CPF is widely applied to the US food stock. Epidemiological studies report that children who are exposed *in utero* to OP pesticides, including CPF, present higher incidence of cognitive impairments, attention deficit/hyperactivity disorder, and autism spectrum disorders than non-exposed children. Cognitive impairments have also been demonstrated in rats and mice following *in utero* exposure to doses of CPF that do not induce overt maternal toxicity and significant acetylcholinesterase (AChE) inhibition. The current study was designed to test the hypothesis that *in utero* exposure of guinea pigs to CPF impairs synaptic transmission in the hippocampus, a major brain region involved in cognitive processing. Guinea pigs were the model

of choice because like primates, they are precocious. *In utero* development of the brain of guinea pigs parallels that of primates. Dunkin Hartley guinea pig dams were injected subcutaneously with CPF formulated in peanut oil (0.05 LD50, 25 mg/kg/day, 0.5 ml/kg) or peanut oil (0.5 ml/kg) once daily on days 50 to 60 of presumed gestation (GDs 50 to 60). Adult male pups were euthanized on postnatal days 60 to 72 (PNDs 60 to 72). Their hippocampi were harvested, and inhibitory (IPSC) and excitatory (EPSC) postsynaptic currents were recorded by means of the whole-cell patch-clamp technique from CA1 pyramidal cells in hippocampal slices. The internal pipette solution was such that the reversal potential of Cl^- was approximately -45 mV. Thus, IPSCs and EPSCs were isolated utilizing voltage manipulation. The frequency and transferred charge of IPSCs were significantly elevated (2.11x and 2.16x, respectively) in CPF-exposed male guinea pigs (8 animals; 1 to 3 cells per animal, 15 cells) as compared to control (6 animals, 1 to 4 cells per animal, 14 cells). Current analysis discloses no significant difference from control in EPSCs. This augmented CA1 pyramidal inhibitory tone could prove to partially underlie cognitive deficits observed in the morris water maze following *in utero* exposure of guinea pigs to CPF (Mamczarz et al, presented at current meeting).

Disclosures: R.D. Burke: Employment; Division of Translational Toxicology, Department of Epidemiology and Public Health, University of Maryland School of Medicine. **E.X. Albuquerque:** Employment; Division of Translational Toxicology, Department of Epidemiology and Public Health, University of Maryland School of Medicine. **E.F.R. Pereira:** Employment; Division of Translational Toxicology, Department of Epidemiology and Public Health, University of Maryland School of Medicine.

Poster

840. Acetylcholine and Acetylcholinesterase

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Countervail Corp. Contract, NIH SBIR Grant R44NS068049

Title: Effectiveness of galantamine and pyridostigmine to prevent the acute toxicity of the organophosphorus nerve agent sarin: Telemetric EEG recordings and Fluoro-Jade staining

Authors: *Y. ARACAVA[1], M. ADLER[3], G. KULKARNI[1], J. R. HUNT[1], E. F. R. PEREIRA[1], L. DETOLLA[2], E. X. ALBUQUERQUE[1]
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Abstract: The nerve agent sarin, one of the most toxic organophosphorus (OP) compounds, has been used in terrorist attacks against civilians in Japan and in the battlefield. In the event of an acute exposure to nerve agents, treatment with the muscarinic receptor antagonist atropine, the acetylcholinesterase reactivator pralidoxime, and the anticonvulsant diazepam may be necessary to prevent their acute toxicity and lethality. In 2003, the FDA approved the use of pyridostigmine bromide (PB) as pre-treatment for military personnel at risk of exposure to the nerve agent soman. In recent years, galantamine emerged as an effective medical countermeasure against OP poisoning (PNAS103:13220, 2006). Here, we compared the pre-treatment efficacy of galantamine to that of PB in protecting guinea pigs against the acute toxicity and lethality of multiple lethal doses of sarin. Guinea pigs were treated with PB (0.026 mg/kg, im) or galantamine (8-12 mg/kg, im) 30 min prior to their subcutaneous challenge with 3x or 4xLD50 sarin. One min after sarin challenge, guinea pigs were treated with atropine sulfate (0.5 mg/kg, im) and pralidoxime HCl (25 mg/kg, im). Twelve min after the injection of sarin, when motor convulsions became evident, guinea pigs were treated with diazepam (1.25 mg/kg, im). EEG activity was recorded telemetrically from these animals 2 h before and for 24 h after the sarin injection, and neurodegeneration was assessed by Fluoro Jade B (FJB) staining. Analysis of EEG recordings revealed that seizures elicited by 3xLD50 sarin subsided within an h, with no recurrence, regardless of whether the animals that had been pre-treated with galantamine or PB. However, a different profile for pretreatment efficacy emerged with 4xLD50 sarin. In animals pre-treated with 12 mg/kg galantamine, seizures induced by 4xLD50 sarin lasted less than an h; seizures recorded from PB-pretreated guinea pigs that were challenged with this dose of sarin were more severe, lasted longer, and recurred during the 24-h recording. Accordingly, FJB staining showed extensive degeneration of neurons in the piriform cortex, amygdala, and hippocampus of guinea pigs that were pre-treated with PB and challenged with 4xLD50 sarin. No FJB staining was evident 24 h after the challenge with 4xLD50 sarin in guinea pigs that had been pre-treated with galantamine or on those that were exposed to 3xLD50. In conclusion, galantamine is more effective than PB in preventing the lethality, controlling seizures, and limiting neuronal death induced by high levels of sarin. (The views expressed are those of the authors and do not reflect the official policy of the U.S. Department of Army, Department of Defense, or U.S. Government)

Disclosures: Y. Aracava: None. **M. Adler:** None. **G. Kulkarni:** None. **J.R. Hunt:** None. **E.F.R. Pereira:** None. **L. DeTolla:** None. **E.X. Albuquerque:** None.

Poster

840. Acetylcholine and Acetylcholinesterase

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:840.05/A65

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Federal funds from the Biomedical Advanced Research and Development Authority, Office of the Assistant Secretary for Preparedness and Response, Office of the Secretary, DHHS, under contract HHSO100201100030C with Countervail Corp.

Title: Effects of an acute exposure to the organophosphorus poison VX on the pharmacokinetics of galantamine in guinea pigs

Authors: R. H. COOMBES, J. R. HUNT, Y. ARACAVA, *J. BANERJEE, E. F. R. PEREIRA, E. X. ALBUQUERQUE, W. P. FAWCETT
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Abstract: Galantamine, a reversible acetylcholinesterase (AChE) inhibitor that is currently approved for treatment of mild-to-moderate Alzheimer's disease, has emerged as an effective antidotal therapy against the acute toxicity of organophosphorus (OP) nerve agents and pesticides (Albuquerque et al., PNAS 103: 13220, 2006). In the present study, we evaluated the pharmacokinetic profile of galantamine in adult male and female guinea pigs that were injected subcutaneously with saline (0.5 ml/kg) or VX (0.6x or 1xLD50) and 24 h later treated intramuscularly with galantamine (2, 4, 8, or 10 mg/kg). Blood (0.4 ml) was collected from a jugular vein catheter into heparinized ependorf tubes before and 5, 15, 30, 60, 120, 240, 360, and 450 min after the treatment. Plasma concentrations of galantamine were determined by means of high-performance liquid chromatography as described in Albuquerque et al. (2006). At all doses tested, the pharmacokinetic profile of galantamine was not significantly different between male and female guinea pigs. For example, in saline-injected males that were post-treated with 2 mg/kg galantamine (n = 6), maximal concentration of galantamine (Cmax), time to reach Cmax (Tmax), area-under-the-curve (AUC), and half-life (t1/2) were 0.33 ± 0.06 µg/ml, 12.5 ± 5.1 min, 23.8 ± 7.54 µg/ml.min, and 51.5 ± 28.2 min, respectively. In females injected with the same dose of galantamine (n = 6), Cmax, Tmax, AUC, and t1/2 were 0.38 ± 0.03 µg/ml, 11.0 ± 2.45 min, 35.6 ± 2.82 µg/ml.min, and 75.9 ± 13.7 min, respectively. While Cmax and Tmax were not affected by the VX challenge, t1/2 and AUC were significantly different ($p < 0.01$)between control and VX-injected guinea pigs that were post-treated with 2 or 4 mg/kg galantamine. For example, in adult male guinea pigs that were challenged with 0.6xLD50 VX and 24 h later treated with 2 mg/kg galantamine, Cmax, Tmax, AUC, and t1/2 were 0.49 ± 0.15 µg/ml, 10.0 ± 5.48 min, 85.4 ± 19.0 µg/ml.min, and 174.1 ± 36.8 min, respectively. Following the challenge with 1xLD50 VX, AUC and t1/2 were further increased to approximately 190 µg/ml.min and 230 min, respectively. The finding that an acute exposure to VX increases the bioavailabilty of low doses of galantamine may be accounted for by decreased metabolism of the drug due to the hepatotoxic effects of VX.

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Poster

840. Acetylcholine and Acetylcholinesterase

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:840.06/A66

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant NS076429

Title: Effect of prenatal exposure to soman on cognitive performance of guinea pigs

Authors: J. PESCRILLE, L. GAVRUSHENKO, Y. ARACAVA, E. F. R. PEREIRA, E. X. ALBUQUERQUE, *J. MAMCZARZ
Dept Epidemiol Publ. Hlth., Univ. of Maryland Sch. Med., Baltimore, MD

Abstract: The sensitivity of the developing brain to organophosphorus (OP) nerve agents and the safety of antidotes to pregnant women and developing fetuses are unknown. Thus, during the 1995 terrorist attack with the nerve agent sarin in Tokyo, pregnant women who sought medical attention after presenting mild signs of OP toxicity received no therapeutic treatment (J Clin Neurosci 9:17, 2002). The urgency of studies to investigate the developmental neurotoxicity of nerve agents is underscored by the recent report of neurobehavioral teratogenicity of sarin in an avian model (Neurotoxicol Teratol 31:406, 2009). Here, we tested the hypothesis that the developing mammalian brain is exquisitely sensitive to nerve agents, including soman. We used guinea pigs because: (i) their brain development closely resembles that of humans and non-human primates, and (ii) like humans, guinea pigs have low levels of circulating carboxylesterases -enzymes that metabolically inactivate OPs. Pregnant guinea pigs were injected with soman 1xLD50 (12 ug/kg, sc) or saline (0.5 ml/kg, sc) between the shoulder blades around gestation day (GD) 50. Offspring were born at term around GD65-67, weaned from mothers on postnatal day (PND) 20, and housed according to their sexes. Evaluation of cognitive functions started around PND 30-40 in the Morris water maze (MWM). During the acquisition phase, animals were trained to find a hidden platform in a constant location in two 5-day blocks (4 trials a day) interspaced by 2-day break. Memory retention was analyzed in probe tests performed in the pool without the platform three days after finishing each training session. Guinea pigs that had been exposed in utero to soman showed significant learning impairment during the first 5-day block of the acquisition phase of the MWM task; only towards the end of the second training block, animals that had been exposed in utero to soman were able to find the

hidden platform as well as control animals. To further evaluate the nature of the learning impairment of the soman-exposed guinea pigs, all animals were trained to find a hidden platform in a MWM that was located in a testing room completely distinct from the previous one. The acquisition phase consisted of 5-day training (4 trials a day) and was followed three days later by a probe test. The learning impairment of soman-exposed guinea pigs became apparent again in the new testing room. These results demonstrate that prenatal exposure to soman trigers significant leaning impairment and that this impairment is likely due to inability of the animals to process spatial information.

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Poster

840. Acetylcholine and Acetylcholinesterase

Location: Hall F-J

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Program#/Poster#:840.07/A67

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: This work was supported in part by the National Institutes of Health National Institute of Neurological Disorders and Stroke CounterACT Program, Grant U01 NS059344

Title: Cognitive impairment in adult male guinea pigs long after an acute exposure to the organophosphorus poison soman

Authors: E. F. R. PEREIRA[1], J. D. PESCRILLE[1], L. GAVRUSHENKO[1], *R. GAMMANS[2], J. MAMCZARZ[1], E. X. ALBUQUERQUE[1]
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Abstract: Nerve agents, including soman, are compounds chemically related to, but far more toxic than organophosphorus (OP) pesticides. It is well known that exposure to lethal doses of these compounds triggers a cholinergic syndrome that results primarily from the irreversible inhibition of acetylcholinesterase. The syndrome is characterized by excessive chewing, hypersecretions, loss of motor coordination, convulsions, and respiratory arrest. Recently we reported that cognitive impairment develops months after an acute exposure of prepubertal female guinea pigs to 1xLD50 soman (Neurotoxicology 32:785, 2011). Since there is evidence that the acute toxicity of nerve agents in guinea pigs is sex and age dependent (J Pharmacol Exp Ther 328:516, 2009), the present study was designed to determine whether the delayed neurotoxic effects of soman can also be detected in adult male guinea pigs. To this end, adult

male guinea pigs (3 months old) were injected subcutaneously with 1xLD50 soman (26.3 µg/kg) or saline (0.5 ml/kg). Four months later their cognitive performance was evaluated in the Morris water maze. Animals were trained to find a hidden platform in a constant position in two 5-day blocks (4 trials a day) interspaced by 2-day break. Memory retention was evaluated in probe tests that were performed 3 days after finishing each of the training blocks. During the first 5-day training block, soman- and saline-injected animals performed equally well in the MWM. The escape latency and the distance they travelled to reach the hidden platform were comparable between the two treatments. During the second 5-day training block, saline-injected animals continued to learn to reach the platform, as evidenced by the progressive shortening of the escape latency and distance traveled with days of training. On the other hand, learning did not progress with soman-challenged guinea pigs during the second training session. Memory retention was also impaired by soman. To further evaluate the ability of the animals to process information from spatial cues, animals were transferred to a new room, with distinct configuration, and trained in another MWM in a 4-day block (4 trials a day). Memory retention was evaluated 4 days later in a probe test. Soman-challenged guinea pigs were memory impaired when compared to control animals. Thus, exposure of adult male guinea pigs to the same dose of soman affects cognitive processing of spatial cues. This is in contrast to the procedural learning impairment that has been reported to develop months after an acute exposure of prepubertal female guinea pigs to 1xLD50 soman (Neurotoxicology 32:785, 2011).

Disclosures: E.F.R. Pereira: None. **J.D. Pescrille:** None. **L. Gavrushenko:** None. **R. Gammans:** Employment; Countervail Corp.. **J. Mamczarz:** None. **E.X. Albuquerque:** None.

Poster

840. Acetylcholine and Acetylcholinesterase

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:840.08/A68

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant NS25296

Title: Tonically active α7 nicotinic receptors (nAChRs) regulate glutamate synaptic activity in CA3 pyramidal neurons to maintain glutamatergic input to CA1 pyramidal neurons

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Abstract: Recently we reported that in the rat hippocampus glutamatergic transmission to CA1 pyramidal neurons is maintained in part by tonically active α7 nAChRs. The location of these receptors remains elusive, however. The major glutamatergic input to CA1 pyramidal neurons arise from CA3 pyramidal neurons through the Schaffer collaterals. CA3 pyramidal neurons and the mossy fibers that synapse onto these neurons are known to express α7 nAChRs. Thus, this study was designed to test the hypothesis that tonically active α7 nAChRs that contribute to the maintenance of glutamatergic synaptic inputs to CA1 pyramidal neurons are largely in the CA3 field of the hippocampus.To test this hypothesis, action potential-dependent excitatory postsynaptic currents (EPSCs) were recorded from CA1 and CA3 pyramidal neurons in rat hippocampal slices under various experimental conditions. The mean frequency of spontaneous EPSCs recorded from CA3 pyramidal neurons (0.87 ± 0.03 Hz) was found to be higher compared to that from CA1 pyramidal neurons (0.61 ± 0.03 Hz). The α7 nAChR antagonist methyllycaconitine (MLA, 10 nM) suppressed the frequency of spontaneous EPSCs by 29.4 ± 2.1% and 22.4 ± 2.3%, respectively, in CA1 and CA3 pyramidal neurons,while having no significant effect on the kinetics of the events. Surgical ablation of the CA3 region from the slices caused a 30.6 ± 4.1% and 22.2 ± 2.1% reduction in frequency and peak amplitude, respectively, of spontaneous EPSCs recorded from CA1 pyramidal neurons. Moreover, in CA3-free slices, the effectiveness of MLA (10 nM) to suppress the frequency of spontaneous EPSCs recorded from CA1 pyramidal neurons decreased significantly. These results demonstrate that tonically active α7 nAChRs in the CA3 field contribute significantly to the maintenance of glutamatergic synaptic activity in CA1 pyramidal neurons.

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Poster

840. Acetylcholine and Acetylcholinesterase

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Program#/Poster#:840.09/A69

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant NS25296

Title: Kynurenic acid and tonically active nicotinic receptors modulate resting excitability of CA1 stratum radiatum interneurons in rat hippocampal slices

Authors: E. X. ALBUQUERQUE[1], E. F. R. PEREIRA[1], *T. D. GOULD[2], M. ALKONDON[1]
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Abstract: CA1 *stratum radiatum* interneurons (SRIs) express α7 nicotinic receptors (nAChRs) and receive inputs from glutamatergic neurons/axons that express α3β4β2 nAChRs. To test the hypothesis that tonically active α7 and/or α3β4β2 nAChRs control the excitability of CA1 SRIs in rat hippocampal slices, we examined the effects of selective receptor antagonists on spontaneous fast current transients (CTs) recorded from these interneurons under cell-attached configuration or AMPA EPSCs recorded in the whole-cell patch configuration. Hippocampal slices were exposed to the test chemicals by preincubation followed by bath application during the recordings. The frequency of CTs, which represent action potentials, increased in the absence of extracellular Mg^{2+} and decreased in the presence of the α3β4β2 nAChR antagonist mecamylamine (3 μM) and in the presence of kynurenic acid (KYNA, 0.2 to 2 μM). The α7 nAChR antagonist methyllycacotine (10 nM) also reduced the frequency of CTs, but only in a subset of the SRIs. Mecamylamine and KYNA also reduced the frequency of AMPA EPSCs, particularly the large-amplitude events. Thus, α3β4β2 nAChRs present on glutamatergic axons/neurons synapsing onto SRIs are activated by basal levels of acetylcholine and the tonic activity of these receptors contributes to the maintenance of the excitability of the SRIs. The suppression of action potential frequency in SRIs by submicromolar concentrations of KYNA suggests the presence of high-affinity sites for KYNA in hippocampal slices.

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Poster

840. Acetylcholine and Acetylcholinesterase

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Program#/Poster#:840.10/A70

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH grant ES019282

Title: : Long-lasting histone modifications are detected in the brains of young adult guinea pigs exposed *in utero* to the organophosphorus pesticide chlorpyrifos.

Authors: *W. R. RANDALL[1,2], R. XU[1], E. F. R. PEREIRA[2,1], E. X. ALBUQUERQUE[2,1]
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Abstract: Organophosphorus (OP) pesticides such as chlorpyrifos (CPF) and malathion are among the most heavily used pest control agents worldwide. Epidemiological data indicate that OP pesticides are toxic to the developing human brain. *In-utero* exposure to OP pesticides has been linked to increased incidence of abnormal reflexes in the neonate as well as delayed mental development in young children. While the acute toxicity of OP pesticides results primarily from the rapid inhibition of acetylcholinesterase, the delayed toxicity is more likely related to long-term changes in gene expression. Our hypothesis is that *in utero* exposure to CPF alters epigenetic mechanisms, particularly histone modifications, that induce changes in the expression of genes known to control higher brain functions. To test this hypothesis, we used guinea pigs, a precocious rodent species that is considered the best non-primate model of human OP intoxication. Pregnant guinea pigs were injected subcutaneously once daily with 25 mg/kg CPF or with peanut oil (the CPF vehicle) for 10 days starting on gestation day 50-53. Guinea pigs generally delivered their pups at GD65-67. On post-natal day 60, animals were euthanized and their brains were removed. Nuclear and cytosolic fractions of brains of the animals were processed and examined by western blotting for histone modifications and intracellular proteins involved in activating histone acetyltransferases and kinases. Levels of acetyl- and phopho-histone 3 as well as levels of acetyl- and phospho-histone 2a and 2b were found to be higher in the brains of animals developmentally exposed to CPF *in utero* than in the brains of the control whose mothers were given peanut oil diluent. In addition, we observed a significant (2-fold) increase in tri-methylated histone 3 in CPF-exposed animals. The expression of p38 kinase was significantly higher in the brains of animals developmentally exposed to CPF than in the brains of control animals. This is noteworthy because p38 is one of the kinases that activates mitogen- and stress-activated kinases 1 and 2 (MSK1/2), which have been identified as the principal histone H3 kinases. Together these results suggest that exposure to CPF during early development produces epigenetic modifications in the acetylation, phosphorylation and methylation of histones 2a, 2b and 3 that could lead to long-term changes in gene expression accompanying the neuronal abnormalities observed in the deficits in cognition.

Disclosures: W.R. Randall: None. **R. Xu:** None. **E.F.R. Pereira:** None. **E.X. Albuquerque:** None.

Poster

840. Acetylcholine and Acetylcholinesterase

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:840.11/B1

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Federal funds from the Biomedical Advanced Research and Development Authority, Office of the Assistant Secretary for Preparedness and Response, Office of the Secretary, DHHS, under contract HHSO100201100030C with Countervail Corp.

Title: An acute exposure to the organophosphorus poison VX shortens sleep episodes in guinea pigs: An electroencephalographic (EEG) analysis.

Authors: J. R. HUNT, E. ALEXANDROVA, Y. ARACAVA, E. F. R. PEREIRA, *E. X. ALBUQUERQUE
Div. of Translational Toxicology, Dept Epidemiology and Publ. Hlth., Univ. Maryland Sch. Med., Baltimore, MD

Abstract: The acute toxicity of lethal doses of the organophosphorus (OP) nerve agents soman, sarin, and VX is well characterized by a cholinergic crisis that culminates on recurrent seizures. More subtle, long-lasting EEG alterations have been seen in rats intoxicated with 1xLD50 soman and in mice injected multiple times with sub-lethal doses of soman (Pharmacol Biochem Behav 42:711, 1992; Toxicology 199:59, 2004). Here, we analyzed cortical EEG recordings from guinea pigs exposed to a sub-lethal dose of VX, and/or treated with galantamine, a drug that has recently emerged as an effective therapeutic countermeasure against OP toxicity (PNAS 103: 13220, 2006). Adult male guinea pigs were implanted with a radio-telemetric electrode system (Data Sciences Intl, DSI) and maintained under light-dark 12:12 h cycle until full recovery from the surgery (10 days). On the 11th day, recordings of cortical EEG and temperature were obtained telemetrically for at least 2 h before and 6 h after with the subcutaneous injection of $0.6xLD_{50}$ VX (3.81 µg/kg) or saline (0.5 ml/kg). On the next day, recordings were continued for 2 h before treatment of the guinea pigs with galantamine (4 mg/kg, im) or saline (0.5 ml/kg, im) and for 6 h thereafter. Recordings were also obtained up to 7 days after treatments. No seizure activity was detected in EEG recordings from VX-injected guinea pigs before or after their post-treatment with saline or galantamine. Prior to the injection of VX, animals spent 36.3 ± 2.7% of the time sleeping. During the first 6 h after the VX challenge, animals spent only 14.8 ± 2.1% of the time sleeping. The VX challenge also decreased the ratio of the spectral density of the delta frequency to the total EEG power density. Twenty four hours after the VX challenge, although the total time the animals spent sleeping was comparable to control, the longest awake time of the VX-challenged guinea pigs was still significantly longer than that of control animals. By 48 h after the challenge, the sleep-wakefulness patterns and the EEG spectrum were similar to control. When animals that had been with injected with saline or VX were treated with galantamine, there was a transient reduction of the sleeping time; the effect of galantamine subsided within 1 h after the treatment. No changes were observed after injections guinea pigs with saline only. These results demonstrate that a single exposure of guinea pigs to a sub-lethal dose of VX significantly shortens sleeping time and this effect is accompanied by reduction of the delta activity. We suggest that EEG sleep-wakefulness pattern changes may be used clinically for evaluation of the effectiveness of therapeutic interventions to treat low level OP exposure.

Disclosures: J.R. Hunt: None. **E. Alexandrova:** None. **Y. Aracava:** None. **E.F.R. Pereira:** None. **E.X. Albuquerque:** None.

Poster

840. Acetylcholine and Acetylcholinesterase

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:840.12/B2

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: This work was supported in part by the National Institutes of Health National Institute of Neurological Disorders and Stroke CounterACT Program Grant U01 NS059344

Title: Acetylcholinesterase inhibition increases the activity of both α7 and non-α7 nAChRs in hippocampal slices

Authors: *C. R. SOURS[1], M. ALKONDON[2], E. F. R. PEREIRA[2], Y. ARACAVA[2], E. X. ALBUQUERQUE[2]
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Abstract: It is well known that inhibition of acetylcholinesterase (AChE), the enzyme that breaks down the neurotransmitter acetylcholine (ACh), leads to overactivation of most nicotinic receptors (nAChRs). However, there have been reports that the activity of synaptically located α7 nAChRs is not increased upon AChE inhibition (Neuron 17:1231, 1996). Studies from our laboratory have demonstrated that the excitability of CA1 stratum radiatum interneurons (SRIs) is regulated by α7 and non-α nAChRs (J Neurophysiol 86:3043, 2001). Thus, the present study was designed to determinehow the activity of hippocampal α7 and non-α7 nAChRs changes in response to AChE inhibition *in vivo* and *in vitro*. To this end, spontaneous glutamatergic excitatory postsynaptic currents (EPSCs) were recorded from CA1 SRIs in hippocampal slices obtained from 12-19-day old male guinea pigs after their subcutaneous injection with the irreversible AChE inhibitor soman (28 µg/kg). EPScs were also recorded from CA1 SRIs in rat hippocampal slices superfused *in vitro* with artificial cerebrospinal fluid containing the reversible AChE inhibitor donepezil. All recordings were obtained in the presence of the muscarinic receptor antagonist atropine (0.5 µM). In hippocampal slices obtained from guinea pigs 24 h after the injection of soman (28 µg/kg, sc) the frequency of EPSCs recorded from the SRIs was higher than that recorded from neurons in slices of control animals. *In vitro* exposure of rat hippocampal slices to donepezil (100 nM) caused a significant and sustained increase in the

frequency of EPSCs. Bath application of donepezil also increased the proportion of short (20-100 ms) inter-event intervals, indicating the presence of burst-like EPSCs. The effects of donepezil were significantly reduced by mecamylamine (10 μM) in all slices tested (n = 11). On the other hand, the α7 nAChR antagonist methyllycaconitine (10 nM) decreased the effects of donepezil in only half of the slices tested. These results suggest that enhanced levels of ACh resulting from AChE inhibition are sufficient to activate both mecamylamine-sensitive (α3β4β2) and MLA-sensitive (α7) nAChRs on glutamate neurons/axons, and thereby increase glutamate drive to the interneurons.

Disclosures: C.R. Sours: None. **M. Alkondon:** None. **E.F.R. Pereira:** None. **Y. Aracava:** None. **E.X. Albuquerque:** None.

Poster

840. Acetylcholine and Acetylcholinesterase

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:840.13/B3

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: DTRA contract HDTRA1-10-C-0067

Title: Post-treatment with galantamine effectively counters cognitive impairment induced by exposure of adult male guinea pigs to high levels of the organophosphorus poisons soman or sarin

Authors: J. MAMCZARZ[1], J. D. PESCRILLE[1], L. GAVRUSHENKO[1], Y. ARACAVA[1], *E. J. BEECHAM[2], E. F. R. PEREIRA[1], E. X. ALBUQUERQUE[1]
[1]Div. of Translational Toxicology, Dept. of Epidemiology and Publ. Hlth., Univ. Maryland Sch. Med., Baltimore, MD; [2]Drug Develop., Countervail Corp., Charlotte, NC

Abstract: It is well known that, in the absence of any pre-treatment, an aggressive treatment with the muscarinic receptor antagonist atropine and an anticonvulsant such as diazepam or midazolam is necessary to curtail the acute toxicity of high doses of the organophosphorus (OP) poison soman and sarin. Here, we addressed the question as to whether cognitive impairment becomes evident in guinea pigs that are exposed to 1.2xLD50 soman or 2.0xLD50 sarin and post-treated with atropine, midazolam, and 2-PAM, and, if so, whether this impairment can be counteracted by inclusion of galantamine, a drug that recently emerged as an effective antidote against the acute toxicity of OP compounds (PNAS103:13220, 2006), in the post-treatment regimen. Adult (3-month-old) male guinea pigs were subcutaneously injected with 1.2xLD50 soman (29.3 μg/kg) or 2.0xLD50 sarin (63.4 μg/kg). One min after the OP challenge animals

were post-treated with atropine (2mg/kg, i.m.) and pralidoxime (25 mg/kg, i.m.), and, then, 40 min later, with midazolam (1.8 mg/kg, i.m.). Twenty four h later guinea pigs were treated with galantamine (5 mg/kg, i.m.) or saline. Three to four months after the treatments, animals were trained to find a hidden platform in a constant position in the Morris water maze (MWM). Training consisted of four trials a day over five consecutive days. Memory retention was evaluated three days later in a probe test. One week after training in the MWM, animals were transferred to a new room and received five days of reference memory training consisting of 4 trials/day in a radial 8-arm water maze (RWM). After a 2-day break, the reference memory training was continued in a one-trial-a-day paradigm for two more days. On the 8th day of RWM training, the platform was moved to the opposite arm and training continued in the one-trial-a-day paradigm. When compared to control (animals injected with saline only), guinea pigs that had been challenged with soman or sarin and post-treated with atropine, midazolam, and 2-PAM showed learning impairment in the MWM. These animals also presented with deficits in the formation of new memories in the RWM. Inclusion of galantamine in the post-treatment regimen completely abolished the adverse effect of soman on cognition. These results demonstrate that cognitive impairments do become evident long after an exposure of guinea pigs to high doses of soman or sarin, despite the post-treatment with atropine, midazolam, and 2-PAM, and are effectively countered by inclusion of galantamine in the post-treatment regimen.

Disclosures: J. Mamczarz: None. **J.D. Pescrille:** None. **L. Gavrushenko:** None. **Y. Aracava:** None. **E.J. Beecham:** Employment; Countervail Corp. **E.F.R. Pereira:** None. **E.X. Albuquerque:** None.

Poster

840. Acetylcholine and Acetylcholinesterase

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:840.14/B4

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Canadian Institutes of Health Research

Capital District Health Authority Research Fund

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Dalhousie Medical Research Foundation

Title: Butyrylcholinesterase and cholinergic neural elements in the mouse brain

Authors: *S. DARVESH[1,2], G. A. REID[2], N. CHILUKURI[3]
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Abstract: Introduction: The cholinergic neurotransmitter acetylcholine is synthesized by choline acetyltransferase (ChAT) and its action terminated by the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). While the role of AChE in cholinergic regulation has been studied extensively, that of BuChE remains to be explored. The present work was therefore undertaken to map the distribution of BuChE and examine its colocalization with cholinergic neural elements.
Methods: 129S1/SvImJ mice were used to map the distribution of BuChE and determine its relationship to the cholinergic neurotransmitter system. Brain tissue was stained for BuChE activity, using a modified Karnovsky-Roots histochemical method, and ChAT, using immunohistochemistry. Immunofluorescent double labeling was used to demonstrate colocalization of BuChE and ChAT.
Results: In the mouse, BuChE staining labeled neurons, neuropil, white matter and glial cells, while ChAT labeled neurons and neuropil. Areas with BuChE-positive neurons included the anterior and lateral thalamic groups, medial amygdala, lateral hypothalamus and several cranial nerve nuclei. BuChE and ChAT neuronal colocalization was largely confined to the brainstem, with the dorsal motor nucleus of the vagus nerve, hypoglossal nucleus, facial nucleus, inferior salivatory, and the noradrenaline cell group A5 all having numerous neurons with both markers. A close relationship was also observed between BuChE positive neuropil and ChAT positive perikarya in the tegmentum. ChAT positive neuropil was also found in areas with BuChE positive neurons such as the medial entorhinal cortex.
Discussion & Conclusions: BuChE staining of neural elements was found in a number of regions of the mouse brain. There was a close relationship between the distribution of BuChE and ChAT in certain areas of the neuraxis. The staining patterns between these two enzymes included neuronal colocalization, BuChE neuropil enveloping cholinergic neurons, and BuChE neurons found in areas with ChAT neuropil. This close relationship between BuChE and cholinergic neural elements implies that BuChE is involved in cholinergic neurotransmission.

Disclosures: S. Darvesh: None. **G.A. Reid:** None. **N. Chilukuri:** None.

Poster

840. Acetylcholine and Acetylcholinesterase

Location: Hall F-J

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Program#/Poster#:840.15/B5

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: U.S. Army's in-house Laboratory Independent Research (ILIR) program

Title: An *in vitro* neuronal model to study organophosphorus (OP) nerve agent mechanisms of action and countermeasures

Authors: B. KEYSER, B. KANJILAL, D. ANDRES, E. NEALLEY, B. BENTON, A. APPELL, A. GRIGOROVITCH, *R. RAY
USA Med. Res. Inst. Chem Def., Aberdeen Prv Gr, MD

Abstract: The OP nerve agents (NA) soman, sarin, tabun and VX act principally as potent cholinesterase inhibitors; this inhibition causes a cholinergic crisis due to a buildup of excess acetylcholine (ACh) at both peripheral and central nervous system (CNS) synapses. In the CNS, this cholinergic abnormality is believed to trigger a glumatergic response, which contributes to convulsions and subsequent damage to postsynaptic neurons. The limitations of the current therapeutic regimen, i.e., atropine, 2-PAM (2-pyridine aldoxime methyl chloride) and an anticonvulsant compound (diazepam) are that (a) these compounds do not offer an adequate protection against both central and peripheral effects and (b) there is no neuroprotection to prevent the subsequent neuronal damage. However, the progress in developing an ideal therapeutic approach has been hindered by the lack of an appropriate in vitro model that can be used to investigate the possible mechanisms of toxicity and the prospective therapeutic interventions prior to their validation in a suitable animal model. Since nerve agents target neurons in both the central and the peripheral nervous systems, using a cell line that incorporates properties of both central and peripheral neurons would be advantageous. We, therefore, studied the cultured human neuroblastoma N18TG2 and motor neuron-enriched embryonic mouse spinal cord hybrid NSC-34 cell line as a potential neuronal model. NSC-34 cells possess cholinergic properties, e.g., action potential generation, expression of choline acetyltransferase, synthesis and storage of ACh, and neurotransmitter release function. Moreover, differentiating these cells by serum deprivation or altering medium composition makes these cells sensitive to glutamate stimulation. Our results show that differentiated NSC-34 cells undergo cell death due to glutamate toxicity/exposure (0.1 – 20 mM, 24 hrs) as determined by caspase-3 activation and annexin V/propidium iodide flow cytometric analyses. We have also studied the effects of the nerve agent VX on these cells. Results show that NSC-34 cells exposed to VX (10 – 100 nM, 18-24 hrs) exhibit a significant ($p<0.05$) increase in caspase-3 activation, a key enzyme in apoptotic cell death. In addition, VX-treated cells exhibit some morphological changes such as enhanced levels of vacuole formation, which is indicative of cell death. Taken together, these results demonstrate that NSC-34 cells are sensitive to both VX and glutamate; however, whether glutamate is directly involved in the VX effect needs to be established.

Disclosures: B. Keyser: None. **B. Kanjilal:** None. **D. Andres:** None. **E. Nealley:** None. **B. Benton:** None. **A. Appell:** None. **A. Grigorovitch:** None. **R. Ray:** None.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.01/B6

Topic: B.03. G-Protein Linked Receptors

Support: BBSRC

Merck & Co.

Title: Distinguishing the distinct contributions of the Group II metabotropic glutamate receptor subtypes (mGlu2 & mGlu3) in modulating somatosensory transmission *in vivo*

Authors: *T. E. SALT, S. A. NEALE, C. S. COPELAND
UCL Inst. Ophthalmology, London, United Kingdom

Abstract: Group II metabotropic glutamate (mGlu) receptor (mGlu2/3) modulation of somatosensory transmission in the rat ventrobasal thalamus (VB) [1] was recently identified by ourselves to have an mGlu2 component to the overall Group II effect on sensory inhibition [2]. However, due to a paucity in mGlu3 selective pharmacological tools, it has not been possible to draw a concurrent conclusion regarding the role of mGlu3 subtype in modulating this sensory inhibition, until now. Extracellular single-neurone recordings were made in vivo with multi-barrel iontophoretic electrodes in the VB of urethane-anaesthetized male Wistar rats (365-420g). Procedures were Home Office (UK) approved and accorded with the Animals (Scientific Procedures) Act 1986. Single vibrissae were stimulated and mGlu agents were applied while stimulating principal and secondary vibrissae and recording from corresponding single VB neurones.
By using the dual mGlu2 agonist/mGlu3 antagonist LY395756 (5mM, pH 8, 75mM saline) we can hypothesize that the Group II effect on sensory inhibition is largely mediated through activation of mGlu3: when applied alone, the predominant of effect of LY395756 was that of antagonism as responses to short-duration stimulation (10-30ms) of the principal vibrissa were reduced (59% ±8% of control) along with a concurrent increase in sensory inhibition evoked by stimulation of a secondary vibrissa (12% ±2% increase in sensory inhibition) - an effect similar to the Group II antagonist LY341495 under the same conditions [2]. This also intimates that mGlu3 is activated by ‘glutamate spillover’ upon physiological sensory stimulation. In addition, we have provided further evidence that there is indeed an mGlu2 component to the Group II effect on sensory inhibition as, when co-applied with the mGlu2 selective positive allosteric modulator (PAM) LY487379 (1mM, pH 6, 1% DMSO, 75mM saline), there was an apparent

reversal in the action of LY395756 as an increase in responses to short-duration stimulation (117% ±2% of control) was observed, along with a concurrent decrease in sensory inhibition (21% ±2% decrease in sensory inhibition). This is an effect similar to the Group II agonist LY354740 under the same conditions [2] and can be attributed to the PAM revealing the underlying mGlu2 agonism component of LY395756.
Thus we suggest Group II receptor modulation of somatosensory transmission in VB is largely mediated by mGlu3, with only a small mGlu2 component, and that selective mGlu3 compounds might modify function of sensory, attentional and cognitive processes.
1. Turner & Salt (2003) Neuroscience 122:459-469.
2. Copeland et al., (2012) J Physiol 590:937-951.

Disclosures: T.E. Salt: None. **S.A. Neale:** None. **C.S. Copeland:** None.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.02/B7

Topic: B.03. G-Protein Linked Receptors

Title: Effects of xanthurenic acid on synaptic transmission in the mouse hippocampus

Authors: *S. A. NEALE[1], C. S. COPELAND[2], T. E. SALT[2]
[1]Neurexpert, Kent, United Kingdom; [2]Visual Neurosci., UCL Inst. of Ophthalmology, London, United Kingdom

Abstract: Xanthurenic acid (XA), a molecule arising from tryptophan metabolism, has been proposed to act as a signalling molecule in the CNS, perhaps via modulation of Group II metabotropic glutamate (mGlu) receptors[1,2]. It has previously been demonstrated that mGlu receptors modulate synaptic transmission in many brain regions, including the hippocampus. We have, therefore, investigated the effects of XA on synaptic transmission in the *in vitro* hippocampal slice preparation of adult C57BL/6 mice with extracellular field recording. In the dentate gyrus, stimulation of the perforant path resulted in excitatory post-synaptic potentials (EPSPs) which were depressed in amplitude upon application of the Group II mGlu receptor agonist LY354740 (10 nM-1 μM; with 1 uM reducing EPSP amplitude to 24 ± 2% of control levels) and this depression was associated with a clear change in the EPSP2:1 paired-pulse ratio from 93 ± 2% to 126 ± 5%. Application of XA (0.1-3 mM) resulted in a concentration-dependent depression of EPSP amplitude (3 mM; 75 ± 2% of control amplitude) which reversed on washout. This depression persisted in the presence of GABA receptor antagonists and was

associated with a small, albeit statistically significant change in the paired-pulse ratio (91 ± 3% to 95 ± 3%; n = 6; P = 0.001), suggesting a presynaptic effect on glutamate release. The effects of XA were modest in comparison to LY354740 and, in contrast to LY354740, were not reversed in the presence of the Group II antagonist LY341495 (300 nM). Furthermore, co-application of XA (0.1-0.3 mM) with LY354740 (10-30 nM) showed no increase in effect compared to the EPSP depression evoked by LY354740 alone. In the CA1 region, the amplitudes of EPSPs evoked by stimulation of the Schaffer collateral/commissural pathway were unaffected by LY354740 (1 μM; 101 ± 2% of control amplitude), whereas XA produced a depression (3 mM; 80 ± 6% of control amplitude) which fully reversed on wash. We have previously demonstrated an effect of XA on sensory processing in the thalamus via modulation of GABA receptor-mediated neurotransmission[3]. Consistent with XA modulating GABAergic signalling, in the CA1 recordings XA (3 mM) produced changes in the EPSP waveform indicative of a reduction in inhibition. In summary, these data suggest XA may indeed be a potential neuromodulator; however, in the hippocampus this action appears to be via mechanisms independent of Group II mGlu receptor activation.

1 Mauro *et al*., 2010 Society for Neuroscience Annual Meeting
2 Fazio *et al*., 2012 Curr. Neuropharmacol., 9, suppl 1, 19
3. Copeland *et al*., 2012 Neuropharmacology, in press

Disclosures: S.A. Neale: None. **C.S. Copeland:** None. **T.E. Salt:** None.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.03/B8

Topic: B.03. G-Protein Linked Receptors

Support: BBSRC

Merck & Co.

Title: Group II metabotropic glutamate receptor (mGlu2 & mGlu3) modulation of limbic processing in the mediodorsal nucleus of the thalamus.

Authors: *C. S. COPELAND, S. A. NEALE, T. E. SALT
Visual Neurosci., Univ. Col. London, London, United Kingdom

Abstract: The mediodorsal thalamic nucleus (MD), which relays information from the amygdala to the prefrontal cortex (PFC) and limbic system, has been heavily implicated in the

pathophysiology of schizophrenia, as has gating within the thalamic reticular nucleus (TRN), a GABAergic nucleus located between the thalamus and cortex, with which the MD makes reciprocal connections. Group II metabotropic glutamate (mGlu) receptor agonists and positive allosteric modulators have proved efficacious in a number of animal models of schizophrenia and a phase II clinical trial, and have been shown to modulate somatosensory transmission within the rat ventrobasal thalamus (VB) by reducing inhibition originating from the TRN [1,2]. We have therefore examined whether Group II mGlu receptor activation can modulate neurotransmission in the MD upon stimulation of the PFC and amygdala. Extracellular single-neurone recordings were made in vivo with multi-barrel iontophoretic electrodes in the MD of urethane-anaesthetized male Wistar rats (320-420g). Procedures were Home Office (UK) approved and accorded with the Animals (Scientific Procedures) Act 1986. The PFC and amygdala were electrically stimulated using concentric bipolar electrodes, and mGlu agents were iontophoretically applied whilst recording from single MD neurones.
Inhibition evoked in the MD upon stimulation of the amygdala was reduced upon iontophoretic application of the Group II mGlu receptor agonist LY356740 (5mM, pH 8, 75mM saline) (27%±16% reduction in inhibition), and was increased upon application of the Group II mGlu receptor antagonist LY341495 (5mM, pH 8, 75mM saline) (77% increase in inhibition). This action of Group II mGlu receptor modulation of thalamic inhibition is similar to that observed in the VB upon principal vibrissa stimulation (1,2). In addition, the duration of long-latency inhibition evoked upon stimulation of the PFC was reduced upon application of LY354740 (80ms ±20ms reduction in long-latency inhibition duration).
Group II mGlu receptor modulation of inhibition evoked in thalamic relay nuclei may therefore represent an over-arching principle for all thalamic nuclei. In addition, Group II mGlu receptor modulation of neurotransmission within the MD may prove to be important in selective attention and cognitive processing.
1. Turner & Salt (2003) Neuroscience 122:459-469.
2. Copeland et al., (2012) J Physiol 590:937-951.

Disclosures: C.S. Copeland: Research Grant; BBSRC. Other Research Support; Merck & Co. **S.A. Neale:** None. **T.E. Salt:** None.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.04/B9

Topic: B.03. G-Protein Linked Receptors

Title: Allosteric potentiation of mGlu5 receptors reduces spike and wave discharges in the WAG/Rij rat model of absence epilepsy

Authors: V. D'AMORE[1], I. SANTOLINI[1], C. M. VAN RIJN[2], F. BIAGIONI[1], G. MOLINARO[1], A. PRETE[3], P. J. CONN[4,5], C. LINDSLEY[4,5], S. ZHOU[4,5], P. N. VINSON[4,5], A. L. RODRIGUEZ[4,5], C. K. JONES[4,5], S. R. STAUFFER[4,5], *M.-A. SORTINO[6], F. NICOLETTI[7], G. VAN LUIJTELAAR[2], R. T. NGOMBA[1]
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Abstract: Metabotropic glutamate (mGlu) receptors regulate the activity of the thalamic-cortical-thalamic network that is involved in the generation of absence seizures. We have shown that allosteric potentiation of mGlu1 receptors reduces spike and wave discharges (SWDs) in the WAG/Rij rat model of absence epilepsy, whereas activation of mGlu2/3 and mGlu4 receptors enhances SWD frequency. Here, we extended the study to mGlu5 receptors using the novel mGlu5 receptor enhancer, VU0360172.6. WAG/Rij rats showed a reduction in the mGlu5 receptor protein levels and in the mGlu5 receptor signalling in the ventrobasal thalamus, and an increased expression of mGlu5 receptors in the somatosensory cortex, as compared with non-epileptic control rats. At least some of these changes were already present in young WAG/Rij rats, at an age that preceded the onset of absence seizures. Pharmacological blockade of mGlu5 receptors with MTEP (10 or 30 mg/kg, i.p.) did not affect SWDs in WAG/Rij rats. In contrast, treatment with the mGlu5 receptor enhancer, VU0360172-6 (3 or 10 mg/kg, s.c.), significantly reduced absence seizures without affecting motor behaviour. The action of VU0360172-6 was antagonized by MTEP. These findings suggest that changes in mGlu5 receptors contributes to the pathophysiology of absence seizures in WAG/Rij rats, and that drugs that potentiate the activity of mGlu5 receptors are potential candidates to the treatment of absence epilepsy.

Disclosures: V. D'Amore: None. **I. Santolini:** None. **C.M. van Rijn:** None. **F. Biagioni:** None. **G. Molinaro:** None. **A. Prete:** None. **P.J. Conn:** None. **C. Lindsley:** None. **S. Zhou:** None. **P.N. Vinson:** None. **A.L. Rodriguez:** None. **C.K. Jones:** None. **S.R. Stauffer:** None. **M. Sortino:** None. **F. Nicoletti:** None. **G. van Luijtelaar:** None. **R.T. Ngomba:** None.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.05/B10

Topic: B.03. G-Protein Linked Receptors

Title: Role of the metabotropic glutamate receptor mGluR7 at a thalamic reticular synapse

Authors: *F. BERTASO, V. TASSIN, L. FAGNI
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Abstract: The metabotropic glutamate receptor, mGluR7, is a presynaptic receptor that inhibits neurotransmitter release upon elevated synaptic activity. We have shown that inactivation of this receptor causes absence-like epileptic seizures in rodents, which result from thalamo-cortical loop dysfunction. The present study aimed at understanding the specific function of the receptor in synaptic transmission. To this end we used in vitro mouse thalamo-cortical slices and patch-clamp recording in wild-type and transgenic knock-in mice bearing a non functional mGluR7 receptor (mGluR7AAA). We found that the mGluR7/4/8 agonist D,L-AP4 (1.2 mM) inhibited excitatory post-synaptic currents (EPSCs) between thalamic relay cells and thalamic reticular neurons in wild type, but not in mGluR7AAA neurons. This result suggests that mGluR7 is functionally expressed in this synapse. The majority of thalamic inputs on wild-type reticular neurons displayed paired-pulse depression of EPSCs, behaving as classical "drivers" of the reticular neurons. Conversely, paired-pulse facilitation of synaptic transmission was recorded in the majority of mGluR7AAA knock-in reticular neurons. These data suggest that reticular activity can be amplified in the absence of a functional mGluR7, thus inducing a hyper-inhibition of thalamic relay neurons, which in turn may trigger thalamo-cortical loop oscillation. Induction of absence epilepsy by mGluR7 inactivation may therefore results from defects of this pathway.

Disclosures: F. Bertaso: None. **V. Tassin:** None. **L. Fagni:** None.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.06/B11

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant NS048884

Title: mGluR5 signaling in cortical glutamatergic neurons - Regulate cortical excitability

Authors: *H. SUN, C. J. BALLESTER ROSADO, C.-S. WU, C. P. JEW, D.-H. YU, H.-C. LU
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Abstract: Signaling by the group I metabotropic glutamate receptor 5 (mGluR5) in cortical glutamatergic neurons is required for the development of whisker-related patterns in the primary somatosensory cortex (REF: Carlos's paper in JN). Proper excitation/inhibition balance is crucial for sensory perception. Deleting mGluR5 in glutamatergic neurons affects the functional development of GABAergic circuits projected onto glutamatergic neurons. We hypothesis that mGluR5 signaling in cortical glutamatergic neurons is required to regulate cortical excitability. We explored this hypothesis using a mouse genetic approach with electrophysiological experiments in acute brain slices and intact mice as well as a range of behavioral paradigms. At the cellular level, we found an increase in the frequency in spontaneous events in cortical layer IV glutamatergic neurons with cortex-glutamatergic mGluR5 conditional knock-out (Cx-Glu-cKO) and their littermate control mice. Consistent with the increased excitability at the neuronal level, the threshold to induce status epilepticus by kainic acid was reduced in Cx-Glu-cKO mice. At the behavioral level, Cx-Glu-cKO mice exhibit enhanced novelty-induced locomotion, with no obvious deficits in either contextual or auditory-cue conditioned fear. mGluR5 deletion has been found in a subset of patients with Attention Deficit Hyperactivity Disorder (ADHD) (REF: Nat. Genetics paper by Hankonarson). Our finding suggests that the genetic disabling of mGluR5 in glutamatergic neurons leads to an overall increase in cortical excitability, which may contribute to ADHD behaviors.

Disclosures: H. Sun: None. **C.J. Ballester Rosado:** None. **C. Wu:** None. **C.P. Jew:** None. **D. Yu:** None. **H. Lu:** None.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.07/B12

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant MH84233 (to Z.Y.)

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Title: Snare proteins are essential in the potentiation of NMDA receptors by group II metabotropic glutamate receptors

Authors: *J. CHENG, W. LIU, Z. YAN
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Abstract: The group II metabotropic glutamate receptor (mGluRII) has emerged as a new drug target for schizophrenia treatment. To understand the potential molecular mechanisms underlying the antipsychotic effects of mGluRII, we examined its impact on NMDA receptors, since NMDAR hypofunction has been implicated in schizophrenia. We previously found that application of APDC, a highly selective mGluRII agonist, caused a potent enhancement of NMDAR-mediated currents in cortical pyramidal neurons. Here we examined whether this effect of mGluRII involves the exocytosis of NMDA receptors mediated by SNARE proteins, such as SNAP-25 (synaptosomal-associated protein of 25 kDa) and Syntaxin 4. We found that the enhancing effect of APDC on NMDAR currents was abolished when SNARE complex was disrupted by delivering Botulinum toxin or SNAP-25 C-terminal blocking peptide into the neurons. Moreover, knockdown of Syntaxin 4 blocked mGluRII potentiation of NMDAR currents. Syntaxin 4 is a postsynaptic component interacting with Rab4, a small Rab GTPase mediating fast recycling from early endosome to the plasma membrane. The effect of APDC on NMDAR currents was abolished by dominant negative Rab4, and occluded by constitutively active Rab4, suggesting the involvement of Rab4-mediated NMDAR exocytosis to the cell membrane. Taken together, these results have revealed the key molecules involved in mGluRII enhancement of NMDA receptor trafficking and function.

Disclosures: J. Cheng: None. **W. Liu:** None. **Z. Yan:** None.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.08/B13

Topic: B.03. G-Protein Linked Receptors

Title: WIN55, 212 attenuates NMDA- but not kainate-induced hippocampal seizures via mGluR1α and CB1 receptors during a critical period

Authors: *L. K. FRIEDMAN, V. RUDENKO
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Abstract: In adult rats, the synthetic cannabinoid agonist, WIN55212, suppresses spontaneous seizure activity and IPSCs of DHPG-sensitive hippocampal neurons. Anticonvulsant effects were attributed to reducing pre-synaptic release of glutamate via G-protein coupled CB1 receptors. In postnatal (P) day 20 rats, we found that kainate (KA)-induced epileptic activity is inversely related to WIN55212 dosage after peripheral injection. Higher doses were proconvulsant and injurious whereas lower doses were anticonvulsant and neuroprotective. To determine whether

this inverse relationship on the seizure threshold occurs in the P20 hippocampus, stereotaxic microinfusions of WIN55212 (0.5 µg) were delivered to the CA1 followed by NMDA (0.1 µg) or KA (0.3 µg) in the presence and absence of LY367385, MPEP or AM251. Electroencephalography (EEG) recordings were simultaneously determined with a bipolar/cannula assembly. CB1 and NMDA receptor proteins were examined. Unilateral intrahippocampal WIN 55,212 alone caused rapid sedation and low amplitude synchronous oscillations in the EEG. In the presence of WIN55212, NMDA-induced epileptic currents were delayed and attenuated. Blockade of mGluR1α receptors with LY367385 or CB1 receptors with AM251 (but not MPEP) prevented the attenuation. In contrast, microinfusion of a saturating concentration of KA after WIN55212, led to increased periodic oscillations containing high frequency spikes. Immunohistochemistry revealed a dose-dependent reduction in CB1 receptor expression with increasing concentrations of WIN55212 in presence or absence of KA seizures. There were steady or increased levels of mGluR1α subunits within interneurons and variable distribution of NR1 and NMDA C2 terminal splice variant expression within pyramidal cells. Differential responses of NMDA and KA on seizure threshold subsequent to WIN55212 application suggests selective cooperation whereby NMDA is expected to co-activate postsynaptic mGluR group I receptors to synergistically activate release of endocannabinoids to further attenuate EEG spiking. Consistent with studies showing LY367385 increases short-term facilitation, particularly in young, we illustrate that mGluR1α subunits and CB1 receptors appear required for the efficacious inhibitory actions of WIN5521 on NMDA currents and that KA receptor activation is not coupled to the cannabinoid/mGluR pathway. CB1 receptor spatiotemporal re-distributions caused by seizures as a function of age may also impart different action on regulatory mechanisms of transmitter release to control the seizure threshold and associated brain injury.

Disclosures: L.K. Friedman: None. **V. Rudenko:** None.

Poster

841. Metabotropic Glutamate Receptors II

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.09/B14

Topic: B.03. G-Protein Linked Receptors

Support: CONICET

UBA

ANPCyT

Title: Astroglial metabotropic glutamate receptor 3 promotes the non-amyloidogenic pathway by modulating alpha and beta secretase expression

Authors: D. DURAND[1], L. CARNIGLIA[1], C. CARUSO[1], *M. I. LASAGA[2]
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Abstract: Alzheimer's disease is characterized by the presence of amyloid β (Aβ) plaques in the brain. Aβ derives from proteolytic cleavage of amyloid precursor protein (APP) by β- (BACE) and γ-secretases. On the other hand, α-secretase cleaves APP within the Aβ domain generating a soluble fragment (sAPPα) which shows neuroprotective effects and improves memory. ADAM-10 and ADAM-17 metalloproteases have been identified as the main α-secretases expressed in the brain. Group II metabotropic glutamate receptors (mGluR) include mGlu3R and mGlu2R, being mGlu3R expressed in astrocytes. Astroglial mGlu3R activation reduces neuronal degeneration induced by Aβ in vitro. Although it has been shown that a non-selective agonist of mGluR promotes the non-amyloidogenic pathway in astrocytes, most likely by activating group II mGluR, this issue has not been further explored. Therefore, we studied the involvement of mGlu3R in the non-amyloidogenic shedding of APP in primary cultured rat astrocytes.
The mGlu3R-selective agonist LY379268 increased sAPPα release from astrocytes in a dose-dependent manner (LY0.01 μM $p<0.05$; LY0.1 μM and LY10 μM $p<0.01$ versus control). In congruence with these results, ADAM-10 and ADAM-17 gene and protein expression was induced by LY379268 (0.1-10 μM), as assassed by qRT-PCR and western-blot (ADAM-17: LY 0.1 and 1 μM $p<0.01$; LY 10 μM $p<0.05$; ADAM-10: LY 0.1 and 10 μM $p<0.05$; LY 1 μM $p<0.001$ versus control). Conversely, BACE1 protein levels were significantly reduced after LY379268 treatment (LY 0.1 and 10 μM $p<0.001$; LY 1 μM $p<0.05$ versus control). The effect of LY379268 on sAPPα production was not observed in the presence of an antagonist of the peroxisome proliferator activated receptor (PPAR)-γ (GW9662 2 μM). Surprisingly, LY379268 reduced PPAR-γ protein expression (LY 0.1 μM $p<0.05$; LY1 and 10 μM $p<0.001$), thus further study is needed to clarify the involvement of this nuclear receptor in the non-amyloidogenic shedding of astroglial APP.
Our data indicate that mGlu3R activation promotes the non-amyloidogenic pathway, shifting APP proteolysis towards sAPPα production in astrocytes, through ADAM-10 and ADAM-17 up-regulation and BACE1 down-regulation. This study provides evidence for a new molecular mechanism accounting for the neuroprotective effects of astrocytic mGlu3R on Aβ neurotoxicity.

Disclosures: D. Durand: None. **L. Carniglia:** None. **C. Caruso:** None. **M.I. Lasaga:** None.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.10/B15

Topic: B.03. G-Protein Linked Receptors

Support: Marie Curie Initial Training Network "Edu-GLIA" PITN-GA-2009-237956

Title: Functional expression of metabotropic glutamate receptors in mouse optic nerve astrocyte

Authors: *I. VANZULLI, A. M. BUTT
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Abstract: Metabotropic glutamate receptors (mGluRs) are seven transmembrane domain G-protein-coupled receptors for L-glutamate, the main excitatory neurotransmitter in the mammalian brain. Here, we analyzed the functional expression of mGluRs in astrocytes of the mouse optic nerve, a white matter tract of the central nervous system that contains neither neuronal cell bodies nor synapses. Calcium imaging experiments were performed in optic nerves from P9-13 wild-type CFLP mice; all procedures were in accordance with the Animal Scientific Procedures Act (1986). Optic nerves were isolated intact and loaded with fluo-4 AM. Nerves were visualized using a Zeiss LSM 5 Pascal Axioskop 2 confocal microscope; images were collected in optical z-sections and changes in fluorescence intensity were measured. The application of mGluR agonists ACPD and DHPG showed that group I mGluRs, which are coupled to the $G_{q/11}$ protein, mediate a rise in glial $[Ca^{2+}]_i$. The action of the group I mGluR antagonist AIDA versus ACPD indicated that the rise in $[Ca^{2+}]_i$ also involves group II and group III receptors, which are negatively coupled to adenylate cyclase through the G_i family of G-proteins. Immunohistochemistry showed that group I subunit mGluR5 and group II subunits mGluR2 and mGluR3 colocalize to both astrocytes and pericytes at the gliovascular interface. The results demonstrate functional mGluRs in optic nerve astrocytes and support a role in neurovascular coupling. Further experiments are required to determine the role of these receptors in driving glial cell coupling of blood flow to axonal activity in the optic nerve white matter.

Disclosures: I. Vanzulli: None. **A.M. Butt:** None.

Poster

841. Metabotropic Glutamate Receptors II

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Program#/Poster#:841.11/B16

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant T32 CMA-SIB

NIH Grant RO1MH085798

NIH Grant K08NS069809

Title: Impaired activity-dependent fmrp translation in fragile x premutation mice

Authors: A. J. RENOUX[1], A. J. ILIFF[2], A. KRANS[3], K. USDIN[5], P. K. TODD[3], *M. A. SUTTON[4]
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Abstract: Neuronal function and cognition are dependent on normal synaptic responses to activity and impairments in synaptic plasticity are thought to underlie disorders such as Fragile X Syndrome (FXS) and autism. One protein important for the regulation of synaptic plasticity is the RNA-binding fragile X mental retardation protein (FMRP), which acts to inhibit translation the synapse. Activation of metabotropic glutamate receptors (mGluRs) triggers FMRP degradation, resulting in rapid synthesis of several proteins, including FMRP itself. Ultimately, mGluR activation produces a temporally constrained window of local protein synthesis. FMRP is known to play an important role in mGluR-dependent plasticity, but the specific role of activity-dependent translation of FMRP in synaptic plasticity is unknown. To approach this question, we utilized a CGG knock-in mouse model of the Fragile X premutation. This model has 120 CGG repeats in the mouse FMR1 5'UTR resulting in increased Fmr1 mRNA production but impaired FMRP translational efficiency, resulting in lower basal expression of FMRP. Cultured hippocampal neurons and cortical synaptoneurosomes derived from CGG KI mice demonstrate impaired FMRP translation in response to the group I mGluR agonist DHPG. The consequences of this impaired FMRP translation are currently under investigation. Together, these studies support a role for activity-dependent synthesis of FMRP in synaptic function and Fragile X premutation associated disorders.

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Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.12/B17

Topic: B.03. G-Protein Linked Receptors

Support: Canadian Institutes of Health Research, #MOP-8580

Title: Reduction of prodynorphin gene expression prevents memory impairment related to aging via group 1 metabotropic glutamate receptor function

Authors: *C. MENARD[1], Y. C. TSE[1], J.-G. CHABOT[1], H. HERZOG[2], C. SCHWARZER[3], T. P. WONG[1], R. QUIRION[1]
[1]Neurosci., DMHUI, McGill Univ., Montreal, QC, Canada; [2]Garvan Inst. of Med. Research, Univ. of New South Wales, Sydney, Australia; [3]Dept. of Pharmacology, Innsbruck Med. Inst., Innsbruck, Austria

Abstract: Expression of dynorphin, an endogenous opioid peptide, increased with age in the hippocampus of rodents. This up-regulation has been associated with memory impairment. In human, elevated dynorphin expression has been reported in Alzheimer's disease. Moreover, prodynorphin (Pdyn) gene polymorphisms have been linked to cognitive function in the elderly. Using Pdyn knockout (KO) mice, we investigated the impact of dynorphin expression on memory during aging. Group 1 metabotropic glutamate receptor (mGluR) function is essential for successful cognitive aging. We compared mGluR1α and mGluR5 levels in Pdyn KO and wild-type (WT) mice hippocampal formation. While protein levels are similar in young animals (5-7 months), mGluR1α and mGluR5 were increased by 63% and 110% in old Pdyn KO mice (18-25 months) with no changes for WT. Synaptic plasticity is crucial to maintain cognitive abilities and long-term depression (LTD) induced by group 1 mGluR activation has been linked to memory in the aging brain. Dihydroxyphenylglycine, a selective mGluR1α/mGluR5 agonist, was applied to hippocampal slices from old Pdyn and WT mice. mGluR-LTD was significantly enhanced in Pdyn KO mice CA1 region (30% vs 4% for WT). mGluR-mediated plasticity has been highly implicated for hippocampal memory formation, suggesting Pdyn KO should display better cognitive function. In young mice, no differences were observed in the Morris Water Maze (WM) task. However while old WT mice developed memory deficits, aged Pdyn KO mice performances were similar to young subjects, suggesting intact cognition. Reverse memory and inhibitory learning, processes known to be affected by aging, were also preserved in old mice lacking Pdyn gene. Immediate early gene translation is induced by mGluR-LTD and necessary to consolidate memories. Following WM training, Homer 1a and Arc expression was 1.4 fold higher in aged Pdyn KO mice compared to WT, supporting intact memory processes despite aging. In an object recognition test, old Pdyn KO animals show intact reference memory as well. Anxiety which affects cognition generally increases with age. Dynorphin peptides enhance stress responses and anxiety-related behaviors. In line with high Pdyn expression in aged WT mice, we observed an anxiolytic phenotype for Pdyn KO mice with longer distance travelled in the elevated plus maze open arms. Low anxiety level for mice lacking Pdyn gene was confirmed with the novelty-suppressed feeding test. Indeed, Pdyn KO mice took 50% less time than WT to start feeding. Our results suggest that an increase in mGluR expression in Pdyn KO mice contributes to successful aging by enhancing cognitive abilities and reducing psychiatric burdens.

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Poster

841. Metabotropic Glutamate Receptors II

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Program#/Poster#:841.13/B18

Topic: B.03. G-Protein Linked Receptors

Support: The Luncbeck Foundation

Partially supported by projects PSI2009-10532, Fundació La Maratò TV3 (092630) and 2009SGR-0051

Title: Differences in serotonergic and glutamatergic receptor binding between the Roman Low- (RLA-I) and High (RHA-I) avoidance rat strains

Authors: *A. B. KLEIN[1], L. ULTVED[1], M. SANTINI[1], A. TOBEÑA[2], A. FERNANDEZ-TERUEL[2], P. FLORES[3], M. MORENO[3], G. M. KNUDSEN[1], S. AZNAR[1], J. D. MIKKELSEN[1]
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Abstract: The Roman low- and high-avoidance rat strains (RLA-I vs RHA-I) have been bidirectionally selected and bred in accordance with their performance in the two-way active avoidance response in the shuttle-box test. Subsequently, numerous studies have reported a pronounced divergence in emotionality between the two rat strains including differences in novelty seeking, anxiety, stress coping, and susceptibility to addictive substances. However, the underlying molecular mechanisms behind these divergent phenotypes are not known. Previous reports describe lower levels of serotonin and noradrenaline in the striatum and nucleus accumbens. Here, we determined levels of 5-HT_{2A}, 5-HT_{1A} and serotonin transporter binding using highly specific radioligands (^{3}H-MDL100907, ^{3}H-WAY100635 and ^{3}H-escitalopram) and mGluR2/3 binding (^{3}H-LY341495) using receptor autoradiography in fronto-cortical sections from RLA-I (n=8) & RHA-I (n=8) male rats. In RHA-I rats, 5-HT_{2A}, 5-HT_{1A} and SERT binding in frontal cortex were significantly higher (18%, 15% and 24%, respectively) compared to RLA-I rats. In contrast, mGluR2/3 binding was decreased by 40% RHA-I rats compared to RLA-I rats in the same brain region. To differentiate between mGluR2 and mGluR3, levels of these mRNA transcripts were further studied using RT-qPCR, and showed a marked reduction (63%) in mGluR2 mRNA levels, while no differences was observed in mGluR3 mRNA levels. Collectively, these data show general differences in the serotonergic system and a pronounced

difference in mGluR2/3 binding, which presumably is due to a large decrease in mGluR2 transcription in RHA-I rats. These changes may be mediating some of the phenotypic characteristics in this strain, e.g. increase in impulsivity and susceptibility to drug addiction.

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Poster

841. Metabotropic Glutamate Receptors II

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Program#/Poster#:841.14/B19

Topic: B.03. G-Protein Linked Receptors

Support: AHA postdoctoral fellowship 10POST3430030

Title: Plasticity of group II metabotropic glutamate receptors in regulating hypothalamic excitability in hypertension

Authors: *Z.-Y. YE, H.-L. PAN
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Abstract: Increased glutamatergic input to the paraventricular nucleus (PVN) of hypothalamus plays a critical role in the development of neurogenic hypertension. However, the contribution of metabotropic glutamate receptors (mGluRs) is not clear. Here we determined the role of inhibitory group II mGluRs in controlling excitatory and inhibitory synaptic inputs to retrogradely labeled spinally projecting PVN neurons in spontaneously hypertensive rats (SHRs). Activation of group II mGluRs with the agonist DCG-IV produced a greater dose-dependent inhibitory effect on the frequency, but not the amplitude, of spontaneous excitatory postsynaptic currents (EPSCs) in SHRs than in normotensive Wistar-Kyoto (WKY) rats. This effect was not altered by tetrodotoxin. DCG-IV also caused a greater inhibition of evoked EPSCs in SHRs than in WKY rats, and such an effect was not changed by including GDP-β-s in the pipette recording solution or by blocking the glia activity with fluoroacetate. Bath application of the group II mGluR antagonist LY341495 alone has no significant effect on the baseline EPSCs, although it abolished the inhibitory effect of DCG-IV. Lowering the blood pressure with celiac ganglionectomy in SHRs normalized the potentiated inhibition of EPSCs by DCG-IV. In addition, DCG-IV produced a similar inhibitory effect on GABAergic inhibitory postsynaptic currents of labeled PVN neurons in WKY rats and SHRs. However, DCG-IV inhibited the firing

activity of labeled PVN neurons in SHRs but not in WKY rats. Together, our findings provide new information that the activity of group II mGluRs is increased at the glutamatergic terminals in the PVN, which influences the glutamatergic input and firing activity of PVN presympathetic neurons in hypertension.

Disclosures: Z. Ye: None. **H. Pan:** None.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.15/B20

Topic: B.03. G-Protein Linked Receptors

Title: Early increase in the transcript of mGlu4 receptors in the striatum of mice treated with MPTP or haloperidol

Authors: M. CANNELLA[1], M. MOTOLESE[1], D. BUCCI[1], G. MOLINARO[1], R. GRADINI[1,2], V. BRUNO[1,3], F. NICOLETTI[1,3], *G. BATTAGLIA[1]
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Abstract: In Parkinson's disease (PD) the progressive loss of nigro-striatal neurons leads to a decreased activity of the direct pathway and an increased activity of the indirect pathway, which lead to inhibition of thalamocortical neurons and motor dysfunction. Dopamine released from the nigro-striatal pathway stimulates the direct pathway acting at D1 receptors and inhibits the indirect pathway acting at D2 receptors. Activation of mGlu4 receptors inhibits GABA release at the synapse between stratial projection neurons and neurons of the external globus pallidus. This is the first synapse of the indirect pathway of the basal ganglia motor circuit, which is overactive in PD. Drugs that activate mGlu4 receptors cause a robust depression of inhibitory synaptic transmission in the external globus pallidus, relieve motor symptoms and induce neuroprotective effects in animal models of parkinsonism. It is generally believed that during the early "compensated" phase of PD a number of mechanisms reduce the activity of the indirect pathway. Whether mGlu4 receptors undergo plastic modification during the early "compensated" phase of PD is unknown. We examined the transcripts of mGlu4 receptors and other proteins involved in the regulation of basal ganglia motor circuit in mice challenged with the parkinsonian toxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and with the dopamine receptor blocker, haloperidol. We found an up-regulation of mGlu4 receptor, CB1 receptor, and preproenkephalin-A mRNA at early times following injection of MPTP (30 mg/kg, i.p.) which kills about 50% of

nigro-striatal dopaminergic, as well as following a single injection haloperidol (1 mg/kg, s.c.). No changes were found in the transcripts of mGlu5 receptors, A2A receptors and the potassium/chloride co-transporter, KCC2. The increase in the number of c-Fos-expressing neurons in the external globus pallidus seen at early times following MPTP injection was absent in mGlu4 receptor knockout mice. These data suggest that an up-regulation of mGlu4 receptors in striatal projection neurons contributes to restrain the activity of the indirect pathway in the early phases of parkinsonism.

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Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.16/B21

Topic: B.03. G-Protein Linked Receptors

Support: MRC funded

Title: Effects of mGluR8-selective compounds in the lateral and medial perforant path inputs to the dentate gyrus

Authors: *M. MERCIER, Z. BORTOLOTTO, G. COLLINGRIDGE, D. LODGE
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Abstract: Metabotropic glutamate receptors (mGluRs) are classified into three groups (I-III) based on sequence homology, transduction mechanisms and pharmacological profiles. Group III mGluRs (mGluR4/6/7/8) have received little attention relative to either group I (mGluR1/5) or II (mGluR2/3), despite being linked to a variety of neurological and psychiatric conditions including anxiety and epilepsy. This may in part be due to the paucity of selective pharmacological tools available for these receptors. However, important advances have recently been made in the development of group III mGluR subtype-selective compounds. Namely, the actions of an mGluR8-selective agonist, (*S*)-3,4-dicarboxyphenylglycine (DCPG), and antagonist (*RS*)-α-methyl-3,4-dicarboxyphenylglycine (MDCPG), have been described in the rat spinal cord (Thomas *et al*., 2001).
Here, we sought to further characterise the effects of these compounds in the lateral perforant path (LPP) input to the dentate gyrus, a hippocampal pathway known to express high levels of mGluR8. For comparison, DCPG effects were also studied in the medial perforant path (MPP), where group II mGluRs are thought to play a more prominent role in regulating transmission.

Using field recordings in rat hippocampal slices, we found that DCPG induced a dose-dependent reduction in the amplitude of field excitatory postsynaptic potentials (fEPSPs) in the LPP, consistent with the known role of mGluR8 as a presynaptic autoreceptor. Interestingly, the concentration-response curve was biphasic, with EC_{50} values of 0.2µM and 12.4µM for the high and low affinity components, respectively. 30µM MDCPG antagonised the high affinity component of the curve only, having no effect on DCPG concentrations >3µM. These higher concentrations of DCPG also reduced fEPSPs in the MPP, producing a curve with an EC_{50} value of 15.4µM.
Further experiments using hippocampal slices from mGluR4, 7 and 8 knock-out ($^{-/-}$) mice revealed that only the high affinity component of the curve in the LPP is mediated by mGluR8, whilst the low affinity component is non-selective and does not appear to be mediated by a group III mGluR. Instead, preliminary experiments with mGluR2$^{-/-}$ animals suggest that DCPG may activate this group II mGluR at higher concentrations, consistent with the reduction of excitatory transmission observed in the MPP at these concentrations. These findings indicate that whilst MDCPG, and lower concentrations of DCPG (<3µM) may prove useful in determining the functional role of mGluR8 in the hippocampus, care must be taken when employing higher concentrations of the agonist which may additionally be activating group II mGluRs.

Disclosures: M. Mercier: None. **Z. Bortolotto:** None. **G. Collingridge:** None. **D. Lodge:** None.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.17/B22

Topic: B.03. G-Protein Linked Receptors

Support: MRC Grant

Title: DHPG-induced long-term depression and E-S potentiation in transgenic mice lacking mGluR1 or mGluR5

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Abstract: Activation of group I metabotropic glutamate receptors (mGluRs, comprising mGluR1 and mGluR5) by the agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) induces long-term depression (LTD) of glutamatergic transmission in the CA1 region of the rodent

hippocampus. Although some of the key signaling cascades involved in DHPG-LTD have been identified, precise mechanisms remain elusive with conflicting results being reported. In addition to LTD, DHPG application in hippocampal slice preparations can induce long-lasting changes in various measures of neuronal excitability, including the prolongation of epileptiform bursts and a potentiation of EPSP-spike coupling (E-S potentiation). Using transgenic mice lacking mGluR1 or mGluR5, the aim of the present study was to assess the role of these two receptors in the induction of DHPG-LTD and DHPG-induced E-S potentiation. Experiments were performed on parasagittal hippocampal slices (with the CA3 region removed) from knockout mice lacking either mGluR1 or mGluR5 or from age-matched wild-type littermates (both male and female mice were used; age range: 3 - 18 weeks). The GABAA receptor antagonist picrotoxin and the NMDA receptor antagonist L-689,560 were present in the perfusate throughout all experiments. Field EPSPs were recorded from CA1 stratum radiatum in response to Schaffer collateral stimulation and EPSP-spike (E-S) coupling was examined by simultaneously recording population spikes from the stratum pyrimadale and constructing input-output curves. In slices from wild-type mice, a 10 minute application of 100 µM DHPG induced LTD of f-EPSPs recorded in the stratum radiatum and a long-lasting potentiation of E-S coupling (LTD and E-S potentiation were assessed 40-60 minutes after DHPG washout). LTD was absent or reduced in slices from both mGluR1 and mGluR5 knockout mice. Conversely, E-S potentiation was preserved in slices from mGluR1 knockout mice, but absent in slices from mGluR5 knockout mice. These results indicate that activation of both mGluR1 and mGluR5 is required for the full induction of DHPG-LTD, whereas activation of mGuR5 alone is sufficient for the induction of DHPG-mediated E-S potentiation.

Disclosures: P. Tidball: None. **A. Volianskis:** None. **S.M. Fitzjohn:** None. **G.L. Collingridge:** None.

Poster

841. Metabotropic Glutamate Receptors II

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Topic: B.03. G-Protein Linked Receptors

Support: NIH GRANT PN2EY018241

Title: Optical control of metabotropic glutamate receptors for high resolution probing of gpcr signaling

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Abstract: G-protein coupled receptors (GPCRs) are the largest family of membrane signaling proteins and the most common drug targets in biology. They respond to a wide-array of stimuli and contain a seven transmembrane domain that couples to G-protein signaling pathways. These pathways control second messenger levels and regulate other signaling proteins, such as ion channels and kinases. Despite the importance of these receptors, specific biochemical,physiological, and behavioral roles of many GPCRs are not well-understood. In order to overcome the limitations of classical pharmacological and genetic techniques, we have developed an approach to elucidate the function of Class C GPCRs by chemically re-engineering them to be light sensitive. The group II metabotropic glutamate receptor, mGluR2, which couples to the Gi/o pathway, was derivatized with photoswitchable ligands to generate both light-agonized (LimGluR2) and light-antagonized ("LimGluR2-block") receptors. LimGluR2 activates and deactivates quickly, shows bistability which allows the receptor to remain on or blocked for extended periods in the dark, and supports multiple reproducible rounds of on/off switching with superior fidelity and speed compared to Rhodopsin. In neurons, LimGluR2 is a powerful tool for optical inhibition through hyperpolarization and presynaptic inhibition of neurotransmitter release. Furthermore, we have demonstrated that these tools function in intact hippocampal slices and in vivo, in zebrafish, to control vertebrate behavior. Recently, we have extended optical control to a variety of mGluRs with distinct G-protein coupling profiles. These designed receptors provide excellent tools for the dissection of the specific roles of different mGluRs in physiological functions with high spatiotemporal precision and subtype specificity that is lacking with traditional ligands. In addition the high resolution control of ligand-binding allows these tools to be excellent biophysical probes of receptor cooperativity and activation mechanism. Finally, future experiments aim to probe the mechanism and behavioral role of mGluR-mediated plasticity in rodents using virus delivery and knock-in mice.

Disclosures: J. Levitz: None. **C. Pantoja:** None. **B. Gaub:** None. **H. Janovjak:** None. **P. Stawski:** None. **D. Trauner:** None. **E. Isacoff:** None.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.19/B24

Topic: B.03. G-Protein Linked Receptors

Support: RO1DA029814.01

Title: Influence of the group II metabotropic glutamate receptor antagonist LY341495 on nicotine sensitization

Authors: *K. TAM[1], W. HUANG[1], M. E. HEFFERNAN[1], S. M. CZERNIAK[1], J. R. DIFRANZA[2], J. A. KING*[1]
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Abstract: The increase in dopamine transmission observed after the administration of nicotine has been supported in numerous studies. Though this neurotransmitter is a vital component of nicotine's addictive properties, recent work has also focused on glutamate, a major excitatory neurotransmitter in the brain, and its role in the neurobiology of nicotine dependence. Glutamate transmission is mediated by ionotropic and metabotropic receptors located both presynaptically and postsynaptically. Of interest to this study are the group II receptors (mGlu2/3) that inhibit glutamate release. By blocking these receptors, glutamate transmission would presumably increase, which would subsequently lead to increased dopamine transmission. A novel group II metabotropic glutamate receptor antagonist LY341495, (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl)propionic acid, has been used to explore glutamate's potential role in nicotine dependence.
In the present study, both a behavioral assay (open-field test) and neuroimaging (fMRI) were used to study the effects of LY341495 on nicotine sensitization. Male Sprague-Dawley rats were administered nicotine (0.4 mg/kg per day) for 7 consecutive days to induce sensitization, and their locomotor activity was immediately recorded. The treatment group received LY341495 thirty minutes prior to nicotine administration, while the controls received a mock injection, on Days 6 and 7. We hypothesized that LY341495 would increase locomotor activity, via an increase in glutamate transmission. Unexpectedly, our results supported a decrease in locomotor activity in the treatment group compared to controls on Days 6 and 7.
Within group analysis of fMRI results after the administration of nicotine, in LY341495 pre-treated rats, reveal strengthened connectivity between the anterior cingulate area (ACA) and the agranular insular area, hypothalamus, and hippocampus. Additionally, the lateral orbitofrontal cortex (ORBl) showed increased connectivity to the retrosplenial area and the hippocampus. Results from the behavioral assay and imaging suggest that while LY341495 appears to attenuate the nicotine-induced increase in locomotor activity, it strengthens the connectivity to brain regions associated with memory and reward. These findings support the notion that glutamate's role in nicotine dependence is complex, and glutamatergic interventions can result in different behavioral and neurobiological effects.

Disclosures: **K. Tam:** Research Grant; RO1DA029814.01. **W. Huang:** Research Grant; RO1DA029814.01. **M.E. Heffernan:** Research Grant; RO1DA029814.01. **S.M. Czerniak:** None. **J.R. DiFranza:** Research Grant; RO1DA029814.01. **J.A. King*:** Research Grant; RO1DA029814.01.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.20/B25

Topic: B.03. G-Protein Linked Receptors

Title: Safety, pharmacokinetic and pharmacodynamic profile of BCI-632, a selective metabotropic glutamate 2/3 receptor antagonist, in healthy human subjects

Authors: *R. A. GADIENT[1], P. C. WEDEL[1], V. M. FRISBIE[1], A. F. LEUCHTER[2], S. D. TARGUM[1], C. T. TRUONG[1], J. H. HUTCHINSON[1]
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Abstract: Treatment resistant depression (TRD) is defined as the failure to fully respond to an antidepressant treatment despite having had an adequate dose and duration of treatment. There is an unmet need to develop medications to effectively treat TRD. The finding that a single administration of a sub-anesthetic dose of ketamine can lead to rapid and long lasting efficacy in TRD patients has strongly influenced depression research. The mechanism by which ketamine confers this activity has been studied by several groups. It seems to involve a burst in synaptic glutamate followed by potentiation of post-synaptic AMPA receptors, increased brain derived neurotrophic factor (BDNF) signaling and downstream activation of mTOR, leading to increased synaptogenesis. An identical signaling cascade is triggered by selective antagonists of the metabotropic glutamate receptor (mGlu) 2/3, such as LY341495 or BCI-632.
Here we report Phase 1 data for BCI-838, the oral prodrug of BCI-632, dosed to healthy male and female subjects in 2 studies: a single ascending dose (SAD) and a multiple ascending dose (MAD) study. In both, safety and tolerability were monitored and plasma was collected for pharmacokinetic (PK) analysis. In the MAD study, electroencephalograms (EEGs) were collected to assess pharmacodynamic (PD) effects on the central nervous system. In the SAD study (n=25), single doses of BCI-838 up to 900 mg were given under fasted conditions. BCI-838 was well tolerated. PK analysis revealed dose-dependent increases in BCI-632 and BCI-838 plasma levels which were slightly less than dose proportional. A food effect sub-study was included in the SAD protocol which showed an increase in BCI-632 plasma levels when BCI-838 was dosed with food. In the MAD study (n=30), BCI-838 was given under fed conditions for

7 days at doses up to 600 mg QD. At 100 and 300 mg, BCI-838 was safe and well tolerated. At 600 mg, a dose well above the estimated therapeutic range, an increase in drug-related AEs of mild to moderate severity was noted. PD analysis of the qEEG data indicates dose-dependent effects on the EEG signal indicating brain penetration. In summary, the compelling clinical data obtained for BCI-838 support the compound's progression into a proof-of-concept study in TRD patients.

Disclosures: R.A. Gadient: Employment; BrainCells Inc. **P.C. Wedel:** Employment; BrainCells Inc. **V.M. Frisbie:** Employment; BrainCells Inc. **A.F. Leuchter:** Consultant/Advisory Board; BrainCells Inc. **S.D. Targum:** Consultant/Advisory Board; BrainCells Inc. **C.T. Truong:** Employment; BrainCells Inc. **J.H. Hutchinson:** Employment; BrainCells Inc.

Poster

841. Metabotropic Glutamate Receptors II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:841.21/B26

Topic: B.03. G-Protein Linked Receptors

Title: Analgesic activity of ceftriaxone in humans

Authors: A. MACALUSO[1], M. BERNABUCCI[2], A. TRABUCCO[2], L. CIOLLI[1], *R. GRADINI[1,2], G. BATTAGLIA[2], F. NICOLETTI[1,2], F. TROISI[1], S. COLLINI[1]
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Abstract: We examined the analgesic action of the antibiotic, ceftriaxone, in 43 patients with carpal tunnel syndrome who underwent surgical release of the carpal tunnel ligament under local anesthesia. Two additional patients underwent surgery for ulnar neuropathy. Two groups of fifteen patients received i.m. injection of ceftriaxone (2 g) or cefazoline (1 g) prior to surgery. An additional group of patients served as control. Mechanical allodynia was assessed with the Von Frey filaments prior to, and 6 hours after drug injection. Pain thresholds were unchanged in control patients and in the group of patients receiving cefazoline. In contrast, treatment with ceftriaxone caused analgesia in all patients. Ceftriaxone is known to up-regulate the glial glutamate transporter, GLT-1 (EAAT2), in the spinal cord, a mechanism that was demonstrated in animal models of chronic pain following repeated doses of ceftriaxone. Using different mouse models of inflammatory and post-surgical pain, we have shown that a single injection of ceftriaxone was able to induce GLT-1 expression in the dorsal horns of the spinal cord after 6 hours, whereas cefazoline was inactive. In all these models, a single injection of ceftriaxone

caused analgesia and its effect was additive to that produced by the mGlu5 receptor antagonist, MPEP. Thus, a combination of ceftriaxone and mGlu5 receptor blockade might be valuable in the treatment of acute and chronic pain. These data encourage the use of ceftriaxone in surgical prophylaxis in order to combine antimicrobical and analgesic activities with the same drug. It will be interesting to examine the action of ceftriaxone combined with mGlu5 receptor antagonists in patients with severe pain refractory to conventional medications.

Disclosures: A. Macaluso: None. **M. Bernabucci:** None. **A. Trabucco:** None. **L. Ciolli:** None. **R. Gradini:** None. **G. Battaglia:** None. **F. Nicoletti:** None. **F. Troisi:** None. **S. Collini:** None.

Poster

842. Calcium Channels III

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Program#/Poster#:842.01/B27

Topic: B.04. Ion Channels

Support: RO1-HL56180

RO1-NS049442

Title: Leaky neuronal ryanodine receptors in stress-induced cognitive dysfunction

Authors: *X. LIU[1], M. J. BETZENHAUSER[1], S. REIKEN[1], B.-X. CHEN[1], O. ARANCIO[2], A. R. MARKS[1]
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Abstract: The type 2 ryanodine receptor/calcium release channel (RyR2), required for excitation-contraction coupling in the heart, is the major isoform in the brain. Chronic stress induces catecholamine biosynthesis and release, stimulating β-adrenergic receptors and activating cAMP signaling pathways in neurons. In a murine chronic restraint stress model neuronal RyR2 were phosphorylated by protein kinase A (PKA), resulting in dissociation of the stabilizing subunit calstabin2 (FKBP12.6) from the channel macromolecular complex, and intracellular ER calcium leak. Chronic restraint stress impaired learning and memory, increased anxiety and reduced spontaneous exploratory activity, and decreased long-term potentiation (LTP) at the hippocampal CA3-CA1 connection. Stress-induced cognitive dysfunction and impaired post-synaptic plasticity of hippocampal neuron were rescued by oral administration of S107, a novel compound developed in our laboratory that stabilizes RyR2-calstabin2 interaction,

or by genetic ablation of the RyR2 PKA phosphorylation site at serine 2808. Thus, RyR2 remodeling in hippocampal neuron contributes to stress-induced cognitive dysfunction. Leaky RyR2 could be a therapeutic target for treatment of stress-induced cognitive dysfunction and post-traumatic stress disorder (PTSD).

Disclosures: X. Liu: Employment; Clyde and Helen Wu Center for Molecular Cardiology, Department of Physiology and Cellular Biophysics, College of Physicians and Surgeons of Columbia University. Research Grant; RO1-HL56180. **M.J. Betzenhauser:** Employment; Clyde and Helen Wu Center for Molecular Cardiology, Department of Physiology and Cellular Biophysics, College of Physicians and Surgeons of Columbia University. **S. Reiken:** Employment; Clyde and Helen Wu Center for Molecular Cardiology, Department of Physiology and Cellular Biophysics, College of Physicians and Surgeons of Columbia University. **B. Chen:** Employment; Clyde and Helen Wu Center for Molecular Cardiology, Department of Physiology and Cellular Biophysics, College of Physicians and Surgeons of Columbia University. **O. Arancio:** Employment; Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Department of Pathology and Cell Biology; 3Department of Medicine, College of Physicians and Surgeons of Columbia University. Research Grant; RO1-NS049442. **A.R. Marks:** Employment; Clyde and Helen Wu Center for Molecular Cardiology, Department of Physiology and Cellular Biophysics, College of Physicians and Surgeons of Columbia University. Research Grant; RO1-HL56180.

Poster

842. Calcium Channels III

Location: Hall F-J

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Program#/Poster#:842.02/B28

Topic: B.04. Ion Channels

Support: NCRR Grant 2P20 RR0017699

Title: Acidic store-operated calcium entry in primary cultured neurons

Authors: *L. HUI[1], X. CHEN[1], B. SINGH[2], J. GEIGER[1]
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Abstract: Endolysosomes are intracellular ‘acidic’ calcium stores that contain high concentrations of readily releasable calcium. Although ‘acidic calcium stores’ are present functionally in neurons, little is known about how calcium is released from the acidic stores and how this release contributes to calcium signals both spatially and temporally. In primary cultured hippocampal neurons, we found that calcium released from acidic calcium stores triggered

calcium influx across the plasma membrane, a phenomenon we termed ‘acidic store-operated calcium entry’ (aSOCE). Because so little is know about how acidic calcium stores communicate with plasma membrane calcium channels, we tested here the hypothesis that calcium released from acidic calcium stores increases calcium influx through plasma membrane N-type calcium channels using a variety of methods including calcium imaging, electrophysiology, surface protein labeling, immunoprecipitation, and RNA interference. Similar to classical thapsigargin-induced store-operated calcium entry (SOCE), calcium released from acidic calcium stores, as induced by either inhibition of vacuolar H+-ATPase with bafilomycin or selective disruption of endolysosome membranes with GPN, triggered a dramatic influx of extracellular calcium. This aSOCE was attenuated significantly by blocking N-type calcium channels. Consistent with our calcium imaging data, our electrophysiological data demonstrated that both bafilomycin and GPN increased voltage-gated calcium currents. Biochemically, we demonstrated that bafilomycin and GPN both increased cell surface expression of N-type calcium channel and LAMP1, a lysosome associated membrane protein that plays an important role in lysosomal exocytosis. Furthermore, we observed a physical linkage between LAMP1 and N-type calcium channels. In addition, knocking down either LAMP1 or N-type calcium channels with RNA interference attenuated bafilomycin- or GPN- induced aSOCE. Collectively, our findings suggest that exocytotic insertion of N-type calcium channels might underlie this novel process of aSOCE in neurons. Such findings could provide a new insight into the spatiotemporal complexity of calcium signals and fundamental calcium-dependent cellular processes in neurons.

Disclosures: L. Hui: None. **X. Chen:** None. **B. Singh:** None. **J. Geiger:** None.

Poster

842. Calcium Channels III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:842.03/B29

Topic: B.04. Ion Channels

Support: NSERC Discovery Grant 238373-2010

Title: Acute application of retinoic acid to neurons *in vitro* results in hyperpolarization of the current-voltage relationship of voltage-gated calcium channels

Authors: *T. F. DAWSON, N. D. VESPRINI, G. E. SPENCER
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Abstract: Spontaneous neuronal firing is known to be critically involved in the processes of neuritogenesis and axonal pathfinding, and both waveform and frequency of firing can be

manipulated pharmacologically. Retinoic acid (RA), a metabolite of dietary vitamin A, can cause a significant change in neuronal firing properties following acute application to cultured neurons. In addition, it has been shown that RA can influence neural development and regeneration in vertebrates through its roles in patterning, axonal pathfinding and neuritogenesis. The classical role of RA in the nucleus has been well studied, and it is known that RA binds to nuclear receptors, which then act as transcription factors. Our laboratory has demonstrated a variety of novel, non-nuclear roles for RA during the regeneration of central neurons *in vitro*. These include actions as a chemoattractant during axonal pathfinding and as a modulator of neuronal firing properties. Prior studies have shown RA's ability to induce positive growth cone turning in cultured neurons and neurites isolated from the soma. Intriguingly, the aforementioned growth cone turning corresponds temporally to the observed changes in the shape of the action potential and firing frequency induced by the acute application of RA. Specifically, RA can convert tonic firing into bursting behaviour in cultured neurons in as few as 15 minutes after application, followed by a transient period of electrical quiescence approximately 50 minutes post-application. The molecular mechanisms that underlie the electrophysiological effects of RA currently remain unknown, and so we have employed whole-cell voltage clamp to determine which ion channel(s) might be modulated by RA. It is hypothesized that RA modulates voltage-gated calcium channels (VGCCs), since it is known to alter the intracellular Ca^{2+} concentration of these neurons. Preliminary recordings of VGCCs from cultured neurons suggest that acute application of RA can transiently hyperpolarize the current-voltage relationship, potentially leading to the previously observed increase in firing probability and bursting behaviour. However, by 50 minutes the current-voltage relationship becomes depolarized, perhaps explaining the observed electrical silencing that was previously noted. Future studies will aim to determine if RA affects inactivation kinetics of VGCCs, as well as to decipher the roles of individual VGCC isoforms and retinoid receptors in the above phenomena.

Disclosures: T.F. Dawson: None. **N.D. Vesprini:** None. **G.E. Spencer:** None.

Poster

842. Calcium Channels III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:842.04/B30

Topic: B.04. Ion Channels

Support: National Honor Scientist Program in Korea 2N35110

Title: Ethanol-induced loss of consciousness is modulated by Cav3.1 channels in Medialdorsal Thalamic nucleus

Authors: *C.-F. V. LATCHOUMANE, S.-W. KIM, H.-S. SHIN
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Abstract: The thalamus is known to play a central role in the modulation of cortical states, hence affecting perception, learning, cognition and up to consciousness states. We induced loss of consciousness using acute sedative dose of ethanol (3.0g/kg, 4.0g/kg) monitoring mice state changes in a forced walking task. General knock out Cav3.1 -/- mice and mediodorsal thalamus (MD) knock-down Cav3.1 -/- mice showed resistance to ethanol sedation and particularly a significant decreased in duration of loss of motion after 3.0g/kg injection of ethanol. The unconscious state of the mice was accompanied with increase low frequency (4-5Hz) quasi periodical EEG signal. Cav3.1 Knock out and MD knock-down mice exhibited lower delta (1-4Hz) and theta (4-8Hz) power, and higher alpha (8-12Hz) power during the unconscious phase. Our results suggest that tonic firing mode in absence of Cav3.1 mediated burst in MD can promote resistance to ethanol-induced loss of consciousness.

Disclosures: C.V. Latchoumane: None. **S. Kim:** None. **H. Shin:** None.

Poster

842. Calcium Channels III

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Program#/Poster#:842.05/B31

Topic: B.04. Ion Channels

Support: Conacyt Grant 128707

PAPIIT UNAM IN221011

Title: T-type calcium channel upregulation by insulin may involve increased recycling.

Authors: *A. TOLEDO GONZÁLEZ
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Abstract: Growth factors and hormones have both short- and long-term regulatory effects on the functional expression of voltage gated calcium (Cav) channels. In particular, chronic treatment with insulin upregulates T-type channel membrane expression, leading to an increase in current density in clonal pituitary GH3 cells. Though this action may result from alterations in gene expression, recent studies have demonstrated also that endosomal trafficking provides a mechanism for dynamic changes in Cav channel membrane density. Therefore, in the present work we sought to determine whether the actions of insulin on T-channel functional expression

are mediated by transcriptional and/or post-transcriptional mechanisms. Using real-time RT-PCR and semi-quantitative Western blot we found no changes after treatment in the transcript and protein levels of Cav3.1, the T-channel isoform preferentially expressed in the GH3 cells. Consistent with this, transcriptional studies using a luciferase reporter system suggested that insulin treatment does not affect the Cav3.1 promoter activity. In contrast, patch-clamp recordings on HEK-293 cells stably expressing Cav3.1 channels showed a significant increase in current density after treatment, suggesting that the effects of insulin may require a post-transcriptional regulation. In line with this, disruption of the endosomal recycling pathway using Brefeldin A and a dominant negative mutant of the small GTPase Rab11 prevented the stimulatory effects of insulin on Cav3.1 channels in HEK-293 cells. These results may help explain the effects of insulin on T-channels and contribute to our understanding of how endosomal recycling impacts the functional expression of Cav channels.

Disclosures: A. Toledo González: None.

Poster

842. Calcium Channels III

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FONCyT PICT 2008-2019

FONCyT PIDRI-PRH 2007

Title: High-speed visualization of calcium mediated sub-threshold oscillations in the parafascicular nucleus

Authors: *J. R. HYDE[1], F. J. URBANO[2], E. GARCIA-RILL[1]
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Abstract: The parafascicular nucleus (Pf) is an ascending target of the pedunculopontine nucleus (PPN) and is part of the “non-specific” intralaminar thalamus modulating arousal. The PPN is involved in waking and REM sleep. Cortical EEG during these periods shows gamma band activity (~30-80Hz). In vitro, gamma oscillations are evident in all Pf neurons and are mediated by high threshold voltage-dependent P/Q- and N- type calcium channels. These studies tested the hypothesis that calcium sensitive dyes can be used to visualize sub-threshold calcium oscillations and that these visual recordings match electrical oscillations recorded using whole cell patch clamp.
Electrical responses were recorded using whole cell patch clamp electrodes using 9-18 day old sagittal rat brain slices. Slices were recorded at 37°C while perfused with oxygenated aCSF in an immersion chamber containing synaptic blockers: APV, CNQX, Gabazine, Strychnine, and Tetrodotoxin. Recording electrodes also contained Fura-2, Indo-1, or Fluo-4 calcium sensitive dye. Cadmium was added to the aCSF to block voltage sensitive calcium channels as a control. The dendrites were visualized using a high speed, dual camera imaging system (Evolve 128, Photometrics).
Calcium-mediated imaging recordings in the proximal dendrites showed a marked increase in activity as Pf neurons were depolarized using ramps. This occurred using all fluorescent calcium labels. Spectral power analysis of the electrical and calcium recordings showed time-coinciding peaks in both signals. The increase in calcium signal and the ramp-induced oscillations disappeared with the addition of cadmium. Cadmium also abolished the peaks in both the power spectra of both signals.
Overlaying calcium and electrical recordings showed highly coincident oscillation peaks. Preliminary data suggests the presence of distributed oscillatory sub-domains. Each domain oscillates with a local phase and timing while the oscillations seen in electrical recordings in the soma are an average of the activity in all sub-domains. This study shows that high-speed calcium imaging is a viable method for measuring sub-threshold calcium oscillations with high spatial and temporal resolution.

Disclosures: J.R. Hyde: None. **F.J. Urbano:** None. **E. Garcia-Rill:** None.

Poster

842. Calcium Channels III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:842.07/B33

Topic: B.04. Ion Channels

Title: The effects of Conus striatus venom on the T-type calcium channel ($Ca_v3.2$)

Authors: G. FROST[1], M. WEAVER[1], *L. B. FRENCH[2]
[1]Neurosci., [2]Allegheny Col., MEADVILLE, PA

Abstract: The low voltage activated T-type calcium channel**,** $Ca_V3.2$, has been implicated in the progression of prostate cancer towards an androgen independent stage (AI), for which there is no effective treatment. Calcium influx through this channel stimulates the synthesis and secretion of mitogenic factors that cause cell proliferation in adjacent cells. Therefore, finding a specific inhibitor of the $Ca_V3.2$ channel could potentially prevent the uncontrolled proliferation of prostate cells that characterizes the AI stage of prostate cancer. *Xenopus laevis* oocytes expressing this channel were exposed to crude *Conus striatus* venom, and the current through these channels before and after exposure was monitored by two-electrode voltage clamp. *Conus striatus* is a species of predatory marine snails whose venom contains a cocktail of peptides that target ion channels, known as conotoxins. Exposure to the crude venom significantly ($p<0.05$) decreased current through the $Ca_V3.2$ channel indicating inhibition. The venom was separated into components by HPLC and the effect of the fractions was tested to identify the specific conotoxin responsible for decreasing calcium current. The active component of the venom was successfully narrowed down to 1 of 6 venom components. However, further investigation is required to isolate the specific $Ca_V3.2$ channel antagonist.

Disclosures: G. Frost: None. **M. Weaver:** None. **L.B. French:** None.

Poster

842. Calcium Channels III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:842.08/B34

Topic: B.04. Ion Channels

Support: National Honor Scientist Programme in Korea 2N35110

Title: Cav3.1 T-type calcium channels in the medial septum controls cholinergic theta oscillations and novel objects exploration behaviors

Authors: *G. GANGADHARAN[1], J. SHIN[2], S.-W. KIM[1], M.-Y. KIM[1], D.-S. KIM[3], M. WATANABE[4], Y. YANAGAWA[5], Y.-S. KIM[6], D. KIM[7], H.-S. SHIN[1]
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machi, Maebashi, Japan; [6]Dept. of Smart Foods and Drugs and Indang Inst. of Mol. Biology, Inje Univ., Seoul, Korea, Republic of; [7]Dept. of Biol. Sciences, Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: The continued concernment resting on the heterogeneity of hippocampal rhythmic oscillatory activities at theta frequency (4-12Hz) is due to their implication in diverse brain functions. However, the underlying neural mechanism for behavioral basis of hippocampal theta oscillations remains unclear. Here we provide the evidence for the functional role of Cav3.1 T-type calcium channel in modulating hippocampal theta oscillations and novel objects exploration behavior. We found that mice with a null mutation for the Cav3.1 channels (Cav3.1-/- mice) showed increased theta power under urethane anesthesia (atropine-sensitive type-2 theta rhythm) and also during wheel running (atropine-resistant type-1 theta rhythm) . Interestingly, Cav3.1-/- mice showed enhanced response to novel objects behavior, increased locomotor activity and alleviated anxiety. To verify the connection between Cav3.1 channels highly expressed in the medial septum and these diverse behaviors, we specifically knock down the endogenous Cav3.1 expression in medial septal neurons by Lentiviruses-mediated RNAi. The mice with selectively reduced Cav3.1 expression (shCav3.1) in the medial septal neurons show increased type 2 theta power without any change in the type1 theta power. Also these mice showed enhanced response to novel objects behavior similar to that of Cav3.1-/- mice with no change in locomotor activity and anxiety behavior. There was a strong correlation between type-2 theta power and enhanced novelty behavior observed for shRNA control mice and shCav3.1 mice. Cav3.1 channels in the medial septum are strongly expressed in the septo-hippocampal GABAergic neurons which are known to modulate the amplitude of type-2 theta rhythm. We found that the septo-hippocampal GABAergic neurons without Cav3.1 channels in the mutant mice showed marked increased discharge activity which explains the increased type-2 theta rhythm via the disinhibition of hippocampal local GABAergic neurons. Taken together, these findings suggest that Cav3.1 T-type calcium channel in the medial septum is critically involved in controlling the hippocampal type 2 theta oscillations and enhanced novelty behavior.

Disclosures: G. Gangadharan: None. **J. Shin:** None. **S. Kim:** None. **M. Kim:** None. **D. Kim:** None. **M. Watanabe:** None. **Y. Yanagawa:** None. **Y. Kim:** None. **D. Kim:** None. **H. Shin:** None.

Poster

842. Calcium Channels III

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Program#/Poster#:842.09/B35

Topic: B.04. Ion Channels

Support: MIUR-PRIN 2007SYRBBH_001

Compagnia di San Paolo grant ORTO11RRT5

Title: Cav 3.2 T-type channels are up-regulated by chronic hypoxia: Involvement in pain signaling pathways

Authors: *C. CALORIO[1], V. COMUNANZA[2], A. MARCANTONI[1], S. MARCHIO[2], F. BUSSOLINO[2], E. CARBONE[1]
[1]Univ. of Torino, Dept of Neuroscience, NIS Ctr. of Excellence, CNISM, INN, Torino, Italy; [2]Inst. for Cancer Res. and Treatment, Candiolo, Turin, Italy

Abstract: T-type calcium channels (TTCCs) are well established mediators of pain signals in primary afferent neurons (DRG neurons). Recent studies indicate that in parallel with the development of diabetes-induced pain, T-type current density increase by twofold in medium-size cells from DRG neurons (Jagodic et al., 2007, J Neurosci, 27:3305).
Moreover, the hyperexcitability of peripheral nerves observed during diabetic neuropathies is associated with a decrease of blood flow that leads to nerve hypoxia (Chavez et al., 2005, Neurosci Lett, 374:179).This issue is of particular interest since chronic hypoxia (CH) up-regulates Cav3.2 TTCCs both in adrenal chromaffin cells (Carabelli et al., 2007, J Physiol, 584:149-65) and PC12 cells .
Furthermore, chronic responses to hypoxia are regulated by the hypoxia- inducible transcription factors (HIFs), whose accumulation is mediated by a ROS-dependent Ca^{2+}-signaling pathway (Yuan et al, 2008, J Cell Physiol, 217 :674).
Here, by combining immunostaining and electrophysiological recordings we show that CH (15 h, 3% O_2) up-regulates TTCCs in small DRG neurons via the HIF-1alpha pathway.
We found that CH significantly increases the percentage of cells expressing TTCCs (from 66 to 84%) while the T-type current density increases by a factor two in hypoxic small DRG neurons, but not in medium-size DRGs. The pharmacological and kinetics properties of CH-up-regulated currents did not change substantially, suggesting a selective up-regulation of the same Cav3.2 subunit available at control. Interestingly,

CH potentiates also DRG excitability manifested by a more prominent after depolarizing potential (ADPs) phase (from 9% to 24%) during the repolarization phase of evoked action potentials.
Voltage-clamp
recordings showed that the up-regulatory effects of CH on TTCCs are mimicked by overnight incubation with the iron chelating agent deferoxamine (DFX), which activates HIFs, while immunostaining revealed an enhanced expression of HIF-1alpha in small-size DRG neurons following CH. These data strongly suggest the involvement of HIF-1alpha in TTCCs up-regulation. Finally, we found that TTCCs recruitment occurred as
a results of ROS generation and PKC/IP_3 pathway activation.
Our data reinforce the idea that
TTCCs are pro-nociceptive and can be recognized as potential therapeutic targets for the treatment of chronic pain associated with CH.

Disclosures: C. Calorio: None. **V. Comunanza:** None. **A. Marcantoni:** None. **S. Marchio:** None. **F. Bussolino:** None. **E. Carbone:** None.

Poster

842. Calcium Channels III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:842.10/B36

Topic: B.04. Ion Channels

Support: AG041360

Title: Rapid inhibition of voltage-dependent calcium and sodium currents by estrogen receptor agonists in rat basal forebrain cholinergic neurons

Authors: *D. W. DUBOIS, D. MURCHISON, A. S. FINCHER, B. ASWIN, K. A. PEEBLES, W. H. GRIFFITH
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Abstract: Estrogen receptors on the plasma membrane mediate a variety of molecular signaling events in many cell types. In neurons, estrogenic signaling can rapidly enhance or inhibit synaptic transmission and voltage-gated channel function, depending on brain region. Previously, we reported that 17β-estradiol (17βE) and other estrogen receptor agonists, potently reduced spontaneous frequency of IPSCs in rat basal forebrain (BF) cholinergic neurons, and that this inhibition was reduced in middle-aged males and reproductively senescent (acyclic) females.

These results implicated at least two classes of receptor in this rapid estrogenic modulation: first, the G-protein coupled estrogen receptor (GPER) and second, the estrogen receptor beta (ERβ) located on the plasma membrane. Membrane-delimited (non-nuclear) classical estrogen receptors are thought to exert their rapid effects by direct or indirect coupling to G-protein cascades. The purpose of this study is to test the hypothesis that estrogen receptor agonists rapidly modulate voltage-gated channel function in BF neurons. We used conventional whole-cell patch clamp recording in acutely dissociated BF neurons from both male and female rats (2-4 months). Cholinergic neurons (choline acetyl-transferase positive) were identified by single-cell RT-PCR. In preliminary studies, high voltage-activated (HVA) Ca^{2+} currents in Sprague-Dawley rats were sensitive to17βE (100 nM) with 57% inhibition (12 of 12 cells) and 21% inhibition by the GPER agonist G-1 (100 nM, 4 of 4 cells). Low voltage-activated (LVA) Ca^{2+} currents also were reduced 34% by 17βE (11 of 13 cells) and 20% inhibited by G-1 (4 of 5 cells). In identified cholinergic neurons from F344 rats, in males, HVA currents were inhibited 24% by 100 nM 17βE (5 of 6 cells) and in females by 42% (3 of 3 cells). LVA currents were inhibited by 24% in males (3 of 5) and 19% in females (2 of 4). The estrogen receptor agonists (100 nM) PPT (ERα agonist) and DPN (ERβ) had little effect. DPN modulated HVA currents by < 5% in both males (n=4) and females (n=3). PPT inhibited HVA currents by < 10% in 6 of 7 cells in both sexes. Voltage-gated Na^{+} currents were inhibited 13% by 100 nM 17βE (5 of 5 cells) and 24% by 500 nM (9 of 13 cells). G-1 (100nM) inhibited Na^{+} currents by 17% (7 of 10). In mature adult females (9-10 months), Na^{+} currents were inhibited 21% by 17βE 500 nM (4 of 5 cells). Experiments are in progress to complete this pharmacological profile and extend this investigation to reproductively senescent females. These results support the interpretation that rapid estrogenic modulation of function in rat cholinergic BF neurons has GPER inhibition of voltage-gated channels as a major component.

Disclosures: D.W. Dubois: None. **D. Murchison:** None. **A.S. Fincher:** None. **B. Aswin:** None. **K.A. Peebles:** None. **W.H. Griffith:** None.

Poster

842. Calcium Channels III

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Topic: B.04. Ion Channels

Support: NIH grant DC009433

NIH grant HL087120

NIH grant EY020850

Carver Research Program of Excellence

Title: Characterization of Ca2+-Binding Protein 1 (CaBP1) expression and localization in mouse brain

Authors: *K. Y. KIM[1], A. SHEPHERD[2], X. LIU[1], F. HAESELEER[3], A. LEE[1]
[1]Mol. Physiol. and Biophysics, [2]Pharmacol., Univ. of Iowa, Iowa City, IA; [3]Physiol. & Biophysics, Univ. of Washington, Seattle, WA

Abstract: CaBP1 is a calmodulin-like Ca2+ binding protein that may regulate neuronal functions through interactions with effectors including intracellular and plasma membrane Ca2+ channels. Multiple variants of CaBP1 have been characterized (CaBP1S, CaBP1L, and caldendrin), but little is known about their roles in specific neurological contexts. To gain insights into the cellular functions of CaBP1, we characterized the expression and localization of CaBP1 variants in mouse brain. Although all CaBP1 variants were amplified by RT-PCR, caldendrin, but little CaBP1S or CaBP1L, was detected in western blot analysis of mouse brain lysates with antibodies recognizing all 3 variants. Two isoforms of caldendrin were found (33 and 36 kDa), which were consistent in size with translation from alternative start sites within a single transcript. The western blotting results reflected the expression of CaBP1/caldendrin since they were not reproduced in mice with targeted disruption of the gene encoding CaBP1/caldendrin (CaBP1 KO). By immunoperoxidase labeling, CaBP1/caldendrin was localized in multiple cell-types including pyramidal cells in the cerebral cortex and hippocampal CA3 region and interneurons in the cerebellum of wild-type but not CaBP1 KO mice. Double-labeling indicated that CaBP1/caldendrin was localized in parvalbumin-positive interneurons in the cerebral cortex and cerebellum. CaBP1/caldendrin was not detected in cerebellar Purkinje neurons but strongly colocalized with GAD65 in pinceaux synapses formed between basket cells and the Purkinje cell axon initial segment. We conclude that caldendrin is the dominant CaBP1 variant expressed in mouse brain. Moreover, CaBP1/caldendrin is localized both pre- and post-synaptically and is positioned to regulate both excitatory and inhibitory transmission.

Disclosures: K.Y. Kim: None. **A. Shepherd:** None. **X. Liu:** None. **F. Haeseleer:** None. **A. Lee:** None.

Poster

842. Calcium Channels III

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Program#/Poster#:842.12/B38

Topic: B.04. Ion Channels

Support: NIH Grant MH51106

Develbiss Fund

NSF-PIRE Program (0730255)

Title: Harmaline attenuates voltage-sensitive Ca^{++} currents in inferior olive neurons

Authors: *X. ZHAN[1,2], W. M. GRAF[1]
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Abstract: Harmaline is a tremor-inducing alkaloid which has been reported to interact with several neurotransmitter receptors as well as ion exchangers and voltage-sensitive channels. One site of harmaline action in the brain is the inferior olive. Either local or systemic harmaline application increases action potential firing rates in the inferior olive and this activation is thought to produce tremor and ataxia through activation of target neurons in the cerebellum. We have performed whole cell voltage-clamp and current clamp recordings from inferior olive neurons in brain slices derived from young rats. We found that transient low-voltage activated (LVA) and sustained high voltage-activated (HVA) Ca^{++} currents are significantly attenuated by 125 - 250 μM harmaline applied to the bath and that this attenuation is partially reversible. In current clamp recordings, action potential after-hyperpolarization complexes were evoked by brief positive current injections. Harmaline produced a small attenuation of action potential amplitudes, but also large action potential broadening associated with attenuation of the fast and medium after-hyperpolarization. Our data suggest that one mode of inferior olive activation by harmaline may involve attenuation of both LVA and HVA Ca^{++} conductances with subsequent attenuation of Ca^{++}-sensitive K^{+} conductances resulting in spike broadening and attenuation of the after-hyperpolarization.

Disclosures: X. Zhan: None. **W.M. Graf:** None.

Poster

842. Calcium Channels III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:842.13/B39

Topic: B.04. Ion Channels

Title: Expressional and functional analysis of N-type calcium channel in the hippocampus using Cacna1b gene knockout mice

Authors: *N. MIYAMOTO, C. M. NAKAZAWA
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Abstract: Voltage-gated Ca^{2+} channels (VGCCs) are essential for a wide variety of physiological functions including neural degeneration and hippocampal synaptic plasticity. Chronic hypertension is associated with an increased risk of both vascular dementia and Alzheimer's diseases. Anti-hypertensive dihydropyridin-calcium channel blocker therapies have been associated with a beneficial effect on cognitive function by lowering blood pressure and/or by specific neuroprotect effect. Long-term effects of L-type Ca^{2+} channel blocker nitrendipine protects against dementia in older patients with systolic hypertension. Moreover, a positive effect of nimodipine in AD patients to slow cognitive decline compared to placebo in short-term trials [1]. Furthermore, peripherally and central administration of L-type Ca^{2+} channel blockers facilitate retention of passive avoidance and maze learning in mice [2]. Chronic oral treatment of verapamil in mice facilitates on short-term presynaptic plasticity of dentate gyrus cells [3]. The T-type C Ca^{2+} channel deficient mice are impaired in the context-cued trace fear conditioning, step-down and step-through passive avoidance tasks and long-term potentiation in hippocampal slices [4]. The possibility that N-type Ca^{2+} channel could contribute to synaptic plasticity in the hippocampus was examined in this study. α1 subunit of N-type Ca^{2+} channel is encoded by *Cacna1b* gene that is specifically expressed in nervous system and the gene product is predominantly coupled to the synaptic transmission. To investigate the functional role of this channel in hippocampus, we subjected *Cacna1b* gene homozygous knockout mice and their wild-type littermates to microarray data analysis. Among more than two-fold expressional changed genes in steady-state of hippocampus, greatly affected canonical biological pathways were glutamate receptor signaling, amyotrophic lateral sclerosis signaling, synaptic long-term depression, Huntington's disease signaling, calcium signaling and nitric oxide signaling in the cardiovascular system. Those data suggested that this channel could be related to learning and memory as well as other type of VGCCs. Electrophysiological experiments using mutant hippocampal slices is underway to answer the question. [1] E. Duron and O. Hanon *J. Alzheimers Dis* **20** (2010) 903-914., [2] D. Quartermain et al., *Neurobiol Learn Mem* **75** (2001) 77-90., [3] R. Lashgari et al., *Neurosci Lett* **415** (2007) 174-178., [4] C-C. Chen et al., *PLoS One* **7** (2012) e29384.

Disclosures: N. Miyamoto: None. **C.M. Nakazawa:** None.

Poster

842. Calcium Channels III

Location: Hall F-J

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Program#/Poster#:842.14/B40

Topic: B.04. Ion Channels

Title: Modulation of N-type Ca(2+) currents by moxonidine via imidazoline I(1) receptor activation in rat superior cervical ganglion neurons

Authors: Y. KIM, D. AHN, *S. CHUNG
Physiol., Dept. of Physiology, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Moxonidine, an imidazoline deriviatives, suppress the vasopressor sympathetic outflow to produce hypotension. This effect has been known to be mediated in part by suppressing sympathetic outflow via acting imidazoline I_1 receptors (IR_1) at postganglionic sympathetic neurons. But, the cellular mechanism of IR_1-induced inhibition of noradrenaline (NA) release is still unknown. We therefore, investigated the effect of IR_1 activation on voltage-dependent Ca^{2+} channels which is known to play an pivotal role in regulating NA in rat superior cervical ganglion (SCG) neurons, using the conventional whole-cell patch-clamp method. In the presence of rauwolscine (30μM), which blocks α_2-adrenoceptor ($R_{\alpha 2}$), moxonidine inhibited voltage-dependent Ca^{2+} current (I_{Ca}) by about 30%. This moxonidine-induced inhibition was almost completely prevented by efaroxan (10 μM) which blocks IR_1 as well as $R_{\alpha 2}$. These data suggest that moxonidine inhibit voltage-dependent N-type Ca^{2+} current (I_{Ca-N}) via activating IR_1. Finally, moxonidine significantly decreased the frequency of AP firing in a partially reversible manner. Taken together, our results suggest that activation of IR1 in SCG neurons reduced $I_{Ca\text{-}N}$ in a PTX-and voltage-insensitive pathway, and this inhibition attenuated repetitive AP firing in SCG neurons.

Disclosures: Y. Kim: None. **D. Ahn:** None. **S. Chung:** None.

Poster

842. Calcium Channels III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:842.15/B41

Topic: B.04. Ion Channels

Support: NHMRC

Title: Novel ω-conotoxins potently inhibit N-type voltage-gated calcium channels in sensory neurons and partially reverse pain behavior after systemic dosing

Authors: *M. SADEGHI[1], S. S. MURALI[2], R. J. LEWIS[3], P. F. ALEWOOD[3], M. J. CHRISTIE[2]
[1]Pharmacology, Sydney Univ., [2]Sydney Univ., Sydney, Australia; [3]UNIVERSITY OF QUEENSLAND, BRISBANE, Australia

Abstract: ω-Conotoxins, selective inhibitors of N-type voltage-gated calcium channels, have emerged as a new class of therapeutics in pain management. ω-Conotoxins are usually administered intrathecally to directly inhibit neurotransmitter release at primary afferent synapses. However, the therapeutic indices of intrathecally administered ω-conotoxins are quite narrow. It has been reported that intravenous injection of ω-conotoxin CVID, isolated from *C.catus*, produces dose-dependent antihyperalgesic effects with less serious cardiovascular side effects than those reported for some other ω-conotoxins (Kolosov et al, 2009, Pain Med 11, 262-73). In the present study we investigated the potency, voltage-dependence and reversibility of ω-conotoxins more recently isolated from *C.catus* and several analogues (Berecki et al, 2010, Mol Pharmacol 77, 139-148) in mouse isolated DRG neurons and determined whether systemic administration of these ω-conotoxins can produce inhibition of inflammatory pain behaviors.Whole-cell patch clamp electrophysiology on isolated mouse DRG neurons demonstrated that CVIE is significantly more potent than CVID, CVIE(R10K),CVIF(R10K) and CVIF. ω-conotoxins have higher affinity for the inactive state of VGCCs a strong membrane hyperpolarization facilitates recovery from block. For evaluating the pain alleviating effects of ω-conotoxin following systemic administration, we used a hindpaw inflammatory pain model (Freund's Complete Adjuvant) in mice and tested hind paw weight bearing and von Frey thresholds before and 1,2,4 and 6 hours after s.c. administration of different doses (0.02,0.2 and 2 mg/kg) of each ω-conotoxin. All peptides produced significant partial reversal of inflammatory pain at a dose of 2 mg/kg and the peak effect was observed 2 hours post-injection, with less obvious effects at lower doses. CVIE had the largest effect of all peptides at 2mg/kg but was not more potent that CVID. These findings suggest that systemically administered ω-conotoxin CVIE may be useful in pain management.

Disclosures: M. Sadeghi: None. **S.S. Murali:** None. **R.J. Lewis:** None. **P.F. Alewood:** None. **M.J. Christie:** None.

Poster

842. Calcium Channels III

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:842.16/B42

Topic: B.04. Ion Channels

Title: A mechanism of hypotension induced imidazoline I_2 receptors activation in agmatine

Authors: S. CHUNG, *Y. KIM
physiology, Yonsei Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Agmatine, an imidazoline deriviatives, suppress the vasopressor sympathetic outflow to produce hypotension. This effect has been known to be mediated in part by suppressing sympathetic outflow via acting imidazoline I_2 receptors (IR_2) at postganglionic sympathetic neurons. But, the cellular mechanism of IR_2-induced inhibition of noradrenaline (NA) release is still unknown. We therefore, investigated the effect of IR_2 activation on voltage-dependent Ca^{2+} channels which is known to play an pivotal role in regulating NA in rat superior cervical ganglion (SCG) neurons, using the conventional whole-cell patch-clamp method. In the presence of rauwolscine (30μM), which blocks α_2-adrenoceptor ($R_{\alpha 2}$), agmatine inhibited voltage-dependent Ca^{2+} current (I_{Ca}) by about 30%. This agmatine-induced inhibition was almost completely prevented by BU224 (10 μM) which blocks IR_2 as well as $R_{\alpha 2}$. These data suggest that agmatine inhibit voltage-dependent N-type Ca^{2+} current (I_{Ca-N}) via activating IR_2. Finally, agmatine significantly decreased the frequency of AP firing in a partially reversible manner. Taken together, our results suggest that activation of IR_2 in SCG neurons reduced I_{Ca-N} in a PTX- and voltage-insensitive pathway, and this inhibition attenuated repetitive AP firing in SCG neurons.

Disclosures: S. Chung: None. **Y. Kim:** None.

Poster

843. Functional Modulation: Peptides and Amines

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:843.01/B43

Topic: B.07. Synaptic Transmission

Support: NIH Grant DK073311

Title: Alterations in serotonergic modulation of basolateral amygdala neurons in bdnf conditional mutant mice

Authors: *S. S. DAFTARY, G. CALDERON, M. RIOS
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Abstract: $BDNF^{2L/2LCamk\text{-}cre}$ mutant mice with brain-specific deletion of the *Bdnf* gene are characterized by an increase in aggressive, anxiety and depressive-like behavior. The behavioral

alterations elicited by perturbed BDNF signaling highlight the importance of this pathway in the regulation of affective behavior. One possible mechanism by which BDNF might modulate mood is by regulating serotonergic neurotransmission in regions of the brain traditionally associated with psychiatric disorders, such as the basolateral amygdala (BLA). To investigate this, we examined whether $BDNF^{2L/2LCamk\text{-}cre}$ mutant mice exhibited alterations in 5-HT transmission in this limbic region of the brain.
Immunohistochemical studies found that BLA GABAergic and pyramidal projection neurons contain TrkB receptors, indicating their ability to be influenced by BDNF. Notably, whole cell patch-clamp recordings from BLA projection neurons in wild-type and BDNF mutants did not indicate any differences in passive membrane properties such as resting potential, input resistance, spike amplitude, spike threshold, membrane time constants and spontaneous excitatory/inhibitory postsynaptic currents under basal conditions. However, bath application of serotonin uncovered significant alterations in the modulation of GABAergic and glutamatergic synaptic transmission in BDNF mutant mice compared to wild-type mice resulting in a hyperexcitable circuit. These changes were associated with decreased expression of $5\text{-}HT_2$ receptors in the BLA. Collectively, our findings indicate that 5-HT-mediated transmission in the BLA is altered in BDNF mutants, which might contribute to the behavioral alterations that they exhibit, including increases in anxiety-like behavior.

Disclosures: S.S. Daftary: None. **G. Calderon:** None. **M. Rios:** None.

Poster

843. Functional Modulation: Peptides and Amines

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:843.02/B44

Topic: B.07. Synaptic Transmission

Support: NIH Grant P50 MH078028

NIH Grant R01 NS052446

Title: A novel role for the serotonin transporter in the regulation of neuroendocrine stimulus-secretion coupling

Authors: *R. L. BRINDLEY[1], R. D. BLAKELY[2], K. P. CURRIE[3]
[1]Dept. of Anesthesiol., Vanderbilt Univ. Med. Ctr., Nashville, TN; [2]Dept. of Pharmacol., [3]Departments of Anesthesiol. and Pharmacol., Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: The serotonin transporter (SERT), an important target for anti-depressants, mediates the reuptake and recycling of serotonin (5HT) from serotonergic synapses. SERT is also expressed outside the brain, including prominently in adrenal chromaffin cells. These cells are a crucial output of the sympathetic nervous system and also provide a good model for neurosecretion. They release a variety of transmitters, including epinephrine and norepinephrine, to help maintain homeostatic functions and provide appropriate responses to acute stress. Autocrine / paracrine signaling pathways tightly regulate stimulus-secretion coupling from chromaffin cells and failure to do so can contribute to the development of hypertension and heart failure. Increasing evidence links depression and cardiovascular diseases, and some depressed patients exhibit elevated catecholamine levels, perhaps suggesting common molecular targets or mechanisms. Chromaffin granules store and release 5HT, albeit at > 1000 fold lower concentration than epinephrine. However, the function of 5HT / SERT in chromaffin cells remains unclear. We postulated that 5HT is accumulated through uptake by SERT. Consistent with this, when bovine chromaffin cells were cultured in media with dialyzed fetal bovine serum (to remove extracellular 5HT) there was a dramatic reduction in intracellular 5HT, but no change in catecholamine content. Addition of 5HT to the dialyzed culture medium rescued the intracellular 5HT content and this was blocked by the SERT antagonist citalopram. Similarly, the 5HT content of adrenal glands from SERT knockout (KO) mice was dramatically reduced compared to wild type littermates with no change in catecholamine content. We used carbon fiber amperometry to investigate the effect of 5HT / SERT on secretion from mouse chromaffin cells. In the presence of extracellular 5HT, loss of SERT activity (acute block by citalopram or use of cells from SERT KO mice) reduced catecholamine secretion evoked by KCl. Whole cell patch clamp experiments showed that 5HT / citalopram did not alter voltage-gated calcium channels. Thus our data demonstrate that intracellular 5HT accumulation in chromaffin cells relies upon uptake by SERT. Loss of SERT has no effect on cellular catecholamine content, but does inhibit exocytosis. We postulate that 5HT acts at an intracellular site(s) following uptake by SERT to control exocytosis, rather than extracellularly via G protein coupled receptors. Ongoing work will investigate this novel mechanism for controlling neuroendocrine catecholamine release.

Disclosures: R.L. Brindley: None. **R.D. Blakely:** None. **K.P. Currie:** None.

Poster

843. Functional Modulation: Peptides and Amines

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:843.03/B45

Topic: B.07. Synaptic Transmission

Support: NSC 99-2628-B-002-052-MY3

Title: Presynaptic 5-HT1B receptor-mediated synaptic suppression to the subplate neurons in the somatosensory cortex of neonatal rats

Authors: *L.-J. LEE[1,2,3], C.-C. LIAO[1]
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Abstract: Serotonin (5-HT) is the target of numerous psychiatric medicines and also plays important roles during neural development. To evaluate the influence of 5-HT in the developing brain, we examined the direct effects of 5-HT on the intrinsic and synaptic properties of neurons in the cortical subplate, an early developed structure that plays important roles in the consequential maturation of cortical circuits. Acute brain slices were obtained from neonatal rats and the physiological properties of SPns were evaluated before and after 5-HT bath-application. In all concentrations tested, 5-HT did not affect the intrinsic properties of SPns. However, thalamus-evoked excitatory postsynaptic currents (eEPSCs) to SPn was significantly suppressed by 5-HT in a dose-dependent manner. Because 5-HT did not affect AMPA-induced EPSC, our results did not support a 5-HT-mediated postsynaptic mechanism in reducing EPSCs. Subsequent to 5-HT application, increased paired-pulse ratio and decreased MK-801 blocking rate were noted, indicating the presence of presynaptic 5-HT receptor-mediated suppressive effect in the thalamocortical afferent (TCA)-SPn synapses. To elucidate the type(s) of 5-HT receptor involved in this process, various 5-HT receptor agonists and antagonists were tested. Our results showed that 5-HT1B receptor agonist CP93129 mimicked the effect of 5-HT and only 5-HT1B receptor antagonist SB224289 prevented 5-HT-mediated synaptic suppression. Taken together, our data demonstrated the presynaptic 5-HT1B receptor-mediated suppressive effect on the excitatory synapses between TCAs and SPns in the somatosensory cortex of neonates. Use of drugs that might interrupt 5-HT homeostasis in pregnant women should be concerned.

Disclosures: L. Lee: None. **C. Liao:** None.

Poster

843. Functional Modulation: Peptides and Amines

Location: Hall F-J

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Program#/Poster#:843.04/B46

Topic: B.07. Synaptic Transmission

Support: National Natural Science Foundation of China, 81070899, 81171049

NIH Grant NS040723

Title: Differential roles of 5-HT1A, 5-HT1B and 5-HT1D receptors in modulation of entorhinal-hippocampal synaptic transmission and connections: Multi-electrode array recordings

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Abstract: The hippocampus is a major target of serotonergic afferents arising from the midbrain raphe nuclei. Serotonin (5-HT)-containing fibers project to the dorsal hippocampus innervating the CA1 and dentate gyrus (DG). The effects of 5-HT on regulation of pain, memory and depression are mediated through its 14 receptor subtypes. There exist many subtypes of 5-HT receptors in the hippocampal formation whereby 5-HT produces differential excitatory and inhibitory effects on hippocampal neurons. However, the roles of 5-HT receptors in modulation of the entorhinal-DG and entorhinal-CA1 synaptic connections at the network level have rarely been studied. Using a 64-channel multi-electrode array (Med 64 System), modulatory effects of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors were studied on the entorhinal-hippocampal synaptic transmission and connections. We recorded two types of field excitatory postsynaptic potentials (fEPSPs) in the CA1 and DG evoked by electrical stimulation of the perforant path in hippocampal slices of rats. After stability of network fEPSPs was achieved, hippocampal slides were perfused with the agonist of 5-HT_{1A} receptors, 8-OH-DPAT, antagonist of 5-HT_{1A} receptors, WAY100635, antagonist of 5-HT_{1B}, SB224289, and antagonist of 5-HT_{1D}, BRL15572, respectively, for 15 min. Two-dimensional current source density analysis was transformed by the bilinear interpolation at each point of the 64 electrodes for spatial imaging of the network fEPSP responses. The results show that perfusion of hippocampal slices with the 5-HT_{1A} receptor antagonist resulted in significant enlargements of synaptic network connection size and synaptic efficacy. In contrast, perfusion with the 5-HT_{1A} receptor agonist produced a reversal effect with reductions in synaptic size and magnitude of fEPSPs. However, perfusion of hippocampal slices with the antagonist of 5-HT_{1B} or 5-HT_{1D} receptors resulted in significant reductions in synaptic size and synaptic efficacy. Thus, we suggest that endogenous release of 5-HT causes an inhibition of entorhinal-CA1 and entorhinal-DG synaptic transmission and connections via acting on 5-HT_{1A} receptors, whereas 5-HT_{1B} and 5-HT_{1D} receptors may mediate an opposite role in synaptic transmission and connections of the entorhinal-CA1 and entorhinal-DG.

Disclosures: J. Jin: None. **J. Chen:** None. **Q. Lin:** None.

Poster

843. Functional Modulation: Peptides and Amines

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Program#/Poster#:843.05/B47

Topic: B.07. Synaptic Transmission

Support: GIST Grant SBIE (JMC)

KMEST Grant NRF (JMC)

Title: Presynaptic inhibition of glutamate release by serotonin in the lateral habenula

Authors: *E.-K. HWANG, J.-M. CHUNG
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Abstract: The lateral habenula nucleus (LHb), known to regulate dopamine and serotonin (5HT) systems in mammalian brain, involves in the regulation of several mental functions such as reward, stress, pain, maternal behavior and cognition. Anatomically LHb where binding sites as well as mRNAs for various 5HT receptor subtypes are present reciprocally connects with 5HT resources such as raphe nuclei, implying that synaptic responses of LHb neurons may be modulated by 5HT. In this study, we have examined, using a whole cell voltage-clamp recording, how 5HT modulates glutamatergic transmission in LHb. We observed that 5HT directly suppressed glutamatergic transmission by decreasing the probability of glutamate release from presynaptic terminals. This inhibitory action of 5HT onto CNQX-sensitive glutamatergic EPSCs was further confirmed by the applications of $5HT_{1B}$ receptor agonist and/or antagonist. The 5HT-induced suppression of glutamate release was also found to be mediated mainly by the activation of a voltage-gated K^+ channel sensitive to both 4-aminopyridine and α-dendrotoxin. Therefore, all these results indicate that 5HT reduces excitatory signals onto an LHb neuron by hyperpolarizing its presynaptic terminals through the activation of $5HT_{1B}$ receptors.

Disclosures: E. Hwang: None. **J. Chung:** None.

Poster

843. Functional Modulation: Peptides and Amines

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Program#/Poster#:843.06/B48

Topic: B.07. Synaptic Transmission

Support: NSERC

FRQS

Title: Dopamine facilitates evoked excitatory synaptic currents in layer II of the lateral entorhinal cortex through D1-family receptors

Authors: *I. GLOVACI, C. A. CHAPMAN
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Abstract: The entorhinal cortex receives dopaminergic inputs from the ventral tegmental area and substantia nigra that may modulate its responsiveness to inputs from other cortical areas. Previous research has shown that dopamine produces concentration-dependent changes in synaptic transmission in layer II neurons of the lateral entorhinal cortex, such that high concentrations of dopamine suppress evoked EPSPs, and lower concentrations of dopamine facilitate EPSPs. Our studies of the enhancement of EPSPs induced by dopamine have shown that it depends on cell type; Layer II entorhinal fan cells, characterized by a sag in the voltage response to hyperpolarizing current steps, typically show a larger dopamine-induced facilitation of mixed EPSPs evoked by layer I stimulation as compared to pyramidal and multiform cells. Presently, we have used voltage-clamp recordings of electrophysiologically differentiated fan and pyramidal neurons to investigate the underlying signalling mechanisms that mediate the dopaminergic facilitation of excitatory synaptic responses in submerged slices of the lateral entorhinal cortex. We have found that dopamine facilitates pharmacologically-isolated AMPA glutamate receptor-mediated EPSCs, but does not significantly affect pharmacologically-isolated NMDA receptor-mediated EPSCs. To determine the dopaminegic receptor subtype responsible for the facilitation of EPSCs, either the D1 receptor blocker SCH-23390 (50 µM) or the D2 receptor blocker sulpiride (50 µM) was bath applied prior to dopamine application. Application of SCH-23390 blocked the facilitation of EPSCs, whereas application of sulpiride had no significant effect on the facilitation of synaptic currents induced by dopamine. Therefore, we suggest that dopaminergic enhancement of EPSCs is likely to be mediated primarily through D1-family receptors. D1 dopamine receptors can act through a variety of intracellular signalling pathways dependent on protein kinase A to modulate synaptic strength. In the present study, we have tested the role of signalling via protein kinase A by including the PKA inhibitor H-89 in the recording pipette solution. Cells filled with H-89 did not show a significant facilitation of EPSCs in response to dopamine application. Thus, the dopamine-induced facilitation of AMPA receptor-mediated synaptic responses in layer II neurons of the lateral entorhinal cortex appears to be mediated via a D1 receptor-dependent increase in PKA activity.

Disclosures: I. Glovaci: None. **C.A. Chapman:** None.

Poster

843. Functional Modulation: Peptides and Amines

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Topic: B.07. Synaptic Transmission

Support: Smith Family Foundation Award

Brown-Coxe Postdoctoral Fellowship

Alfred P. Sloan Research Fellowship

Title: DIfferential noradrenergic modulation of synaptic AMPA- and NMDA-type glutamate receptors in the prefrontal cortex

Authors: G. LUR, *M. J. HIGLEY
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Abstract: Cognitive functions such as working memory and attention are strongly influenced by noradrenergic modulation of neuronal activity. Emerging evidence suggests that distinct subtypes of adrenergic receptors play differential roles in neuropsychiatric illnesses such as attention deficit and anxiety disorders. Noradrenaline (norepinephrine) is thought to regulate glutamatergic synaptic transmission in the prefrontal cortex. However, the cellular targets and mechanisms of this modulation remain poorly understood. To investigate noradrenergic modulation of excitatory synapses in the mouse prelimbic and infralimbic cortices, we first utilized afferent-specific expression of Channelrhodopsin2. Our experiments revealed that noradrenaline negatively regulates inputs to prefrontal layer 5 pyramidal neurons arising from both the ventral hippocampus and medial thalamus. We then used 2-photon laser scanning microscopy and focal 2-photon glutamate uncaging to analyse the postsynaptic actions of noradrenaline on individual dendritic spines. We stimulated glutamatergic synapses in three subcellular regions: basal dendrites, proximal apical oblique dendrites, and the apical tuft. Activation of α1 adrenergic receptors (A1Rs) had minimal effect on uncaging evoked excitatory postsynaptic potentials (uEPSPs) but significantly reduced the evoked synaptic calcium transient in all three regions. In contrast, activation of α2 receptors (A2Rs) significantly reduced both uEPSPs and calcium transients in basal dendrites and the apical tuft but had no effect in apical oblique dendrites. Subsequent experiments revealed that A1Rs specifically reduce synaptic currents and calcium influx through NMDA-type glutamate receptors, while A2Rs reduce currents through AMPA-type receptors. Our data demonstrate that noradrenergic modulation of synaptic transmission occurs via distinct mechanisms that are compartmentalized within dendritic subregions of pyramidal neurons. This heterogeneity likely permits the selective modulation of specific circuits contributing to different aspects of cognitive function.

Disclosures: G. Lur: None. **M.J. Higley:** None.

Poster

843. Functional Modulation: Peptides and Amines

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Topic: B.07. Synaptic Transmission

Support: NIH Grant DA025143

OD008761

AFAR New Investigator Award

Title: Effects of nano-molar Abeta 1-40 on excitatory synaptic transmission

Authors: *R. M. LAZARENKO, L. L. ROUHANA, B. EDBERG, X. GAO, Q. ZHANG
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Abstract: Alzheimer's is a chronic disease of elderly and aggregated amyloid beta (Aβ) is the major component of plaques found in patients' brains. Aβ is known to disrupt synaptic transmission and likely contributes to neurodegeneration. Among various isoforms of Aβ, Aβ(1-40) is the most abundant and less aggregative. Production of Aβ correlates with synaptic activity and can reach tens of nano-molar in vivo. Though, there is a documented role of Aβ on postsynaptic site, little is known about effect of Aβ on presynaptic terminals. Combining live cell imaging and patch clamp electrophysiology, we examined how elevated Aβ(1-40) levels impact presynaptic function.
We found that acute application of 100 nano-molar Aβ(1-40) leads to cell membrane instability, swelling and fragmentation of neurites, which is unlikely caused by insoluble Aβ(1-40) aggregates as dynamic light scattering measurement shows no proteinous particles larger than 100 nm. At 10 nM, Aβ leads to increase of mEPSC amplitude and decrease of frequency without measurable changes in cell morphology. At 1 nM - effects are similar to 10 nM but milder.
The reduced frequency of minis may reflect the lower vesicle release probability (Pv), availability of fewer synapses, impaired vesicle recycling, etc. By comparing normalized evoked responses to 30 Hz/20 pulses trains of local electric stimulation of presynaptic inputs, we found decreased Pv in the presence of Aβ(1-40). NMDA/AMPA ratio in the presence of 10 nM beta amyloid was slightly increased, likely corresponding to the increase of mEPSC amplitude. Using single Qdot imaging, we have identified that the decrease of Pv is due to the lack of fast vesicle recycling not the decrease of readily releasable pool. Using simultaneous FM, we have confirmed that fast release and retrieval of synaptic vesicles are impaired. Slow vesicle recycling is unaltered. By labeling three representative synaptic vesicle proteins, synaptobrevin II,

synaptophysin and vesicular glutamate transporter 1, with pH-sensitive GFP variant (a.k.a. pHluorin), we have identified that the proportional retrieval of vesicular proteins remain the same despite significantly slower vesicle recycling. In addition, we did not detect any significant elevation of baseline intracellular Ca2+ concentration or Ca2+ channel conductance. Therefore, we concluded that the likely cause of presynaptic impairment is the interaction between soluble Aβ(1-40) and presynaptic membrane. Although moderate, this effect may be countable for neurite degradation or neuronal death as prolonged or dramatic surge of Aβ(1-40) in vivo can break down membrane homeostasis, especially at axonal terminals.

Disclosures: R.M. Lazarenko: None. **L.L. Rouhana:** None. **B. Edberg:** None. **X. Gao:** None. **Q. Zhang:** None.

Poster

843. Functional Modulation: Peptides and Amines

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Program#/Poster#:843.09/B51

Topic: B.07. Synaptic Transmission

Support: NSERC Discovery Grant

CIHR New Investigator

Title: Leptin depresses excitatory synaptic transmission onto dopamine neurons of the ventral tegmental area

Authors: *J. L. THOMPSON[1], S. L. BORGLAND[2]
[1]Anesthesiology, Pharmacol. and Therapeut., [2]Anesthesiology, Pharmacol. & Therapeut., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Leptin is an adipocyte-derived cytokine that can act in the brain to suppress feeding and maintain energy homeostasis. Leptin regulates food intake via activation of leptin receptors in the hypothalamus. Additionally, leptin activates its receptors in the ventral tegmental area (VTA), a critical site for neuroadaptations to rewarding stimuli, and decreases responding for food, heroin, and intracranial self-stimulation. While leptin can decrease intrinsic excitability of dopamine neurons in the VTA, it is unknown if leptin can modulate excitatory synaptic transmission in this brain region. Because plasticity of glutamatergic synapses onto VTA neurons can encode predictive information about reward, we tested how leptin modulates excitatory synaptic transmission onto dopamine neurons. Using whole-cell patch clamp electrophysiology in mouse midbrain slices, we tested the effects of leptin on firing and evoked

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) or N-methyl-D-aspartate receptor (NMDAR)-mediated excitatory post synaptic currents (EPSCs) onto VTA dopamine neurons. Consistent with previous reports, we found leptin (100 nM) modestly depressed firing rate. Interestingly, leptin (15 min, 100 nM) caused a long-lasting reduction of NMDAR EPSCs reaching a maximum of 26 ± 2.9 % . Similarly, leptin (15 min, 100 nM) inhibited AMPAR EPSCs to a maximum of 20 ± 3.1%. Taken together, these results indicate that while leptin modestly and transiently inhibits firing, it causes a long-lasting suppression of glutamatergic synaptic transmission onto VTA dopamine neurons. Leptin-induced weakening of synaptic strength may underlie the inhibitory effects of leptin on appetitive behavior for rewarding stimuli.

Disclosures: J.L. Thompson: None. **S.L. Borgland:** None.

Poster

843. Functional Modulation: Peptides and Amines

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:843.10/B52

Topic: B.07. Synaptic Transmission

Support: CIHR

AI-HS

Title: CCK triggers plasticity of GABA synapses in the dorsomedial hypothalamus through astrocytic ATP release

Authors: *K. M. CROSBY, J. S. BAINS, Q. J. PITTMAN
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Abstract: The dorsomedial hypothalamus (DMH) plays a key role in integrating and relaying satiety signals within neural networks that regulate energy intake and expenditure. We have previously demonstrated that GABA synapses in the DMH are plastic, such that food deprivation-induced activation of the stress response shifts the polarity of inhibitory plasticity from endocannabinoid-mediated LTD to nitric oxide-mediated LTP. Cholecystokinin (CCK), a satiety peptide and neuromodulator, suppresses food intake via actions in the DMH. Thus, we asked how CCK would impact the plasticity of DMH GABA synapses. We conducted experiments on horizontal hypothalamic slices containing the DMH from male Sprague-Dawley rats (postnatal day 21-30). Whole cell recordings were obtained from DMH neurons and an extracellular stimulating electrode was positioned inside the DMH to activate synaptic inputs to

these neurons. Consistent with previous findings, high frequency stimulation elicited a robust, long-term depression of GABA synapses. Interestingly, in the presence of CCK, the same stimulus resulted in a long term potentiation at these synapses.
This plasticity is mediated by CCK_B receptors that are localized on nearby astrocytes. Activation of these receptors triggers a functional up-regulation of the group I metabotropic glutamate receptor 5 (mGluR5), also localized on astrocytes. This results in release of the gliotransmitter, ATP, which subsequently acts on purinergic receptors at the presynaptic terminal to trigger an increase in GABA release. Overall, our results provide new insights into synaptic mechanisms that may contribute to the central
satiety actions of CCK in the DMH.

Disclosures: K.M. Crosby: None. **J.S. Bains:** None. **Q.J. Pittman:** None.

Poster

843. Functional Modulation: Peptides and Amines

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Topic: B.07. Synaptic Transmission

Support: FAPESP 01/138735

CAPES

Title: Hypothalamic projections from suprachiasmatic nucleus according to vasoactive intestinal peptide and vasopressin immunoreactivity in the Cebus apella monkey.

Authors: *L. M. CAMPOS[1], L. PINATO[2], M. I. NOGUEIRA[1,2]
[1]Anat., Inst. of Biomed. Sci., Saõ Paulo, Brazil; [2]Speech-Language and Hearing Therapy, São Paulo State Univ., Marília, Brazil

Abstract: The suprachiasmatic nucleus (SCN) of the hypothalamus, considered the endogenous master clock, in the most of studied species, contains a variety of different neurons that tend to form two major subpopulations within the nucleus, one that produces vasoactive intestinal peptide (VIP) and other vasopressin (VP). The immunoreactive (IR) fibers derived from the VIP and VP IR cells of the SCN present morphological characteristics that allow their specific tracking in long distances within the hypothalamus. In the present investigation we aimed first map VIP and VP IR terminals in hypothalamic areas of the primate *Cebus apella* using immunohistochemistry for the neuropeptide VIP and VP. Our second aim was the identification of hypothalamic recipient areas from SCN using the mapping distribution of fibers IR associated

with morphological analysis of these two neuroactive substances. The VIP and VP IR fibers from SCN showed a specific morphology compared to other sources. The fibers are thinner and varicose with bulbs and buttons dilated irregularly spaced along their trajectories. VIP and VP IR fibers with characteristics from SCN were identified in the rostral anterior hypothalamic area and medial preoptic area, laterally to the lateral hypothalamic area, and more caudally in SPZV and retrochiasmatic tuberal area. In the morphological analysis of the intrinsic neurons to SCN, we found VP IR neurons grouped in two neuronal subpopulations with different measures (area, perimeter) in the shell portion of the nucleus. There was a higher density of VP IR fibers located in areas at the same level of SCN in coronal sections. These fibers proceeding toward the posterior hypothalamus and the medial periventricular areas compared with the VIP IR fibers. We also verified the coexistence of VIP and VP IR fibers in the hypothalamic areas analyzed. The results indicate that in the primate *Cebus apella* there is a similarity in the pattern of distribution of VIP and VP fibers in the hypothalamic areas and also in areas recipients from SCN projections when compared with nocturnal rodent species described in the literature.

Disclosures: L.M. Campos: None. **L. Pinato:** None. **M.I. Nogueira:** None.

Poster

843. Functional Modulation: Peptides and Amines

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Topic: B.07. Synaptic Transmission

Support: NIH Grant GM088213

American Heart Association 0635421N

Title: Investigating the neuromodulatory role of VAV-1, a Rho family guanine nucleotide exchange factor, in Caenorhabditis elegans

Authors: A. FRY[1], J. LABOY[1], *K. NORMAN[2]
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Abstract: *C. elegans* VAV-1, a conserved guanine nucleotide exchange factor that activates Rho/Rac GTPases, is the sole worm homolog of mammalian Vav proto-oncogenes. VAV-1 regulates a variety of rhythmic activities in *C. elegans*, and we suspected a role in the rhythm of locomotion since *vav-1* null worms are hyperactive. By utilizing neuronal GFP constructs, we found that *vav-1* mutants do not have any gross developmental defects. Using genetic and pharmacological techniques, we have determined that VAV-1 acts specifically in the nervous

system to regulate neuronal excitation and locomotory speed. To learn what neurons express *vav-1*, we generated transgenic strains expressing fluorescent *vav-1* reporters, and have found that a limited number of neurons, comprising multiple subtypes, including an interneuron and both GABA- and cholinergic motor neurons, express these reporters. We then generated transgenic strains in which VAV-1 is re-expressed specifically in the different neuronal subtypes identified. By expressing VAV-1 only in cholinergic neurons, we found that the hyperactive locomotion of *vav-1* null worms can be rescued to wild type levels. Additionally, *vav-1* null worms display a more robust full body contraction than wild type animals upon activation of the cholinergic neurons using channel rhodopsin, suggesting that the cholinergic neurons are hyperexcitable in the *vav-1* null background. Our data suggest that VAV-1 affects the nervous system through dense core vesicles (DCVs), which carry neuromodulatory molecules such as insulins, neuropeptides, and biogenic amine neurotransmitters. We found that VAV-1 is upstream of UNC-31/CAPS (Calcium-activated protein required for secretion), a known regulator of DCV release. Moreover, we have determined that EGL-3/PC2 (Proprotein convertase 2), required for neuropeptide processing, is not downstream of VAV-1. These results together suggest that VAV-1 exerts its effect on the nervous system by regulating a molecule(s) secreted by DCVs that is not a neuropeptide. We have preliminary evidence that VAV-1 negatively regulates insulin-like signaling-mediated processes, and thus are currently investigating whether *vav-1* null worms have altered insulin release from the nervous system. Specifically, we are investigating whether deregulated insulin release from cholinergic neurons is responsible for the *vav-1* null worm hyperactivity and pharmacological response. Our studies provide novel evidence for Vav proteins in regulation of dense core vesicle-released molecules, and may reveal an interesting role for insulins in modulating neural activity.

Disclosures: A. Fry: None. **J. Laboy:** None. **K. Norman:** None.

Poster

843. Functional Modulation: Peptides and Amines

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Topic: B.07. Synaptic Transmission

Support: MDA 175744 (MMF)

NIH R01 5064263 (MMF)

Title: Modulation of neuromuscular excitability by *C. elegans* neuropeptides

Authors: *D. TOUROUTINE[1], R. BHATTACHARYA[2], M. FRANCIS[2]
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Abstract: Neuronal excitability can be shaped by the actions of a host of neuromodulators including neuropeptides. Neuropeptides are known to play important roles in intercellular signaling across a wide range of organisms and cellular contexts. However, the mechanisms by which neuropeptides regulate cellular excitability remain poorly understood. We have begun to explore mechanisms underlying neuropeptide signaling using the model organism *C. elegans*, in which powerful genetic tools can be paired with electrophysiological and behavioral analysis. To identify potential neuromodulators, we generated a mutant form of the levamisole receptor (L-AChR), an ionotropic acetylcholine receptor (iAChR) responsible for excitatory signaling at the neuromuscular junction, that carries a gain-of-function leucine to serine mutation. Specific expression of the L-AChR(*gf*) transgene in muscles caused prolonged postsynaptic current responses. Importantly, L-AChR(*gf*) animals also exhibit easily identifiable locomotory phenotypes, including decreased speed and exaggerated muscle flexures during movement. We found that these behavioral effects required the activity of genes involved in dense vesicle biogenesis and secretion. To test whether neuropeptides may be required for the behavioral effects of L-AChR(*gf*) expression, we reduced the levels of specific pro-neuropeptide family members using RNAi. We found that five neuropeptide-like genes, including the cholecystokinin-like peptide NLP-12, are required to achieve the effects of chronic muscle hyperactivation in our model. The kinetics and amplitude of photo-evoked postsynaptic currents were not changed by loss of NLP-12, suggesting that this neuropeptide does not act directly on iAChRs to alter their function or localization. We are currently conducting additional electrophysiological experiments to elucidate mechanisms of action for NLP-12 and the other peptides identified from our screen, and the results of these experiments will be presented. Our results to date indicate that a network of neuropeptide signals act to modulate neuromuscular excitability and locomotion. By characterizing the neuropeptides and signaling pathways involved in motor control, we hope to elucidate conserved mechanisms for neuropeptide regulation of cellular excitability that will be relevant for organisms ranging from nematodes to humans.

Disclosures: D. Touroutine: None. **R. Bhattacharya:** None. **M. Francis:** None.

Poster

844. Pharmacological Modulation: General

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:844.01/B56

Topic: B.07. Synaptic Transmission

Support: AHFMR

CIHR

Title: Prenatal lipopolysaccharide-induced immune activation alters nociception in the adult rat

Authors: *S. A. RANA, A. C. KENTNER, Q. J. PITTMAN
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Abstract: Peripheral inflammation experienced during early development can have profound effects on subsequent pain sensitivity. Lipopolysaccharide (LPS)-induced immune activation during the prenatal and neonatal periods of life produces long-term changes in febrile responses to a similar immunogen exposure in adulthood. Adult LPS exposure, which induces sickness behaviors, also produces hyperalgesia. In fact, previous research from our lab has shown that rats exposed to LPS on postnatal day 14 displayed enhanced baseline responses to nociceptive stimuli and elevated spinal cord cyclooxygenase-2 (COX-2); they also failed to mount the expected hyperalgesic response following adult LPS exposure and showed a reduction in spinal cord COX-2 expression compared with neonatally saline-treated rats. To determine whether prenatal immune activation will have similar effects on adult pain sensation, adult male Sprague-Dawley rats, born to dams exposed to 100 µg/kg LPS, 50 µg/kg LPS, or saline on gestational day (GD) 12, were examined for nociceptive responses to thermal and mechanical stimuli using the Hargreaves' Method (plantar test) and von Frey filament test, respectively; as well as, tumor necrosis factor (TNF) -α and COX-2 mRNA expression in the hypothalamus, and TNF-α, COX-2 and cannabinoid receptor type 1 (CB1) mRNA expression in the dorsal horn of the spinal cord, following adult LPS challenge. Preliminary results suggest, unlike neonatally-treated rats, GD 12 LPS-treated offspring do not exhibit altered baseline nociceptive (behavioral) responses and spinal COX-2 levels. Further, basal hypothalamic TNF-α and COX-2 transcript levels were elevated in the GD12 LPS-treated rats; and spinal TNF-α and COX-2 levels were elevated in GD12 LPS-treated rats only following adult LPS challenge compared to GD12 saline-treated rats. Spinal CB1 levels remained unaffected by both prenatal- and adult- LPS exposure. The present findings suggest that not only is adult pain sensation differentially affected by timing of early immune system activation, but also patterns of TNF-α and COX-2 mRNA expression differ across brain regions.

Disclosures: S.A. Rana: None. **A.C. Kentner:** None. **Q.J. Pittman:** None.

Poster

844. Pharmacological Modulation: General

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:844.02/B57

Topic: B.07. Synaptic Transmission

Support: CIHR

Title: The effect of prenatal maternal inflammation on the cerebellum of mouse offspring

Authors: *T. AAVANI[1], R. HAWKES[2], Q. J. PITTMAN[1]
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[2]Cell Biol. and Anat., Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Autism Spectrum Disorders (ASDs) are characterized by abnormal development in social, language, and sensorimotor behaviours. Systemic infection during pregnancy is known to be a possible non-genetic factor for the development of ASDs in offspring. Individuals with ASD have been shown to have not only cognitive deficits, but also deficits and delays in movement in which cerebellar function might play an important role. In previous studies, prenatal exposure to a viral-like agent has indicated Purkinje Cell (PC) loss in the cerebellum of offspring. The present study was designed to examine the effect of maternal inflammation on the organization of PCs as well as the behaviour of the offspring. Pregnant C57Bl/6 mice were injected with the viral mimetic poly(I:C) (20 mg/kg) or saline on gestational days 13, 14 and 15. Male and female pups from the prenatally-treated dams were sacrificed on postnatal day 18 for wholemount immunohistochemistry which was carried out using a monoclonal antibody raised against zebrin II (ZII). In another group of pups, rotarod and social interaction tests were tested on day 30. Our findings revealed that the pattern of PC stripes in the cerebellum changed subtly as a result of the prenatal immune challenge. Independent of sex, offspring of poly(I:C)-exposed dams showed an increase in the distance between the ZII immunopositive PC stripes in the anterior vermis relative to controls. In addition, the lateral extent of the cerebellum as well as the width of the ZII negative stripes were greater in the offspring of poly(I:C)-exposed mice. Also, the number of PCs revealed by the pan-PC marker calbindin and the circumference of lobules III and VII were also increased in the poly(I:C) group. Furthermore, these animals had motor deficits as revealed by the rotarod test. However, there was no significant difference in the social interaction test between the offspring of the poly(I:C)-treated dams compared to the control group. These preliminary findings therefore suggest that cerebellar development is abnormal in the offspring of dams that have been exposed to prenatal immune challenge.

Disclosures: T. Aavani: None. **R. Hawkes:** None. **Q.J. Pittman:** None.

Poster

844. Pharmacological Modulation: General

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:844.03/B58

Topic: B.07. Synaptic Transmission

Title: The effects of early life peripheral immune challenge on amygdala synaptic functioning

Authors: *A. SZUBRA
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Abstract: Compelling evidence now suggests that early life immune challenges during key developmental stages can have long lasting effects on brain excitability, neurochemistry, and behaviour. Experimentally, perinatal and neonatal rat exposure to the inflammatory molecule lipopolysaccharide (LPS) has been shown to result in long term alterations in memory formation and fear response. Of particular interest in the study of amygdala functioning is the latter. There exists a growing body of work examining the way in which the amygdala shapes its circuitry in conjunction with fear association formation. As there may be synaptic changes in the amygdala as a consequence of neonatal inflammatory insults, the present research sought to examine the effect of early life peripheral inflammation on amygdala synaptic functioning in order to provide a mechanistic explanation for the ostensible behavioural changes previously described. Young C57Bl/6 rats were injected with LPS (100 µg/kg) or saline on post natal day 14, and sacrificed at 1 month of age. Whole cell voltage and current clamp recordings were taken in basolateral amygdala neurons while stimulating afferent pathways to these cells. Cell responses between neonatal LPS- and saline-pretreated animals were compared. Our findings revealed that cells in LPS-treated rats are more excitable with alerted glutamatergic inputs and more easily depolarized cell membranes when compared with saline treated controls. These findings provide a basis for future work, which will examine the mechanistic basis for these changes.
Supported by CIHR
Keywords: Inflammation, neonatal, amygdala, synaptic

Disclosures: A. Szubra: None.

Poster

844. Pharmacological Modulation: General

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:844.04/B59

Topic: B.07. Synaptic Transmission

Support: Kumamoto Health Science Univ.

Title: *In vivo* transport of type A2 botulinum toxin

Authors: *M.-C. SHIN, N. AKAIKE
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Abstract: Type A botulinum toxin blocks not only ACh release from motor nerve terminals but also central synaptic transmissions such as glutamate, noradrenaline, dopamine, ATP, GABA, and glycine. In addition, the antero- and retrograde axonal transports of the neurotoxins via either motor or sensory neurons from peripheral tissues to CNS were reported. Futhermore, type A2 neurotoxin, A2NTX, injected into the rat left foreleg muscles was transported to the contralateral ones, suggesting the neurotoxin was transported retrogradely to spinal neurons through motor neuron of toxin-injected (ipsilateral) muscles side, and then transcytosed to contralateral motor neurons within spinal cord, after that the toxin was transported anterogradly via motor nerve from the contralateral spinal neurons to the unilateral soleus muscles as a possible explanation. Present study clearly showed that the toxin injection to rat left soleus muscles induced bilateral muscle relaxization in a dose-dependent fashion, although the contralateral muscle relaxization appeared after the complete inhibition of toxin-injected ipsilateral muscles. The muscle relaxation was faster and stronger in A2NTX than in A1LL. A1LL (BOTOX) was transported via both pathways of nerve and blood circulation to the contralateral muscle, but the contribution of axonal transportation was greater than blood. On the other hand, A2NTX was mainly transported to contralateral muscles via blood. In addition A1LL was more easily transcytosed to contralateral spinal neurons than A2NTX. It is also demonsterated that neurotoxins A1LL and A2NTX were carried from peripheral to CNS and vice versa by not only both antero- and retrograde axonal transports through either motor or sensory neurons but also blood circulation. 1

Disclosures: M. Shin: None. **N. Akaike:** None.

Poster

844. Pharmacological Modulation: General

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:844.05/B60

Topic: B.07. Synaptic Transmission

Title: Intrathecal administration of botulinum toxin A: Antiallodynic action and inhibition of excitatory synaptic transmission in the spinal dorsal horn

Authors: K. NEMOTO[1], *S. YAMAGUCHI[1], R. MATSUZAWA[1], T. TAKASUSUKI[1], T. TERASHIMA[1], T. FUKUSHIMA[2], T. OTSUBO[2], Y. HORI[2]
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Abstract: Botulinum toxin type A (BoNT1A) is known to bind the presynaptic nerve terminal where BoNT1A is internalized into the nerve terminal, cleave synaptosomal-associated protein 25 (SNAP-25), which is a component of the SNARE complex. Thus, BoNT1A interferes with vesicle docking thereby inhibiting transmitter release from the presynaptic nerve terminal. It has recently been shown that intrathecal (IT) administration of BoNT1A attenuates inflammatory and neuropathic pain. In the present experiments, we have investigated the effects of IT BoNT1A on neuropathic pain induced by partial ligation of the sciatic nerve in adult ICR mice, with the final aim of clarifying the synaptic mechanisms of BoNT1A's antinociceptive actions. The sciatic nerve was partially ligated under halothane anesthesia. IT injection of BoNT1A was made according to the method described by Hylden and Wilcox (1980). Tactile allodynia was assessed by counting the frequency of withdrawal responses to the stimulation by von Frey filaments. IT injection of BoNT1A prior to nerve ligation did not exhibit apparent antiallodynic efficacy: in contrast, IT injection of BoNT1A one day after nerve ligation significantly attenuated allodynia and this attenuation was maintained for about ten days. In some experiments, the spinal cord was removed under ketamine-xylazine anesthesia and spinal slices were prepared. Tight-seal whole-cell recordings were made from neurons located in the superficial dorsal horn. In the presence of tetrodotoxin, bicuculline, and strychnine,spontaneously occurring miniature excitatory postsynaptic currents (mEPSCs) were recorded. The frequency of mEPSCs was found to be significantly lower in nerve-ligated BoNT1A-injected mice than in nerve- ligated saline-injected mice. The amplitudes of mEPSCs were not significantly different between these two groups. BoNT1A is known to cleave the SNAP-25 protein that is essential for transmitter release. Our preliminary Western blot analysis of spinal dorsal horn protein exhibited that the expression level of SNAP-25 was only slightly lower in nerve-ligated BoNT1A-injected mice than in nerve-ligated saline-injected mice. Taken all together, the present results seem to indicate that at least some part of antinociceptive effects of BoNT1A is produced by presynaptic inhibition of excitatory synaptic transmission in the spinal dorsal horn and that mechanisms other than cleavage of SNAP-25 are also involved in antinociceptive effects of intrathecally administered BoNT1A.

Disclosures: K. Nemoto: None. **S. Yamaguchi:** None. **R. Matsuzawa:** None. **T. Takasusuki:** None. **T. Terashima:** None. **T. Fukushima:** None. **T. Otsubo:** None. **Y. Hori:** None.

Poster

844. Pharmacological Modulation: General

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:844.06/B61

Topic: B.07. Synaptic Transmission

Support: Dokkyo Medical University Intramural Research funds

Title: The effects of HPC1/Syntaxin 1A gene knockout on the general anesthetic actions of sevoflurane

Authors: S. KOKUBU[1], R. MATSUZAWA[1], K. NEMOTO[1], T. FUKUSHIMA[1], S. YAMAGUCHI[1], *M. MAEKAWA[1], T. FUJIWARA[2], K. AKAGAWA[2], Y. HORI[1]
[1]Dept Physiol Biol Information, Dokkyo Med. Univ. Sch. Med., Tochigi, Japan; [2]Dept of Cell Physiol, Kyorin Univ. Sch. of Med., Tokyo, Japan

Abstract: We reported at the 2010 Society for Neuroscience meeting that the minimum alveolar concentration (MAC) of the volatile anesthetic sevoflurane in HPC-1/Syntaxin 1A-knockout (KO) mice was significantly greater than that in wild-type (WT) mice, although the precise mechanisms for this difference was unclear. Additionally, it is well known that HPC-1/Syntaxin 1A is a membrane protein that plays an integral role in the exocytotic release of neurotransmitter. Based on these, we hypothesize that the activity of HPC-1/Syntaxin 1A affects the potency of general anesthetics and that general anesthetics produce the anesthetic state via interaction with exocytotic machinery in the presynaptic terminals. To evaluate these hypotheses, we investigated the effects of sevoflurane on excitatory synaptic transmission in the spinal superficial dorsal horn.
We used adult (6- to 8-week-old) male KO mice and WT mice. Under anesthesia by ketamine and xylazine, the lumbar spinal cord was removed, and cross-sectional slices were prepared. Tight-seal whole-cell recordings were made from neurons located in the superficial dorsal horn. Excitatory postsynaptic currents (EPSCs) were evoked by electrical stimulation of the dorsolateral funiculus. The recordings were made at the holding potential of -70 mV in the presence of strychnine (1 mM) and bicuculline (10 mM). The perfusates were bubbled with sevoflurane through the use of a calibrated vaporizer. In order to measure the aqueous phase concentration of sevoflurane during experiments, 2 ml of the perfusates were sampled from the recording chamber. Chloroform was added as the solvent for liquid-liquid extraction, and gas chromatographic measurements of sevoflurane were performed.
Sevoflurane at the concentration of 0.5 mM reduced the amplitude of EPSCs to the same extent in both WT and KO mice (40-50% of the control). Sevoflurane at the concentration of 0.75 mM and 1.0 mM reduced eEPSC amplitude to 20-30% and 15-25% of the control, respectively, in WT mice. In contrast, the inhibitory effects of 0.75 mM and 1.0 mM sevoflurane were significantly weakened in KO mice (45-50% and 25-35% of the control, respectively). The

paired-pulse ratio of eEPSCs was consistently increased during the inhibition of the amplitude of eEPSCs.
These results suggest that sevoflurane produces the anesthetic state, at least in part, via interaction with exocytotic machinery in the presynaptic terminals and that the activity of HPC-1/Syntaxin 1A affects the actions of general anesthetics on excitatory synaptic transmission in the spinal dorsal horn and thereby affects the potency of general anesthetics.

Disclosures: S. Kokubu: None. **R. Matsuzawa:** None. **K. Nemoto:** None. **T. Fukushima:** None. **S. Yamaguchi:** None. **M. Maekawa:** None. **T. Fujiwara:** None. **K. Akagawa:** None. **Y. Hori:** None.

Poster

844. Pharmacological Modulation: General

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:844.07/B62

Topic: B.07. Synaptic Transmission

Support: Neuroscent Pty Ltd

Title: Effect of green odours on stress induced up regulation of sympathetic neurotransmission.

Authors: *J. M. SODEN, N. A. LAVIDIS
Sch. of Biomed. Sci., The Univ. of Queensland, St Lucia, Australia

Abstract: Chronic stress results in the up regulation of neurotransmission from sympathetic varicosities. Previously, we reported that specific plant derived odours such as *cis*-3-hexen-1-ol (hexenol), *trans*-2-hexenal (hexenal) and α-pinene are effective at reducing the stress induced increases in sympathetic neurotransmission. In the present study, we examined the effectiveness of the combinations of these plant derived odours in reducing the stress induced increases in sympathetic neurotransmission. Six week old Balb/c mice were exposed for 11 days to minimal stress or concurrent (predator plus social deprivation) stress with and without either vehicle (tri-ethyl citrate), green odour (hexenol (0.03%) with hexenal (0.03%) in tri-ethyl citrate), or Praescent™ (hexenol (0.03%), hexenal (0.03%) and α-pinene (0.015%) in tri-ethyl citrate). The odours were delivered by spraying 0.2 mLs on the bedding material, 3 times per day. N = 16 for each treatment. Following treatment, animals were sacrificed by cervical fracture and both vasa deferentia were dissected. The prostatic end of the vas deferens was stimulated. Sympathetic varicosities were visualised using DiOC2(5)-fluorescence. Excitatory junction currents (EJCs) were recorded using focal extracellular recordings with 10 to 15 µm tip diameter electrodes. The mean EJC amplitude (mEJC) and success rate of recording EJCs was determined. The mEJC for:

minimal stress was 37±16μV; concurrent stress was 118±27μV; concurrent stress with Praescent™ was 16±5μV; and concurrent stress with green odour was 28±9μV. There was a significant increase ($P<0.01$) in mEJC between minimal and concurrent stress of 311±41%. Both Praescent™ and green odour significantly reduced the stress induced increases in mEJC shown in the concurrent stressed vas deferens, ($P<0.01$). Our findings indicate Praescent™ and green odour are effective in reducing the chronic stress induced increases in sympathetic neurotransmission.

Disclosures: J.M. Soden: None. **N.A. Lavidis:** Consultant/Advisory Board; Consultant for Neuroscent Pty Ltd.

Poster

844. Pharmacological Modulation: General

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:844.08/B63

Topic: B.07. Synaptic Transmission

Support: FAPESP

CAPES

CNPq

Title: Effects of A2a adenosine receptor knockdown on neuronal nitric oxide synthase (nNOS) enzyme expression levels in primary cell culture from dorsal medulla oblongata of neonate spontaneously hypertensive and normotensive rats.

Authors: *M. A. COSTA[1], J. P. MATSUMOTO[1], D. C. CARRETTIERO[2], D. R. FIOR-CHADI[1]
[1]Biosci. Institute/University of São Paulo, São Paulo, Brazil; [2]Univ. of ABC, Brazil, Brazil

Abstract: Introduction: Adenosine and nitric oxide (NO) acts as modulator of sympathetic activity, with modulatory action on cardiovascular control. The relationship between NO and adenosine in the peripheral system is well established, but this relation in the nucleus tract solitary (NTS) is still being studied. The purpose of the present study was to analyze the effect of A2a knockdown on the nNOS mRNA in cultured cells from the dorsomedial medulla oblongata of normotensive (WKY) and spontaneously hypertensive (SHR) rats.
Methods: All the procedures were performed in accordance with the Institutional Guidelines for Animal Experimentation (CEA/IB-USP). Cell cultures were made using the dorsomedial

medulla oblongata region of one-day old WKY and SHR (n=15). Cells cultures were infected by GIPZ lentiviral vector containing shRNAmir to A2aR and non-silencing GIPZ lentiviral shRNAmir as control. After three days in culture, cells of the medulla oblongata of WKY and SHR were exposed at approximately $1x10^8$ TU/ml for five hours. Three days after the infections, the RNA was extracted for real time PCR analysis of both A2aR and nNOS mRNA levels. Results were analyzed by unpaired Students t test and a p-value<0.05 was considered to indicate statistically significant differences.

Results: Results are expressed as fold-change ± SEM. Although it is difficult to infect primary neuronal culture, this study demonstrated a percentage up to thirty per cent of infected cells in both strains. This infection level by ADORA2a shRNA produced a significant knockdown of A2aR mRNA in both strain (WKY: 0.75±0.03 and SHR: 0.60±0.09, p<0.05), followed by a concomitant reduction in the levels of the nNOS enzyme when compared to non-silence control (WKY: 0.77±0.03 and SHR: 0.69±0.04, p<0.05).

Conclusion: We suggest that in primary neuronal culture from dorsal medulla oblongata of neonate spontaneously hypertensive and normotensive rats, the effects of A2aR might be mediated by NO, at least in part. These results reinforce the existence of a cross-talk between A2aR and NO in the central control of cardiovascular function.

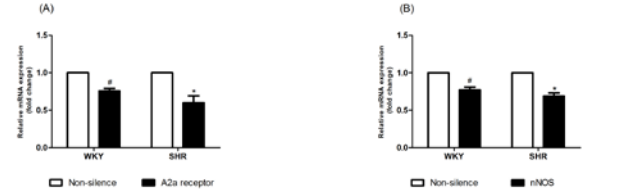


Fig.1. Quantitative analysis adenosine A2a receptor (A) and nNOS (B) mRNA expression evaluated in primary culture cells of the dorsomedial medulla oblongata of SHR and WKY rats after knockdown of A2a adenosine receptor. Both A2a adenosine receptor and nNOS mRNA expression were decrease when compared to respective control. Values are shown as mean ± SEM (n = 3 for mRNA experiments). # p<0.05 compared with WKY non-silence control and * p<0.05 compared with SHR non-silence control. Student's t test was employed in the statistical analysis.

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Disclosures: M.A. Costa: None. **J.P. Matsumoto:** None. **D.C. Carrettiero:** None. **D.R. Fior-Chadi:** None.

Poster

844. Pharmacological Modulation: General

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:844.09/B64

Topic: B.07. Synaptic Transmission

Support: R25 GM072643 (JP)

RL1-MH083269 (AL)

Title: Effects on NMDA receptor activity by positive allosteric modulation of mGluR5 receptors in dysbindin-1 null mutant mice.

Authors: *J. I. PEÑA BRAVO[1], J. M. ROOK[2], P. J. CONN[2], A. LAVIN[1]
[1]Neurosci., Med. Univ. of South Carolina, Charleston, SC; [2]Pharmacol., Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Understanding the mechanism behind N-methyl-D-aspartate (NMDA) receptor hypofunction in prefrontal cortex pyramidal neurons (PFC) is important for the development of novel treatments for schizophrenia and related psychiatric disorders. The activity of cortical pyramidal neurons is regulated by fast-spiking (FS) GABA interneurons. Most recent advances at modulating NMDA receptor activity are the use of allosteric modulators of mGluRs. In our study we are specifically interested in testing the putative enhancing effects of VU0360172, a mGluR 5 positive allosteric modulator (PAM), on NMDA receptor activity. In previous studies done in our lab we have used a mouse carrying a null mutation in the dtnbp I gene as a model for schizophrenia. This gene encodes for dysbindin, a protein involved in vesicular trafficking and exocytosis and expressed in glutamate producing neurons of the PFC.
In order to examine excitatory neurotransmission, we performed whole-cell patch clamp recordings of excitatory post synaptic currents (EPSCs) in brain slices from male wild type (WT), heterozygous (HET) and mutant (MUT) mice (35-60 days of age). We evaluated the contribution of NMDA receptors to the amplitude of evoked excitatory postsynaptic currents (eEPSCs) and amplitude and frequency of spontaneous excitatory postsynaptic currents (sEPSCs) and compared between genotypes. To control for any GABA receptor contribution we added picrotoxin (50 μM), a selective GABA blocker, to a group of recordings. In our preliminary results, we found a trend toward an increase of NMDA-mediated eEPSCs in HET mice following VU0360172 administration; this was not detected in WT or MUT. We detected a significant main effect on NMDA-mediated eEPSC amplitude between the genotypes. Interestingly, when only AMPA currents were blocked, but GABA and NMDA currents were intact, we found that VU0360172 significantly decreased eEPSCs amplitude in MUT mice, suggesting that the drug might be acting on mGluR5 receptors coupled to NMDAR located in GABA neurons.
Our results suggest that the mGluR5 PAM increases NMDA currents in cases in which the population of NMDA receptors is still intact (HET), but also seems to modulate NMDA currents in GABAergic neurons. This mechanism supports results shown by Xi, D. et al (2009) who has demonstrated a differential distribution of NR2A-containing NMDA receptors, known to have distinct agonist sensitivity and deactivation rates, in GABA interneurons in the PFC.

Disclosures: J.I. Peña Bravo: None. **J.M. Rook:** None. **P.J. Conn:** None. **A. Lavin:** None.

Poster

844. Pharmacological Modulation: General

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:844.10/B65

Topic: B.07. Synaptic Transmission

Support: Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.

Title: Comparative studies of nine tertiary oximes on reactivation of OP nerve agent-inhibited AChE in the CNS

Authors: *T.-M. SHIH, I. KOPLOVITZ, R. KAN, J. MCDONOUGH
US Army Med. Res. Inst. Chem Defn, Aber Prov Grd, MD

Abstract: Organophosphate nerve agents irreversibly inhibit the enzyme acetylcholinesterase (AChE), which leads to an excess of the cholinergic neurotransmitter acetylcholine in the synapses, causing numerous toxic effects, such as hypersecretions, tremors, convulsions, respiratory distress and death. The current nerve agent treatment regimen includes 2-pralidoxime (2-PAM) to reactivate inhibited AChE. The quaternary structure of this oxime, however, does not allow it to cross the blood brain barrier to reactivate brain AChE and to mitigate CNS toxicity. We have shown earlier that the tertiary oxime monoisonitrosoacetone (MINA) provided some AChE reactivation in the brain, enhanced survival and mitigated the seizure activity following nerve agent exposure. In this study, the *in vivo* reactivating capabilities of several new tertiary oximes, diacylmonoxime (DAM), N,N-diethyl-3-(2-(hydroxyimino) acetoxy) propan-1-aminium chloride (DHAP), RS194B, JK-3-38, SWRI48A, SWRI53A, Pro-2-PAM, and diethyxime, were compared to each other and to MINA, following subcutaneous nerve agent exposure. Guinea pigs were challenged with a 1.0 x LD_{50} dose of sarin (GB), cyclosarin (GF) or VX 15 min after administration of atropine methylnitrate (2.0 mg/kg, im), followed 15 min later by a test dose of the oxime. Four to eight doses (ranging from 5.6 to 240.0 mg/kg, im) of each oxime were tested. The animal was euthanized 45 min after oxime treatment; blood was collected and target tissues (brain regions, diaphragm, heart, skeletal muscle) were harvested. AChE activity was measured with the Ellman assay. Of these tertiary oximes, pro-2-PAM and JK-3-38 enhanced the toxicity of sarin at doses above 25 and 35.5 mg/kg, respectively. In peripheral tissues pro-2-PAM provided the greatest AChE reactivation (21-64%), while SWRI53A showed reactivation (15%) only at the highest dose (180 mg/kg), with the rank order of pro-2-PAM > RS194B = JK-3-38 > DHAP > MINA > diethyxime > DAM = SWRI48A = SWRI53A. In the CNS tissues, MINA displayed the highest capacity to reactivate AChE (2-31%), while DAM provided minimum reactivation (3%) even at the highest dose (129 mg/kg), with the rank order of MINA > pro-2-PAM > DHAP > SWRI48A = SWRI53A = RS194B = diethyxime > JK-3-38 = DAM. Thus, the findings suggest that like the quaternary oximes,

tertiary oximes show different reactivation capacities against AChE inhibited by specific nerve agent in the periphery or in the CNS. The protective efficacy of these tertiary oximes against nerve agent exposure is currently under investigation.

Disclosures: T. Shih: None. **I. Koplovitz:** None. **R. Kan:** None. **J. McDonough:** None.

Poster

845. LTP: Physiology and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:845.01/B66

Topic: B.08. Synaptic Plasticity

Support: 5520177 AHW

Title: The role of Pcp4 in synaptic transmission of the CA2 region of the hippocampus

Authors: *A. N. PETERSEN, N. Z. GERGES
Cell Biology, Neurobio. & Anat., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: Purkinje cell protein 4 (Pcp4) is a calmodulin-binding protein highly expressed in the CA2 region of the hippocampus but absent from the CA1 and CA3. Calmodulin binding proteins are important for synaptic plasticity due to their ability to regulate the activation of Ca2+/CaM-dependent enzymes (e.g. calcineurin and CaMKII) which modulate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) function and trafficking. Pcp4 is required for long-term depression (LTD) in Purkinje cells, as well as cerebellar-dependent learning. However, the role of Pcp4 in hippocampal synaptic plasticity and learning and memory is unknown. We have investigated the role of Pcp4 in CA2 synaptic transmission using a combination of molecular biology and electrophysiology. We find that AMPAR-mediated synaptic transmission is regulated by Pcp4 in a bidirectional manner, with acute overexpression causing a reduction in AMPAR responses while its knockdown enhances AMPAR responses. These results suggest a role for Pcp4 in the regulation of AMPAR in the hippocampus.

Disclosures: A.N. Petersen: None. **N.Z. Gerges:** None.

Poster

845. LTP: Physiology and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:845.02/B67

Topic: B.08. Synaptic Plasticity

Support: CIHR

CRC

FRQS

Title: Long-term plasticity in identified classes of somatostatin- and parvalbumin - expressing interneurons in the mouse hippocampus

Authors: *O. C. VASUTA[1], I. LAPLANTE[1], K. ELAYOUBI[1], J. HUANG[2], J.-C. LACAILLE[1]
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Abstract: The hippocampus contains a heterogeneous population of interneurons (INs), which play key roles in modulating the physiology of the local circuits, enabling them to engage in coherent activities. The behavioral correlates of these activities in hippocampus are memory acquisition and space representation. The role of each type of IN may be specifically shaped through plasticity mechanisms at their synapses, and we have previously described a hebbian form of long-term potentiation (LTP) induced through mGluR1, at the synapses between CA1 pyramidal cells and oriens/alveus INs. We now examine more specifically which classes of INs express this plasticity taking advantage of two lines of mice, the somatostatin (SOM)-Cre and parvalbumin (PVALB)-Cre lines, crossed with Flox-EYFP reporter mice. In hippocampus, YFP-labeled SOM-INs are mostly detected in CA3 and CA1 oriens/alveus layers and in DG hilus, while YFP-labeled PVALB-INs are mostly present in or around the pyramidal and granular layers. Immunofluorescence analysis in CA1 area of SOM-Cre mice revealed that all YFP-labeled cells expressed SOM, while less than 5% of them were immunoreactive for PVALB. Similarly, in the PVALB-Cre mice, all YFP-labeled CA1 cells were immunoreactive for PVALB, but only 5% of them for SOM. By using electrophysiological recordings and minimal stimulation in YFP-expressing INs of acute hippocampal slice from these mice, we find that LTP is induced by theta-burst stimulation (TBS) paired with depolarization in CA1 SOM-INs, but not in PVALB-INs. Moreover, LTP did not occur when the induction protocol consisted of either TBS or depolarization alone. LTP induced by the paired protocol is mGluR1-dependent and was blocked by an mGluR1 but not an mGluR5 antagonist. A similar pairing protocol induces a depression of synaptic transmission in PARV-INs. These data indicate that theta burst stimulation induces hebbian LTP through the activation of mGluR1s specifically at the excitatory synapses of somatostatin-expressing interneurons in CA1 hippocampus, and provides the

background for further investigating the role of this cell type-specific plasticity in regulating the local neuronal circuit and hippocampal function.

Disclosures: O.C. Vasuta: None. **I. Laplante:** None. **K. Elayoubi:** None. **J. Huang:** None. **J. Lacaille:** None.

Poster

845. LTP: Physiology and Behavior

Location: Hall F-J

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Program#/Poster#:845.03/B68

Topic: B.08. Synaptic Plasticity

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Drs. Chantal and Peritz Scheinberg Research Fund

Title: Chronic nicotine hinders hippocampus-dependent learning and memory in female rats

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Abstract: The rise in the number of female smokers is a major public health concern in the United States. Currently 22 million (22 %) American women smoke cigarettes and even though the detrimental effects of smoking-derived nicotine on health are well-established, giving up a smoking habit is more difficult for women than for men. Women metabolize nicotine faster than men and that influences smoking behavior, causing more dependence and increasing the associated risks. Most prior studies to understand the mechanisms of nicotine dependence were carried out on male experimental animals and were focused on identifying the effects of nicotine on its receptors; however, effects of nicotine unique to females warrant investigation. Recently, we demonstrated that chronic nicotine exposure attenuates short-term synaptic plasticity in the hippocampus of female rats. Hippocampus plays important roles in long-term memory and spatial navigation; therefore, we further investigated effects of chronic nicotine on hippocampus-dependent learning and memory. Twenty female rats were randomly assigned to either a nicotine

(4.5 mg/kg/day) or saline treatment. Starting on day 16 of nicotine/saline treatment, rats were tested for their hippocampus-dependent cognitive capabilities using the Morris water maze, using a 7-day testing paradigm. The first 4 days measured learning capabilities (learning the position of a platform), the 5th day measured memory (memory of platform location when platform is removed), and the 6th and 7th days measured working memory (learning a new platform location). The results are presented as Mean ± SEM of latency time in seconds. During four days of learning paradigm, nicotine exposed rats took longer time to find platform (40±3s, 37±4s, 39±3s and 41±2s) as compared to saline (36±4s, 31±4s, 31±5s and 26±2s). The results of this test demonstrated significant difference in latency period of nicotine and saline-treated female rats on the 4th day ($p < 0.001$). The outcome of the long-term memory testing performed on fifth day demonstrated that there was a distinct difference between the saline and the nicotine-exposed groups. Similarly, the working memory task demonstrated that saline-exposed rats performed better the nicotine-exposed rats. The nicotine-exposed rats took 38 and 36 seconds to find the platform on the first and second trial respectively, as compared to 36 and 29 seconds in saline group. Overall, the significant increase in latency period in the nicotine-exposed group suggested deficits in spatial learning and memory performance.

Disclosures: R. Borges-Garcia: None. **M. Behairy:** None. **M. Perez-Pinzon:** None. **H. Bramlett:** None. **A.P. Raval:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Title: The role of nogo-a in synaptic plasticity and motor (re-) learning

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Abstract: Nogo-A is a well described inhibitor of axonal outgrowth in the CNS. However, the protein may have additional physiological functions which are not yet well characterized. Although the main expression of Nogo-A in the adult CNS is in oligodendroctyes, Nogo-A is also present in subpopulations of neurons and at synapses, e.g. in the hippocampus and the neocortex. In this study, we analyze the influence of the neurite outgrowth inhibitor Nogo-A on

synaptic transmission and long-term synaptic plasticity (long-term potentiation; LTP) by applying function blocking antibodies against Nogo-A or a blocking peptide of the Nogo receptor NgR1 (NEP 1-40) on acute cortical slices of the rat primary motor cortex. Both treatments enhanced LTP significantly. Subsequently, we trained antibody-treated animals in a motor learning task which requires skilled forelimb reaching. Anti-Nogo-A treated rats showed faster learning of the task and reached higher scores than the control antibody infused animals. These results show a new role of Nogo-A as a negative regulator of synaptic plasticity in the sensory-motor cortex and as an influential regulatory factor for motor learning *in-vivo*. The consequences of these findings for functional recovery and rehabilitation after CNS injury will be analysed in further steps.

Disclosures: A. Zemmar: None. **L. Schnell:** None. **M. Gullo:** None. **H. Kasper:** None. **A. Luft:** None. **M. Pedotti:** None. **M. Schwab:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NINDS NS066987

NIGMS MBRS-RISE GM60655

Title: Long-term potentiation of temporoammonic-CA1 synapses in awake, freely moving rats

Authors: *J. GONZALEZ[1], I. S. MORALES[1], D. M. VILLARREAL[1], B. E. DERRICK[1,2]
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Abstract: Although hippocampal memory function traditionally has been viewed to occur through the relay of perforant path (PP) input to the dentate gyrus (DG) and the ensuing activation of the “tri-synaptic loop,” hippocampal area CA1 also receives direct cortical input via the temporoammonic (TA) path. Given the paucity of *in vivo* examinations of TA-CA1 synaptic plasticity, we utilized current source density analysis, paired pulse stimulation and high frequency stimulation (HFS) to characterize CA1 field responses in intact rats following stimulation of the medial aspect of the angular bundle, and investigated long-term potentiation (LTP) at medial TA (mTA)-CA1 synapses in freely moving rats. Our data demonstrate independent current sinks in the *stratum lacunosum moleculare* of areas CA1 and CA3 following stimulation of the angular bundle, indicating locally generated responses. Medial TA-CA1 field

excitatory postsynaptic potentials (fEPSP) exhibited paired pulse facilitation, contrasting the paired pulse depression observed with medial PP responses in the DG and CA3. Medial TA-CA1 fEPSPs displayed an onset of 1.5-3 ms and followed 100 Hz stimulation, indicating mTA-CA1 fEPSPs are monosynaptic. Medial TA-CA1 and contralateral CA3 (cCA3)-CA1 fEPSPs were collected for at least 5 days prior to mTA-CA1 LTP induction. LTP of mTA-CA1 fEPSPs was then induced by 400 Hz theta burst stimulation (TBS) of the medial aspect of the angular bundle in awake rats at current intensities that produce a half maximal CA1 fEPSP. A one hour post-TBS collection of mTA-CA1 and CA3-CA1 fEPSPs demonstrated a sustained LTP of mTA-CA1 fEPSPs (n=9) measuring 144 ± 11 percent of baseline. cCA3-CA1 fEPSPs (n=6) did not potentiate after mTA TBS, indicating mTA-CA1 LTP was input specific. Daily measurement of mTA-CA1 and cCA3-CA1 fEPSP slopes indicate mTA-CA1 LTP, on average, decays to baseline within 6 days after LTP induction, comparable to the 5-7 day LTP observed at PP-DG synapses. Together, these data suggest *in vivo* mTA-CA1 fEPSPs are locally generated, monosynaptic and exhibit long-lasting LTP in behaving rats. Given the proposed novelty detection function of TA-CA1 input, mTA-CA1 LTP may encode novel spatial stimuli and contribute to the proposed comparator (match-mismatch) function of CA1.

Disclosures: J. Gonzalez: None. **I.S. Morales:** None. **D.M. Villarreal:** None. **B.E. Derrick:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: ANR Neurosciences 06-037-01

French Ministere of Research

Fondation pour la recherche medicale

Title: Reelin, an extracellular matrix protein linked to early onset psychiatric diseases, drives postnatal development of the prefrontal cortex.

Authors: *J. IAFRATI, J. OREJARENA, O. LASSALLE, L. BOUAMRANE, P. CHAVIS
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Abstract: Reelin is a large secreted glycoprotein present in the brain extracellular matrix. In the postnatal and adult hippocampus reelin signalling modulates synaptic plasticity and our group has previously shown that reelin controls the maturation of excitatory transmission and regulates the homeostasis of glutamatergic receptors.
Reelin dysfunction has been proposed to contribute to the etiology of psychiatric disorders: levels of reelin are decreased by approximately 50% in the PFC of schizophrenic patients. Moreover disruption of the maturation of the PFC functions participates to several psychiatric diseases such as schizophrenia. However the role of reelin in maturation and plasticity of glutamate connectivity in the prefrontal cortex (PFC) has never been investigated. To study the impact of decreased levels of reelin on the maturation of PFC glutamatergic functions, we took advantage of the Heterozygote Reeler Mice (HRM) which exhibits reduced reelin levels.
We combined ex-vivo electrophysiological recordings, 3D imaging and behavioural approaches to study synaptic plasticity in the PFC from wild-type and HRM from young to adult. We found that long term potentiation, a leading synaptic model for memory storage is abolished in HRM compared to wild-type. This is correlated with a lower dendritic spines density in HRM and to changes in glutamatergic receptors content. Treatment with glutamatergic antagonists restored spine density and synaptic plasticity. We propose that dysregulation of glutamatergic synapses due to a decrease in reelin content could lead to the dysfunction of the PFC.

Disclosures: J. Iafrati: None. **J. Orejarena:** None. **O. Lassalle:** None. **L. Bouamrane:** None. **P. Chavis:** None.

Poster

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Program#/Poster#:845.07/C2

Topic: B.08. Synaptic Plasticity

Support: HHMI

Title: CPEB3 mediates the maintenance of LTP and the perpetuation of memory storage by regulating the translation of key synaptic components

Authors: *L. FIORITI[1], C. MYERS[1], Y. HUANG[1], X. LI[1], P. TRIFILIEFF[1], E. R. KANDEL[1,2,3]
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Abstract: Long-term memories are persistently maintained in the brain through long-lasting changes in the strength and number of synaptic connections. Although the cellular and molecular mechanisms underlying induction of memories have been extensively studied, those which mediate the maintenance of long-term memories and of the associated synaptic plasticity, are less well understood. In Aplysia and Drosophyla the persistence of synaptic plasticity and memory is mediated by the regulated activation of a self-sustaining translational regulator ApCPEB and Orb2 (Si et al, 2003, 2010). These findings raise the question whether such mechanism is also conserved in vertebrates, thus constituting a general system for the perpetuation of memory. To address this issue, we have identified the murine homolog of the memory-related invertebrate CPEB, called CPEB3 (Theis et al, 2003) and studied its role in the induction and maintenance of hippocampus-dependent forms of explicit long-term memory.
We generated a CPEB3 conditional knock-out mouse strain and found that genetic elimination of CPEB3 impairs the persistence of hippocampus-dependent spatial memory, as evidenced by both spatial recognition and Morris water maze tests. We then investigated the electrophysiological and molecular bases for the CPEB3-KO-related deficit in memory maintenance. We also found that the long-term maintenance - but not the induction - of learning-related long-term plasticity of hippocampal synapses is impaired in CPEB3-KO mice. At the molecular level, we found a significant reduction in the learning-induced synthesis of the glutamatergic AMPA receptor subunits GluA1 and GluA2, which have a critical role in hippocampus-dependent synaptic plasticity. Taken together, this behavioral, electrophysiological, and molecular evidence identifies CPEB3 as a key molecular mediator of the persistence of long-term forms of behavioral memory in the mammalian brain through translational regulation of key components of the synaptic machinery.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: NSF Grant IBN-0344559

NIH Grant NS58867

Title: NR2D-containing NMDA receptors regulate cerebellar synaptic plasticity and learning

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Abstract: Fear conditioning can induce a sustained enhancement in GABA release onto cerebellar Purkinje cells, highlighting the involvement of long term potentiation (LTP) of inhibitory transmission. We have shown that activation of NMDA type glutamate receptors (NMDARs) can induce a long-lasting increase in GABA release from stellate cells (I-LTP). Here we investigated the role of NR2D-containing NMDARs in the induction of presynaptic I-LTP and fear conditioning learning.
Burst stimulation of presynaptic parallel fibers (PF, 4 depolarizing pulses at 100 Hz) can activate extrasynaptic NMDARs and evoke NMDAR currents. Application of PPDA which preferentially blocks NR2C and NR2D-containing receptors reduced the synaptic current amplitude. The isolated currents had slower decay kinetics than PPDA-insensitive currents, suggesting the expression of functional NR2D-containing NMDARs in stellate cells.
Repetitive stimulation of PF with either 5 or 15 depolarizing trains (4 pulses at 100 Hz) triggered a lasting increase in mIPSC frequency, inducing an NMDAR-dependent form of presynaptic I-LTP. The presence of PPDA blocked the I-LTP, whereas ifenprodil, a selective NR2B blocker, did not prevent the potentiation. Similarly, a low concentration of CPP, that preferentially blocks NR2A and NR2B-containing NMDARs, also failed to prevent the development of I-LTP. Thus NR2D receptors appear to be required for the induction of presynaptic I-LTP.
We further examined the role of NR2D in I-LTP using NR2D knockout mice. As expected, deletion of NR2D markedly accelerated the decay time kinetics of NMDA currents. We found that 5 trains of depolarizations failed to induce a lasting increase in mIPSC frequency in NR2D knockout mice, indicating NMDARs that contain NR2D subunits are required for the induction of I-LTP. However I-LTP could be induced using a stronger stimulation protocol (15 trains of depolarizations).
We next investigated whether genetic deletion of the NR2D subunit alters cerebellar-dependent learning. NR2D KO mice required more US-CS pairings to exhibit fear conditioning learning than control animals. An open field paradigm indicated that this was due to altered learning rather than a change in locomotor function or in the anxiety state of the animal. Finally, NR2D KO mice exhibited normal motor learning in the rotarod paradigm.
In summary, we show here that functional NR2D-containing receptors are present in stellate cells. These receptors appear to be essential for the induction of I-LTP. These results might explain the elevated threshold for the induction of both I-LTP and fear learning in mice lacking functional NR2D receptors.

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Poster

845. LTP: Physiology and Behavior

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Topic: B.08. Synaptic Plasticity

Support: Canadian Institutes of Health Research

Title: Modulation of hippocampal long-term potentiation by a naturally occurring theta rhythm during REM Sleep

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Abstract: Hippocampal theta rhythm is prominent during behaviours such as walking and rapid eye movement sleep (REM), and plays a role in spatial navigation and learning. Previous studies have shown that the phase of the hippocampal theta rhythm modulates synaptic transmission and plasticity in anesthetized and behaving animals. Synaptic plasticity is a leading model of learning at the cellular level. It has been shown that in awake rats, high frequency electrical stimulation in hippocampal CA1 stratum (str.) radiatum delivered to the peak or trough of the local theta rhythm yields long-term potentiation (LTP) or long-term depression (LTD), respectively. Since REM may be important for learning, we hypothesized that the same may occur for the theta rhythm during REM theta. In addition, we studied the duration of LTP/LTD of tetanized and non-tetanized pathways. Field potentials in CA1 were recorded using a silicon probe with 16 channels at 50 μm intervals, and excitatory postsynaptic potentials (fEPSPs) at CA1 str. radiatum were recorded following stimulation of ipsi- or contralateral CA1 electrodes. After a stable baseline of recording, a stimulation burst consisting of 4 pulses at 400 Hz (~ 50% of maximum fEPSP amplitude) was delivered to one (tetanized) pathway at a particular phase of the REM theta rhythm; a total of 5 bursts were given. The slope of fEPSP was measured and current source density analysis was computed when available. Our results showed that bursts delivered during the positive phase of theta rhythm (at str. radiatum) induced LTP of 110-130% of the baseline fEPSP slope and bursts during the theta negative phase induced LTD of 80-90% of the baseline. The LTP and LTD lasted for 1-2 hr. When LTP developed in a tetanized pathway, a non-tetanized pathway showed LTP, LTD or no change; while LTD in the tetanized pathway appears to be accompanied by no change or LTD in the non-tetanized pathway. These data confirm that synaptic plasticity in the hippocampus can be modulated by naturally occurring REM theta. (Supported by a CIHR grant)

Disclosures: M. Kuo: None. **L.S. Leung:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Title: Subregion specific PKA anchoring in the hippocampus is critical for synaptic tagging and contextual memory formation

Authors: *A. J. PARK[1], R. HAVEKES[1], T. NIE[1], T. HUANG[2], T. ABEL[1]
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Abstract: Synaptic plasticity is an input-specific neuronal process which requires highly coordinated molecular signaling achieved by subdomain-specific localization of signaling molecules. In the hippocampus, long-term potentiation (LTP) has been studied as a cellular model of memory storage. PKA has been reported as a key signaling molecule underlying LTP and memory formation. PKA-anchoring proteins (AKAPs) compartmentalize complex signaling by localizing not only PKA but also other signaling molecules to specific neuronal processes. By using transgenic mice expressing Ht31, a PKA anchoring blocking peptide, we previously showed that significantly reduced Ht31 expression in hippocampal area CA3 rescued deficits in a form LTP and spatial memory observed when Ht31 was highly expressed throughout hippocampal subregions. However, the subregion specific role of PKA anchoring in input-specific nature of LTP and memory formation is still not clear. Here, we adopted two-pathway synaptic tagging paradigm and contextual fear conditioning to further investigate the role of compartmentalized PKA signaling in synaptic plasticity and memory formation. We found that both strong-before-weak and weak-before-strong version of synaptic tagging, and contextual fear memory were impaired only when Ht31 was highly expressed throughout hippocampal subregions but not impaired when Ht31 expression was significantly suppressed in CA3. In addition, we found that forskolin, an adenylate cyclase activator, -induced LTP which relies on CA3 presynaptic neurotransmitter release was not affected only when Ht31 expression level was markedly reduced in CA3. Although postsynaptic PKA function in CA1 is important, these data suggest that presynaptic compartmentalized PKA signaling in CA3 is critical for hippocampal synaptic plasticity and memory formation.

Disclosures: A.J. Park: Employment; University of Pennsylvania. **R. Havekes:** None. **T. Nie:** None. **T. Huang:** None. **T. Abel:** None.

Poster

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Topic: B.08. Synaptic Plasticity

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Senter - Neuro-Bsik

Sensopac

Cerebnet

Title: Impact of NMDA receptor overexpression on cerebellar Purkinje cell activity and motor learning

Authors: *M. SCHONEWILLE[1], E. GALLIANO[2], M. RUTTEMAN[2], M. BOCCHIO[2], D. JAARSMA[2], F. E. HOEBEEK[2], C. I. DE ZEEUW[2]
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Abstract: A basic property of chemical synapses is their ability to modify themselves in response to certain stimulus patterns. This property, known as synaptic plasticity and predicted by Donald Hebb, is thought to be the cellular basis of learning and memory. Hebbian plasticity requires the existence of a coincident detector of pre- and post-synaptic activities, a role that the N-methyl-D-aspartate glutamate receptor (NMDAR) can fulfill.
Interestingly, at the main plastic site of one of the oldest learning circuits in the mammalian brain, the parallel fiber to Purkinje cell synapse (PF-PC) in the cerebellum, NMDAR are absent. Indeed, in Purkinje cells NMDAR are exclusively found facing the other glutamatergic input, i.e. the climbing fibers. However the PF-PC synapse is, as shown by a multitude of studies, plastic. One could therefore wonder what the introduction of functional NMDAR at the PF-PC synapse would imply in terms of synaptic plasticity and, eventually, behavioral learning.
To answer this question we overexpressed the NR2B subunit of the NMDA receptor selectively in murine cerebellar Purkinje cells using the Pcp-2/L7 promoter. Here we report the results from two independent mouse lines that showed normal growth, no sign of ataxia and no gross morphological abnormalities. Western blot analysis and electrophysiological characterization

confirmed respectively that both transgenic lines express significantly more NR2B protein and NMDA-mediated currents than their wild type littermates. Functionally, NR2B transgenic mice show normal motor behavior but impaired eye movement adaptation and synaptic plasticity. Together these data suggest a direct connection between abnormal NMDAR expression in Purkinje cells and impaired learning implying that, to guarantee proper learning, non-Hebbian forms of plasticity are essential.

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Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant 2205503

AA 2207230

AHAF 2207045

Title: Calmodulin targeting regulates long-term potentiation

Authors: *L. ZHONG, N. GERGES
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Abstract: Calcium entry and the subsequent activation of CaMKII trigger synaptic plasticity in many brain regions. The induction of long-term potentiation (LTP) in the CA1 region of the hippocampus requires a relatively high amount of calcium-calmodulin. This requirement is usually explained, based on in vitro and theoretical studies, by the low affinity of CaMKII for calmodulin. An alternative hypothesis, however, is that calmodulin is not randomly distributed within the spine and its targeting within spine regulates LTP. Using post-embedding immunogold electron microscopy, we show that calmodulin gold particles are not randomly distributed, but spatially organized in the spine. Neurogranin regulates calmodulin distribution such that its overexpression concentrates calmodulin closer to the plasma membrane, where a high level of CaMKII immunogold labeling is also found. The targeting of calmodulin by neurogranin is sufficient to lower the threshold for the induction of LTP. These findings help to understand the physiological role of calmodulin targeting within the spine in synaptic plasticity.

Disclosures: L. Zhong: None. **N. Gerges:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NINDS-NS35058

NMSS-RG4280A

Title: Demyelination affects memory function.

Authors: *S. BALTAN, R. DUTTA, S. BRUNET, A. CHOMYK, G. KIDD, B. TRAPP
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Abstract: Multiple sclerosis (MS) is an immune-mediated disease of the human central nervous system (CNS) that demyelinates white and grey matter. While neurological disability is diverse, demyelination of the hippocampus is likely to contribute to the cognitive dysfunction found in greater than 50% of MS patients. We recently reported extensive demyelination in MS hippocampus with minimal neuronal loss accompanied by significant decreases in synaptic density and genes associated with glutamate neurotransmission, memory and synaptic plasticity. To explore if loss of myelin can alter neuronal function, we have generated a mouse model with a combination of cuprizone and rapamycin (C/R) that lead to consistent hippocampal demyelination. Using this treatment paradigm, six (acute) and 12 week (chronic) of C/R administration reduced hippocampal myelin to 20% and to 5% of control levels, respectively. Spontaneous remyelination occurred after withdrawal of the combined treatment, and after 6 week of normal diet, remyelination reached to 80% in the acute model and to 63% of control levels in the chronic model. Demyelination correlated with decrease in key genes involved in AMPA mediated neurotransmission, synaptic plasticity and memory function. In addition, loss of hippocampal myelin was associated with a decrease in synaptic densities and major reorganization of synapses associated with CA1 hippocampal neurons as determined by 3D-EM analysis. Demyelination progressively impaired synaptic transmission leading to complete loss of long term potentiation (LTP) at 5 weeks in hippocampal slices. Chronic demyelination significantly impaired memory function assessed by Morris Water Maze. Interestingly, these changes were reversed by remyelination, establishing a role of myelin in controlling neuronal function. Modulation of synapses and remyelination is therefore an attractive therapeutic target for enhancing memory function.

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Poster

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Topic: B.08. Synaptic Plasticity

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AHW 5520177

Title: Synaptic function of BRAG1

Authors: *J. C. BROWN[1], L. ZHONG[1], A. PETERSEN[1], M. FLORENCE[1], S. LEE[1], R. WALIKONIS[2], N. GERGES[1]
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Abstract: The post-synaptic density (PSD) is a region of concentrated proteins involved in the regulation of excitatory transmission and plasticity in dendritic spines. Glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors mediate the majority of this transmission, and are essential for normal synaptic and cognitive function. A prominent molecule of the PSD, BRAG1 (Brefeldin A-Resistant Arf-GEF (Adenonise di-phosphate (ADP) ribosylating factor- guanine nucleotide exchange factor)-1) is implicated in X-linked mental retardation. Therefore, we have investigated the role of BRAG1 in synaptic function with electrophysiology and confocal imaging in rat organotypic hippocampal slice cultures. We report that BRAG1 is involved in the bidirectional regulation of AMPA receptors, suggesting an important role in synaptic function.

Disclosures: J.C. Brown: None. **L. Zhong:** None. **A. Petersen:** None. **M. Florence:** None. **S. Lee:** None. **R. Walikonis:** None. **N. Gerges:** None.

Poster

845. LTP: Physiology and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:845.15/C10

Topic: B.08. Synaptic Plasticity

Support: R01 NS 037562

T32 MH 067763

R01 NS 064114

Title: Altered levels of endogenous proBDNF regulate hippocampal area CA1 synaptic plasticity

Authors: *L. C. HARTE-HARGROVE[1,2], J. YANG[3], T. MARINIC[3], J. J. LAFRANCOIS[1], B. L. HEMPSTEAD[3], H. E. SCHARFMAN[1,2]
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Abstract: Brain-derived neurotrophic factor (BDNF) modulates hippocampal area CA1 synaptic plasticity including long-term potentiation (LTP) and long-term depression (LTD). The *bdnf* gene is translated as a precursor, proBDNF, that can be cleaved to mature BDNF (mBDNF). Importantly, mBDNF increases hippocampal LTP, whereas application of recombinant proBDNF can enhance LTD. This suggests that relative levels of mBDNF to proBDNF can alter synaptic plasticity. However these conclusions relied upon exogenously-applied proBDNF. Therefore, we examined the impact of endogenous proBDNF on synaptic plasticity in CA1. We generated a knock-in mouse in which one *bdnf* allele was replaced by cleavage resistant *probdnf*, leaving one normal allele (*probdnf*/+ mice). We also studied animals haploinsufficient for *bdnf* (*bdnf*+/-), and controls (*bdnf*+/+). We evaluated area CA1 field EPSPs (fEPSPs) in horizontal hippocampal slices (400 μm-thick) from 4-7 wk old mice. Slices were placed in an interface-style recording chamber and kept at 31-32°C in artificial cerebrospinal fluid (in mM: 126 NaCl, 4 KCl, 2.5 $CaCl_2$, 1.0 $MgSO_4$, 26 $NaHC0_3$, 1.25 NaH_2P0_4, 10 D-glucose). Standard recording procedures were used (Skucas et al., 2011). Following a stable baseline, theta burst stimulation (TBS; 10 bursts of 4 pulses at 100 Hz, 200 ms apart) was triggered. TBS was chosen because it elicits BDNF-dependent LTP. Although *bdnf*+/- mice had significantly reduced fEPSP slopes, indicating a deficit in basal transmission, *probdnf*/+ mice had the greatest deficits in basal transmission. Additionally, while *bdnf*+/+ mice showed LTP 60 minutes after TBS (132% ± 1.6; n=16 slices, 12 mice), *bdnf*+/- mice had neither LTP nor LTD (99% ± 4.5%; n=8 slices, 5 mice) and *probdnf*/+ mice showed LTD (71% ± 11.1; n=9 slices, 6 mice), suggesting that elevated levels of proBDNF have a substantially different effect on synaptic plasticity than reduced levels of mBDNF (due to haploinsufficiency of *bdnf*). The results indicate that levels of endogenous mBDNF and proBDNF can affect basal transmission and plasticity in area CA1. The deficits

may be relevant to cognitive impairments and psychiatric diseases in which BDNF levels have been suggested to play an important role.

Disclosures: L.C. Harte-Hargrove: None. **J. Yang:** None. **T. Marinic:** None. **J.J. LaFrancois:** None. **B.L. Hempstead:** Research Grant; Pfizer, Inc. **H.E. Scharfman:** None.

Poster

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Program#/Poster#:845.16/C11

Topic: B.08. Synaptic Plasticity

Support: CHDI

Title: Shunted TrkB signaling leads to loss of striatal long term potentiation in Huntington's disease

Authors: *J. L. PLOTKIN[1], M. DAY[2], J. D. PETERSON[2], X. ZHONG[2], C. S. CHAN[2], E. ILLIJIC[2], D. J. SURMEIER[3]
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Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder profoundly impacting the major input nucleus of the basal ganglia, the striatum (caudate and putamen). Cognitive and behavioral deficits appear well before frank neuronal degeneration, as do overt abnormalities in striatal and cortical physiology. The integration and prioritization of cortical inputs to the basal ganglia is the charge of striatal spiny projection neurons (SPNs), but their nonlaminar structure and highly convergent afferents have made this an elusive process to study, both in normal and disease states. Here we show, using 2 photon uncaging of glutamate and a novel method to induce long term potentiation (LTP) at individual glutamatergic inputs, that a subset of SPN dendritic spines readily support LTP, and this process is attenuated in the BACHD and Q175 mouse models of HD. This LTP requires both NMDA receptors and BDNF activation of TrkB receptors. Importantly, we show that the loss of LTP in BACHD mice is not due to a loss of TrkB receptors, but rather inhibition of TrkB signaling through an upregulated phosphatase, suggesting a novel therapeutic target for this disorder.

Disclosures: J.L. Plotkin: None. **M. Day:** None. **J.D. Peterson:** None. **X. Zhong:** None. **C.S. Chan:** None. **E. Illijic:** None. **D.J. Surmeier:** None.

Poster

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Program#/Poster#:845.17/C12

Topic: B.08. Synaptic Plasticity

Support: CNRM Grant G1702Z

Title: Effects of multiple isoflurane exposures on long term potentiation in the hippocampus

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Abstract: Service Members wounded in action during Operations Iraqi Freedom and Enduring Freedom, or New Dawn, require multiple administrations of general anesthetics in a short period of time after exposure to a traumatic brain injury (TBI). While the deleterious effects of TBI on cognitive function are the subject of intense scientific investigations, little is known about the effects of multiple exposures to general anesthetics in the brain. By using electrophysiological techniques, we examined the effects of repeated exposures to isoflurane on the induction and maintenance of long-term potentiation (LTP) in the rat hippocampus. Male Sprague-Dawley rats, ages 7-8 weeks (n=5) and 13-14 weeks (n=5), were anesthetized with 1.5% isoflurane and 100% oxygen for one hour every other day, for a total of three exposures in one week. 24 hours or 7 days after the final exposure, CA1 hippocampal field recordings were obtained. 24h after the final anesthetic, hippocampal LTP was significantly increased compared with naïve controls ($p=0.007$), while at 7 days after the final session, hippocampal LTP was inhibited compared to baseline recordings ($p=0.013$). These data suggest that multiple exposures to general anesthesia may produce long-term adverse effects on hippocampal-dependent learning and memory functions. Continued evaluation of the effects of repeated isoflurane exposure will provide insight for the development of rehabilitative medicine strategies.

Disclosures: R. Long: None. **E. Prager:** None. **R. Kenneally:** None. **M. Braga:** None.

Poster

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Program#/Poster#:845.18/C13

Topic: B.08. Synaptic Plasticity

Support: Istituto Italiano di Tecnologia (projects seed)

Title: Reduced D-serine levels in the nucleus accumbens of cocaine-treated rats hinder the induction of NMDA-dependent synaptic plasticity

Authors: L. CURCIO[1], L. LEONE[1], M. V. PODDA[1], R. PIACENTINI[1], P. CAPPELLETTI[2], S. SACCHI[2], L. POLLEGIONI[2], C. GRASSI[1], *M. D'ASCENZO[1]
[1]Inst. of Human Physiol., Catholic Univ., Rome, Italy; [2]Dept. of Biotech. and Life Sci., Univ. of Insubria, Varese, Italy

Abstract: Recent data have shown that D-serine is a physiologically relevant glycine-site ligand of NMDAR in several brain areas. Aim of the present study was to investigate the potential involvement of D-serine signaling in cocaine-induced changes in NMDAR-dependent synaptic plasticity in the nucleus accumbens (NAc). Analysis of the peak amplitudes of AMPAR- and NMDAR-mediated EPSCs revealed a significantly increased AMPA/NMDA ratio in rat slices treated with the selective D-serine degrading enzyme DAAO respect to control slices (6.0 ± 1.1 vs. 1.3 ± 0.1; n=10). Moreover, D-serine degradation markedly reduced the amplitude of NMDAR-dependent LTP and LTD (-92.2%, and -90.2%, respectively). The results indicate that D-serine is the endogenous coagonist of synaptic NMDAR in the NAc, where its presence is essential for NMDAR-dependent synaptic plasticity. We then compared the amplitude of LTP and LTD in slices obtained from cocaine- and saline-treated rats in the absence or in the presence of saturating levels of exogenous D-serine (100 μM). The amplitudes of LTP and LTD were markedly reduced in slices from cocaine-treated animals (-55.3% and -80.4%; respectively), but were completely restored to saline values when slices were perfused with exogenous D-serine thus suggesting that cocaine treatment of rats reduces D-serine levels in the NAc. Exogenous d-serine increased NMDAR currents in slices from cocaine-treated rats to a greater extent than that observed in the saline-treated rats (+196% v.s +78%). This observation further support our hypothesis and prompted us to measure D-serine concentrations in the NAc slices from cocaine- and saline-treated rats, by high performance liquid chromatography (HPLC). This set of experiments revealed that the amount of D-serine decreased significantly from 4.6 ± 0.9 pmol/μg of protein (saline-treated rats) to 2.7 ± 0.4 pmol/μg of protein (cocaine-treated rats). To investigate whether the reduced D-serine levels in the NAc core was due to changes in the expression of the enzymes responsible for its biosynthesis and/or degradation, we performed real time RT-qPCR and Western blot analyses of NAc extracts. DAAO mRNA and protein levels were significantly increased in the NAc core of cocaine treated rats (+68%; and +195%; respectively). On the other hand a significant decrease in SR protein levels (-50.6%) was observed, although no difference in the corresponding mRNA transcript level was detected. Taken together these data suggest an important role for D-serine signaling in the NAc as molecular correlate for cocaine-induced impairment of NMDAR-dependent synaptic plasticity.

Disclosures: L. Curcio: None. **L. Leone:** None. **M.V. Podda:** None. **R. Piacentini:** None. **P. Cappelletti:** None. **S. Sacchi:** None. **L. Pollegioni:** None. **C. Grassi:** None. **M. D'Ascenzo:** None.

Poster

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Program#/Poster#:845.19/C14

Topic: B.08. Synaptic Plasticity

Support: CRCNS/41771-0002

Title: Modulation of plasticity with low amplitude electric fields in rat hippocampal slices.

Authors: *B. LAFON, D. REATO, A. RAHMAN, M. BIKSON, L. PARRA
City Col. of New York, New York, NY

Abstract: Low amplitude electric fields are known to modulate ongoing neural activity as well as induce prolonged excitability changes lasting several minutes and up to an hour. Animal and human studies seeking to explain the effects of weak field stimulation have thus far identified immediate effects on neural activity such as acutely altered spike timing and changes in excitability that may result in network entrainment and changes in firing rate. However, demonstrating the physiologic underpinning of the long term plastic effects has remained elusive.

Here we are particularly interested in the effects of slow-oscillating stimulation during sleep which were shown to boost the consolidation of hippocampus-dependent declarative memory. How synaptic connections are modified resulting in a memory improvement is still unknown. Currently, two main complementary hypotheses exist regarding consolidation of memory during sleep. The synaptic homeostasis hypothesis conjunctures that consolidation is in part a result of synaptic downscaling that improves the signal-to-noise ratio for all synapses that were potentiated during prior waking experiences. The active system consolidation hypothesis proposes an active consolidation process where learning-specific synaptic connections are strengthened by means of increased plasticity-related immediate early gene transcription and induction of LTP.

Slow-oscillating stimulation has been linked to synaptic homeostasis by an effect on the homeostatic downscaling of endogenous slow-waves during sleep. However, the mechanisms by which this low-amplitude stimulation might be modulating LTP processes related to the potentiation of learning related synapses has not yet been studied. Previous studies show that

electrical stimulation coupled with ongoing activity in the brain induces plasticity, here we will focus on the modulation of LTP processes while applying acutely several low-amplitude field configurations. Thus linking the long-term behavioral effects with acute electrical stimulation.

Disclosures: B. Lafon: None. **D. Reato:** None. **A. Rahman:** None. **M. Bikson:** None. **L. Parra:** None.

Poster

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Program#/Poster#:845.20/C15

Topic: B.08. Synaptic Plasticity

Title: Chronic unpredictable stress attenuates synaptic plasticity in the dentate gyrus and increases the incidence of depressive-like behaviours

Authors: *E. R. WHITE[1], P. S. BROCARDO[1], D. F. HAWLEY[2], J. LEIGH LEASURE[2], B. R. CHRISTIE[1]
[1]Div. of Med. Sci., Univ. of Victoria, Victoria, BC, Canada; [2]Psychology, Univ. of Houston, Houston, TX

Abstract: Chronic stress is an epidemic in today's society; approximately 6.5 million adult Canadians experience high or extreme stress most days of their lives. One region of the brain particularly affected by chronic unpredictable stress (CUS) is the hippocampus. The hippocampus is not a uniform structure, as dorsal regions may be more involved in spatial function with ventral more involved in affect. Previous studies have found decreased survival of progenitor cells in the ventral, but not the dorsal, hippocampus. The purpose of this study was to determine if CUS preferentially reduced synaptic plasticity in the ventral dentate gyrus (DG). Twenty-four adult male Long-Evans rats were divided into control or stressed groups. For 14 days, stressed animals were subjected daily to an unpredictable schedule of ethologically relevant stressors, such as altered light cycle, predator sounds, and damp bedding. Twenty-four hours following the last stressor, sucrose preference and splash tests were performed. Both tests indicated that animals were exhibiting signs of stress. CUS animals exhibited a reduction in sucrose preference, and increased latency to start grooming coupled with diminished time spent grooming when compared to controls during the splash test. Immediately after behavioural testing, animals were sacrificed for in vitro electrophysiology field recordings in which long-term potentiation (LTP) was elicited in the dorsal and ventral DG. In contrast to a region specific

effect for neurogenesis, synaptic plasticity was attenuated in both ventral and dorsal slices following CUS.

Disclosures: E.R. White: None. **P.S. Brocardo:** None. **D.F. Hawley:** None. **J. Leigh Leasure:** None. **B.R. Christie:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NIH grant NINDS NS21229

NIH grant NIDA DA09411

Title: Methylphenidate influence over *in vivo* hippocampal plasticity underlying memory

Authors: D. JENSON[1], A. ACEVEDO-RODRIGUEZ[2], J. TANG[2], *J. A. DANI[2]
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Abstract: Attention-Deficit Hyperactive Disorder (ADHD) is the most commonly studied and diagnosed psychiatric disorder in children. Methylphenidate (MPH, e.g., Ritalin) is the most commonly prescribed treatment for ADHD, and it has been used for decades to treat ADHD, but little is known about its therapeutic mechanisms.
The hippocampus is a center for learning and memory, and synaptic plasticity is a measurable underlying mechanism for memory. This project examines the effects of MPH on synaptic plasticity in the hippocampus that correlates with and may contribute to learning and memory. Our hypothesis is that MPH will affect synaptic plasticity in the hippocampus that underlies learning, and the influence of MPH will arise from its ability to boost catecholamine signaling. The object of this proposal is to determine the effects of MPH on synaptic plasticity in the hippocampus of awake animals, and to more clearly elucidate its mechanism of action in the hippocampus.
We measure synaptic plasticity in response to MPH in the perforant path to dentate gyrus pathway in the hippocampus by recording field potentials with permanently implanted electrodes in freely moving mice. When weak theta burst stimulation was applied along the perforant pathway, it did not produce synaptic change. However, when the same stimulation was applied along with MPH (5 mg/kg), then the stimulation produced long-term potentiation (LTP) of 64 ± 18 % ($n=6$, $p<0.01$). These in vivo recordings offer a highly precise measurement of synaptic

transmission, while preserving the overall circuitry and neurotransmitter systems that we ultimately wish to understand.
Supported by grants from the NIH, NIDA DA09411 and NINDS NS21229.

Disclosures: D. Jenson: None. **A. Acevedo-Rodriguez:** None. **J. Tang:** None. **J.A. Dani:** Employment; Baylor College of Medicine. Research Grant; NINDS NS21229, NIDA DA09411.

Poster

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Topic: B.08. Synaptic Plasticity

Support: A*STAR (NC)

NIH (MS)

Simons Foundation (MS)

Title: Astrocytes mediate nucleus basalis enabled, orientation stimulus specific plasticity in V1

Authors: N. CHEN[1], H. SUGIHARA[2], J. SHARMA[2], G. PEREA[2], J. PETRAVICZ[2], C. LE[2], *M. SUR[1]
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Abstract: While sensory experience associated with nucleus basalis (NB) driven, cholinergic activation of the cortex has been shown to instruct cortical plasticity, little is known about the underlying cellular mechanisms. To understand how cortical responses and representations can be altered by experience during cholinergic modulation, it is critical to identify the circuit elements involved and elucidate how their interactions can alter cortical network dynamics. In this work, we show that electrical stimulation of NB, paired with visual stimulation in vivo, can induce significant potentiation of visual responses in excitatory neurons recorded by cell-attached patch in mouse primary visual cortex (V1). We further demonstrate with in vivo two-photon calcium imaging, ex vivo calcium imaging and whole-cell recordings that this pairing induced potentiation is mediated by direct cholinergic activation of V1 astrocytes via muscarinic acetylcholine receptors. The astrocyte-mediated potentiation is stimulus specific, as pairing NB stimulation with a specific visual orientation reveals a highly selective potentiation of responses to the paired orientation compared to unpaired orientations. Collectively, these findings reveal a novel and surprising role for astrocytes in NB-induced stimulus-specifc plasticity in the cerebral

cortex and suggest an intimate organization of astrocytes with the synapses that convey and generate orientation specific responses.

Disclosures: N. Chen: None. **H. Sugihara:** None. **J. Sharma:** None. **G. Perea:** None. **J. Petravicz:** None. **C. Le:** None. **M. Sur:** None.

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Topic: B.08. Synaptic Plasticity

Support: HKRGC 774608

Title: Regulation of synaptic plasticity by the partnership of proheparanase with peri-synaptic heparan sulfate proteoglycan and AMPA-type glutamate receptor

Authors: *C.-W. MA[1,2], Y. L. LAM[2], W. C. CHAM[2], Y. S. CHAN[1], D. K. Y. SHUM[2]
[1]Dept. of Physiol., [2]Dept. of Biochem., The Univ. of Hong Kong, Hong Kong, China

Abstract: Perineuronal heparan sulfates (HS) have been implicated in controlling the open-state of AMPA-type glutamate receptors (AMPARs) which govern excitatory synaptic transmission in the hippocampus. We hypothesize that neuronal mechanisms modulate peri-synaptic HS level and as a result regulate synaptic function. Our finding of neuronal heparanase expression in adult rats led us to test if neuronal heparanase is secreted to act on perineuronal HS, thereby contributing to synaptic plasticity. Following phorbol ester stimulation of hippocampal neurons in culture, Western blot analysis of the secreted product revealed proheparanase but not the active heparanase. Synaptosomes prepared from phorbol ester-treated cortex slices were enriched in proheparanase; co-immunoprecipitation studies further showed association of AMPAR subunits (GluA1 and GluA2/3) with both syndecan-3 (transmembrane HS-proteoglycan) and proheparanase, suggesting their clustering as a functional complex. Treatment of hippocampal neuron cultures with recombinant proheparanase triggered internalization of proheparanase, perineuronal HS-proteoglycans and AMPARs. Heparitinase pre-treated hippocampal neuron cultures showed reduced proheparanase-induced internalization of AMPARs, suggesting that proheparanase binds to syndecan-3 via the HS moiety. Consistent with these findings, treatment of hippocampal slices with exogenous proheparanase resulted in declines in both basal synaptic strength and LTP. Proheparanase treatment also prevented glutamate-induced calcium influx of the hippocampal neurons in culture. These results reveal a novel role of neuronal proheparanase

in regulating synaptic plasticity by resetting AMPARs and perineuronal HS levels at the synapse. [*Supported by HKRGC 774608*]

Disclosures: C. Ma: None. **Y.L. Lam:** None. **W.C. Cham:** None. **Y.S. Chan:** None. **D.K.Y. Shum:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: NSERC Grant 203175

Title: Role of thalamic vs. cortical NMDA receptors in LTP in the primary visual cortex: Shift to a cortical mechanism by visual discrimination training of adult rats

Authors: *A. M. HAGER, H. C. DRINGENBERG
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Abstract: Synaptic plasticity (long-term potentiation, LTP) plays an important role in processes of learning and memory formation and several studies now have identified LTP-like changes at central synapses following various types of learning experiences. The thalamocortical projections from the lateral geniculate nucleus (LGN) to the primary visual cortex (V1) have been shown to reliably produce LTP in adult rats. Here, we examined the role of N-methyl-D-aspartate (NMDA) receptors in the LGN and V1 for LTP in this fiber system. Adult, urethane anesthetized rats exhibited reliable LTP of field post-synaptic potentials (fPSPs) in V1 following theta-burst stimulation of the LGN. Local application of the NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (APV; 10 mM, applied by reverse microdialysis) in the LGN blocked LTP induction, while cortical application was ineffective. Thus, thalamic NMDA receptors appear to be critical for the induction of this type of thalamocortical plasticity.
In a second set of experiments, behavioral (visual discrimination) training was carried out prior to LTP induction. Interestingly, trained rats displayed significantly greater levels of LTP (~50%) relative to naïve or swim-control animals (~25% LTP), indicative of a metaplastic increase in the modification range of V1 synapses. This enhanced potentiation in trained rats was reversed by application of APV to either the LGN or V1, resulting in naïve-like levels of LTP in trained rats (~25% LTP). Thus, visual training can result in the appearance of a novel, cortical locus to induce robust LTP even in the absence of thalamic NMDA receptor contributions. These data are consistent with previous evidence, showing that visual training leads to a metaplastic increase of

LTP in V1 that involves NR2B subunits of NMDA receptors.
In summary, plasticity in the rodent thalamocortical visual system is strongly influenced by experience-dependent factors: naïve LTP depends on thalamic NMDA receptors, while visually trained animals are able to recruit both thalamic and cortical NMDA mechanisms to express LTP. Thus, future work examining anatomically distributed networks will be necessary to further characterize plasticity, particularly for neural circuits shaped by training and other behavioral experiences.

Disclosures: A.M. Hager: None. **H.C. Dringenberg:** None.

Poster

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Program#/Poster#:845.25/C20

Topic: B.08. Synaptic Plasticity

Title: Functionality of paired associative stimulation when the soleus muscle is targeted

Authors: *S. M. KUMPULAINEN[1], N. MRACHACZ-KERSTING[1], M. VOIGT[1], A.-M. ALEXANDROU[2], J. AVELA[2]
[1]Hlth. Sci. and Technol., Aalborg Univ., Aalborg, Denmark; [2]Sport Sci., Univ. of Jyvaskyla, Jyvaskyla, Finland

Abstract: There are several studies showing that paired associative stimulation (PAS) can induce local changes in excitability of cortical projections to the target muscle. However, these studies have not examined the effect on functionality in a related motor performance. The purpose of this study was to measure the functionality of PAS-induced effects when the soleus muscle (SOL) is targeted. Subjects sat on a custom built ankle dynamometer (hip, 110°; knee, 180°; ankle, 90°) with their right foot resting on a pedal. Transcranial magnetic stimulation (TMS) was used to measure motor-evoked potentials (MEPs) of SOL before and following PAS. PAS-intervention consisted of 200 paired stimuli of tibial nerve electrical stimulation and TMS over the soleus area of motor cortex. To identify potential changes in spinal excitability, SOL short latency stretch reflexes (SLR) were elicited with perturbations induced by the ankle dynamometer. Functionality was measured with three different isometric plantar-flexor motor tasks, which assessed rate of force development (RFD), reaction time and accuracy of force control. SLR measurements and the motor tasks were also performed in a control group. Depending of the PAS-effect, the 24 subjects were divided into a facilitation group (16), where there was an increase in the MEP amplitudes (81 ± 92 %) following PAS and an inhibition group

(8), where there was a decrease in the MEP amplitudes (-31 ± 17 %). SLR remained unchanged after the intervention in all groups suggesting no changes in the excitability at the spinal level. Reaction time shortened and accuracy of force control improved significantly in the facilitation group and RFD decreased significantly in the inhibition group ($P < 0.05$). There were no changes in the motor performance in the control group. Our results demonstrate that the leg motor performance of healthy subjects can be enhanced by facilitative PAS - intervention.

Disclosures: S.M. Kumpulainen: Employment; University of Aalborg. **N. Mrachacz-Kersting:** None. **M. Voigt:** None. **A. Alexandrou:** None. **J. Avela:** None.

Poster

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Topic: B.08. Synaptic Plasticity

Support: KAKEN23700449

Title: Neuropsin-dependent synaptic plasticity in the hippocampal CA1 field of freely moving mice.

Authors: *Y. ISHIKAWA, H. YAMASHITA, S. SHIOSAKA
NAIST, Nara, Japan

Abstract: Long-term potentiation (LTP) is supposed to be a cellular mechanism involved in memory formation. Similar to distinct types of memory formation, LTP can be separated into a protein synthesis-independent early phase (early-LTP) and a protein synthesis-dependent late phase (late-LTP). An important question is whether the transformation from early- into late-LTP can be elicited by behavioral conditions such as the attention to novel events.
In our previous study, we found that synaptic plasticity in the hippocampal Schaffer collateral pathway depended on neuropsin (kallikrein-related peptidase 8 or KLK8), a plasticity-related extracellular protease in acute hippocampal slices and in anaesthetized mice. However, neuropsin dependent synaptic plasticity in freely moving mice remains poorly understood. Therefore, we investigated the neuropsin-dependent effect of early-LTP and novelty-exploration with early-LTP in the hippocampal CA1 of freely moving mice. In the present study, we investigated how neuropsin participates in synaptic plasticity in the hippocampal CA1 of freely moving mice. We found that neuropsin-knockout mice were significantly impaired in early-LTP induced by a single tetanus.

Disclosures: Y. Ishikawa: None. **H. Yamashita:** None. **S. Shiosaka:** None.

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Topic: B.08. Synaptic Plasticity

Support: NSERC

CIHR

National Counsel of Technological and Scientific Development, Brazil

Title: The role of the GluN2A subunit of the NMDA receptor in synaptic plasticity and spatial pattern separation in the dentate gyrus.

Authors: *T. S. KANNANGARA[1,3], B. D. EADIE[2], C. A. BOSTROM[2], P. S. BROCARDO[2], B. R. CHRISTIE[2,3]
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Abstract: The different secondary subunits of the *N*-methyl-D-aspartate (NMDA) receptor each convey unique biophysical properties to the receptor complex, and may be key in determining the functional role played by NMDA receptors. In the hippocampus, the GluN2A and GluN2B subunits are particularly abundant, however their exact roles in synaptic plasticity and behaviour remain controversial. Here we show that mice lacking GluN2A subunits exhibit a significant shift in the ratio of NMDA to AMPA (α-amino-3- hydroxy-5-methyl-4-isoxazolepropionic acid) current in granule cells of the dentate gyrus, without impacting mature dendritic morphology. These animals also show significant impairments in both long-term potentiation (LTP) and long-term depression (LTD) of synaptic efficacy in the dentate gyrus. These deficits are not apparent in pyramidal cells of the CA1 subfield. In addition to this decrease in bidirectional synaptic plasticity in the dentate gyrus, GluN2A$^{-/-}$ animals show impaired performance on spatial pattern separation tasks that have been reported to be dentate gyrus-specific. In contrast, GluN2A$^{-/-}$ mice show no deficit in temporal pattern separation, a process associated with CA1 functioning. Our data indicates that the GluN2A subunit may play an important role in synaptic and behavioural plasticity in the dentate gyrus.

Disclosures: T.S. Kannangara: None. **B.D. Eadie:** None. **C.A. Bostrom:** None. **P.S. Brocardo:** None. **B.R. Christie:** None.

Poster

845. LTP: Physiology and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:845.29/C24

Topic: B.08. Synaptic Plasticity

Support: KAKENHI (20300116)

FIRST Program (SI)

Title: Presynaptic netrin-G subtypes differentially control synaptic plasticity and behavior in mice

Authors: *H. MATSUKAWA[1], H. GOTO[1], R. LUJÁN[2], S. AKIYOSHI-NISHIMURA[1], Q. ZHANG[1], K. YAGUCHI[1], T. HASHIKAWA[1], R. SHIGEMOTO[3], S. ITOHARA[1]
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Abstract: Netrin-G1 and netrin-G2, are vertebrate-specific, GPI-anchored membrane proteins of the UNC-6/netrin family. They are expressed on the axons of distinct neuronal circuits within the brain. The pattern of this expression determines the distribution of their dedicated receptors, NGL-1 and NGL-2, within sub-dendritic segments of target neurons, in a cortical layer-specific manner (Akiyoshi-Nishimura et al., PNAS, 2007). The physiological roles of these trans-neuronal interactions, however, are poorly understood. Here, we performed electron microscopy and electrophysiological analysis on the hippocampus, a structure where netrin-G1/NGL-1 and netrin-G2/NGL-2 interactions occur along the distal temporoammonic pathway (TA-CA1) and the proximal Schaffer collateral pathway (SC-CA1), respectively. Immunostaining using antibodies against each netrin-G and its receptor, revealed that netrin G localized to excitatory presynaptic membranes, while receptors localized to postsynaptic membranes of selected pathways. Mice with deletions of either netrin-G1 or netrin-G2 showed opposing alterations in long-term and post-tetanic potentiation. Netrin-G1 knock-out (KO) mice showed attenuated TA-CA1 synaptic plasticity, while netrin-G2 KO mice showed augmented SC-CA1 synaptic plasticity. Furthermore, phorbol-ester induced potentiation of transmitter release was significantly reduced at the TA-CA1 synapse of netrin-G1 KO mice, but normal at the SC-CA1 synapse of netrin-G2 KO mice. This effect was indistinguishable between netrin-G1 KO and wild-type mice, in the presence of protein kinase C inhibitor. These results suggest that

presynaptic netrin-G subtypes play differential modulatory roles in glutamate release, via distinct biochemical pathways. In addition, we characterized the phenotypes of these KO mice in a wide range of behavioral tests. Netrin-G1 KO mice exhibited abnormalities in the fear freezing response, anxiety, locomotion and impulse control, whereas netrin-G2 KO mice showed abnormalities in spatial reference and working memory, procedural learning, anxiety, visual acuity, hearing and motor coordination. Interestingly, there was little overlap in phenotypes between the sets of mutants, reflecting the differential expression pattern of the two genes. Taken together, these findings indicate that trans-synaptic netrin-G/NGL interactions play important roles in modulating synaptic efficacy of specific neuronal circuits, as well as behavioral output.

Disclosures: H. Matsukawa: None. **H. Goto:** None. **R. Luján:** None. **S. Akiyoshi-Nishimura:** None. **Q. Zhang:** None. **K. Yaguchi:** None. **T. Hashikawa:** None. **R. Shigemoto:** None. **S. Itohara:** None.

Poster

845. LTP: Physiology and Behavior

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Program#/Poster#:845.30/C25

Topic: B.08. Synaptic Plasticity

Support: 1|01BX000819

TGS-109219

Title: Recurrent apnea disrupts long-term potentiation (LTP) in the hippocampus in *in vivo* rats

Authors: S. J. FUNG[1,2], M. XI[1,2], *V. B. FENIK[3], J. LOPATTO[1,2], M. H. CHASE[1,2,4]
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Abstract: Cognitive impairments, which commonly occur in humans with obstructive sleep apnea (OSA), are also present when animals are subjected to recurrent periods of apnea. Behavioral studies have demonstrated that rats that are exposed to chronic episodic hypoxia are deficient in Morris water maze learning tests. Since hippocampal long-term potentiation (LTP) is recognized as an important cellular substrate for learning and memory, we hypothesize that recurrent apnea reduces LTP in a monosynaptic excitatory (CA3-CA1) pathway in the hippocampus that has been shown to mediate the processing of cognitive information.
To examine this hypothesis, adult rats, which were anesthetized with α-chloralose and

immobilized with Flaxedil, were subjected to recurrent episodes of apnea (via ventilatory arrest). During each apneic episode, when the SpO2 was desaturated to 60%, re-ventilation was initiated until a baseline level of oxygen saturation (95%) was achieved. Following a 2 hr period of recurrent apnea, CA3 was stimulated and a baseline field excitatory postsynaptic potential (fEPSP) was recorded from CA1 5 min prior to the initiation of LTP. A high-frequency stimulation (400 Hz, 2 s duration, 800 pulses, 0.2 ms pulse width) paradigm was used to induce LTP in the CA1 fEPSP. LTP was then monitored for a period of 60 min. In control animals, LTP was recorded for 60 min while the animals were respired at normal levels of oxygen saturation. In control (normoxic) rats, LTP (220% of baseline fEPSP) was observed immediately after stimulation; thereafter, LTP remained at 180%. In apneic rats, LTP was significantly reduced compared with the response in normoxic animals. It was 130% initially and 125% of its baseline level during the recording period. We conclude that recurrent episodes of apnea have deleterious effects on long-term synaptic plasticity in a hippocampal pathway that is involved in the processing of cognitive information. (Supported by grants 1|01BX000819 and TGS-109219).

Disclosures: S.J. Fung: None. **M. Xi:** None. **V.B. Fenik:** None. **J. Lopatto:** None. **M.H. Chase:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

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Program#/Poster#:846.01/C26

Topic: B.08. Synaptic Plasticity

Support: CIHR Grant MOP 86473

NSERC Grant RGPIN 121698

Title: Low frequency stimulation induced long term potentiation at the accessory olfactory to medial amygdala synapse in Anurans.

Authors: *G. DEROSENROLL, K. DELANEY
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Abstract: Long term potentiation (LTP), an activity-based long-lasting enhancement of the synaptic connectivity between neurons, currently stands as one of the best physiological models for learning in the brain. NMDA glutamate receptor-dependent high frequency stimulation-induced LTP is the most studied form of LTP in the CNS. We made use of an en bloc preparation of the amphibian Rana catesbiana brain that maintains the integrity of the

telencephalon in order to study another form of LTP in the amygdala, which receives direct inputs from many brain areas including the main and accessory olfactory bulbs. Extracellular field recordings reveal a form of LTP in the medial amygdala, induced by repetitive low frequency stimulation of the accessory olfactory tract (AOT) comprised of the axons of mitral cells projecting from the accessory olfactory bulb. Field potential amplitude was tested every 20 seconds with a single stimulation of the AOT that evoked 30% of maximum field. Conditioning trains of 1Hz (900 pulses) or 5Hz (5 x 25 pulses; 15s intervals) delivered to the AOT induced up to threefold increase of field strength that persisted for at least three hours. Additionally, 5Hz conditioning yields a transient post-tetanic potentiation up to sixfold over baseline that decays to the sustained LTP level over the course of 50 minutes. Since the accessory olfactory bulb, receives primary sensory information from the vomeronasal organ, and its target, the amygdala, plays an important role in the expression of mammalian fear the phenomenon reported here could represent a sensitization to predator odours or a mechanism to enhance detection of behaviourally relevant odours that are persistent in the environment.

Disclosures: G. Derosenroll: None. **K. Delaney:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:846.02/C27

Topic: B.08. Synaptic Plasticity

Title: Understanding and optimizing vagus nerve stimulation directed cortical plasticity

Authors: *M. BORLAND, W. A. VRANA, K. C. RAHEBI, N. A. MORENO, B. R. HEATH, M. M. PANTALIA, R. E. BABU, S. ISLAM, M. P. KILGARD
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Abstract: Abstract Vagus nerve stimulation (VNS) is a safe and effective treatment for refractory epilepsy. Map plasticity can be directed in primary auditory or primary motor cortex by pairing a tone or a movement with a brief bursts of VNS. VNS-directed plasticity has been shown to be highly effective in treating an animal model of tinnitus (Engineer et al., 2011). Our VNS-tone pairing paradigm requires one hundred times less VNS than is used to treat epilepsy. The mechanisms by which vagus nerve stimulation directs neural plasticity are not clear and the optimal stimulation parameters to direct plasticity are not known. Earlier studies had suggested that the effect of VNS is an inverted-U function of current intensity with a peak at 0.4 mA. Preliminary analysis of our modified paradigm using 0.5 second bursts of VNS instead of 30

second trains suggests that 0.8 mA and 1.2 mA VNS result in equivalent cortical map plasticity, while 0.4 mA VNS does not generate detectable plasticity. Map plasticity is reduced when the interval between VNS-tone pairing events is decreased from 30 to 8 seconds. Based on earlier evidence that locus coeruleus is required for the effects of VNS, we have injected rats with DSP-4 to destroy the central noradrenergic system and determine whether release of norepinephrine is required for VNS-directed map plasticity. Results from these studies will be used to inform the clinical optimization of VNS-event pairing as a treatment for tinnitus and other neurological disorders.

Disclosures: M. Borland: None. **W.A. Vrana:** None. **K.C. Rahebi:** None. **N.A. Moreno:** None. **B.R. Heath:** None. **M.M. Pantalia:** None. **R.E. Babu:** None. **S. Islam:** None. **M.P. Kilgard:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:846.03/C28

Topic: B.08. Synaptic Plasticity

Title: Cerebellar mechanisms underlying habituation of the acoustic startle response

Authors: X. CHEN[1], H. ADELSBERGER[2], A. STROH[2], C. RUEHLMANN[2], M. BRILL[2], T. MISGELD[2], *A. A. KONNERTH[2]
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Abstract: Habituation, representing the gradual reduction of the organism's response after repeated sensory stimulation, is an important and universal feature in the animal kingdom, which is relevant for the adaption to the surrounding environment (Thompson and Spencer, 1966; Groves and Thompson, 1970). Habituation of the acoustic startle response, one of the most commonly used models for studying this type of simple learning in mammals (Thorpe, 1956), has been considered to involve the cerebellar vermis (Leaton and Supple, 1986; Lopiano et al., 1990; Timmann et al., 1998; Frings et al., 2006). However, the precise identification of neural locus and underlying cellular mechanisms remained unclear. Here by using two-photon imaging-based in vivo electrophysiological recordings, we find that repeated sound stimulation induces both behavioural habituation of the acoustic startle response as well as a reduction of sound-evoked excitatory postsynaptic potentials (EPSPs) and simple spike firing in Purkinje neurons of the mouse cerebellar vermis. The behavioural habituation and the reduction of EPSPs show highly similar time courses. Blocking GABA-A receptor-mediated synaptic inhibition by

pharmacological or molecular manipulations blocks both the reduction of EPSPs and behavioural habituation. This suggests that activity-dependent potentiation of synaptic inhibition in Purkinje neurons, a process that was previously termed rebound potentiation (Kano et al., Nature, 1992), is essential for the habituation of the acoustic startle response. Finally, optogenetically-induced inhibition in Purkinje neurons artificially causes habituation of the acoustic startle response. Together, our result identify essential cellular and circuit mechanisms underlying the habituation of the acoustic startle response in mammals.

Disclosures: X. Chen: None. **H. Adelsberger:** None. **A. Stroh:** None. **C. Ruehlmann:** None. **M. Brill:** None. **T. Misgeld:** None. **A.A. Konnerth:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

Location: Hall F-J

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Program#/Poster#:846.04/C29

Topic: B.08. Synaptic Plasticity

Title: Effects of phosphodiesterase 9 inhibition on synaptic transmission and neurodegeneration

Authors: *A. I. PARACHIKOVA[1], K. PEDERSEN[1], L. GLASDAM JENSEN[1], C. YANG[2], H. SINDAL JENSEN[1], N. SVENSTRUP[1], N. PLATH[1]
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Abstract: Cyclic nucleotides have long been recognized to play a crucial role in regulating synaptic plasticity. Increased levels of cAMP as well as cGMP have been associated with improved memory performance as well as amelioration of experimentally induced deficits in long term potentiation in rodents. Consequently, phosphodiesterase inhibitors, which lead to an increase in cAMP and/or cGMP levels have been viewed as potential drugs for increasing cognitive functioning. The PDE superfamily of enzymes consists of 11 members; the cGMP selective PDE9 is characterized by widespread expression in the brain, albeit at low levels, which could be offset by a particularly low Km for cGMP. Previously, PDE9 inhibition has been demonstrated to reverse learning and memory deficits observed in various models of pharmacological disruptors. The pro-cognitive potential of PDE9 inhibitors has led to the hypothesis that inhibition of this enzyme could be beneficial for the symptomatic treatment of Alzheimer's disease (AD). To date, no evidence however has been presented linking PDE9 inhibition to the underlying pathological hallmarks of the disease.
We have investigated the role of PDE9 inhibition with particular focus on the deficits associated with AD pathogenesis. The pathway resulting in cGMP production has been linked to β-amyloid

and the downstream alterations in neuroinflammation. Based on these data, we employed an APP transgenic model of the disease and evaluated the effect of PDE9 inhibition at both a behavioral and purely mechanistic level. Behavioral characterization of PDE9 inhibition demonstrates reversal of cognitive deficits in the APP transgenic mouse model of AD. Furthermore, we examined the mechanism underlying the amelioration of synaptic functioning by PDE9 inhibition by addressing basic and advanced properties of spontaneous and evoked synaptic transmission.

Disclosures: A.I. Parachikova: None. **K. Pedersen:** None. **L. Glasdam Jensen:** None. **C. Yang:** None. **H. Sindal Jensen:** None. **N. Svenstrup:** None. **N. Plath:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: Grant-in-Aid for Young Scientists

Title: Sex difference of the effect of neonatal social isolation on experience driven synaptic delivery of AMPA receptors in rat barrel cortex

Authors: *H. TADA, K. SUYAMA, T. TAKAHASHI
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Abstract: Although sex differences of the response to stress have been well known, molecular mechanisms are still poorly understood. Here we show that neonatal social isolation disrupts experience-driven delivery of GluR1 containing AMPA receptors to synapse in barrel cortex of males but not females. Furthermore, we found that injection of glucocorticoid disrupts experience dependent synaptic GluR1 trafficking in the barrel cortex of male but not female rats. Moreover, we showed that disruption of GluR1 trafficking with social isolation was not prevented in masuculinized female rats. Our findings suggested that female rats could have molecular machinery which protects social isolation stress induced disruption of synaptic AMPA receptors delivery.

Disclosures: H. Tada: Research Grant; Grants-in-Aid for Scientific Research. **K. Suyama:** None. **T. Takahashi:** Research Grant; Strategic Research Program for Brain Sciences (SRPBS).

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

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Program#/Poster#:846.06/C31

Topic: B.08. Synaptic Plasticity

Support: AA010983

AA0119967

Title: Developmental progression of pyramidal neuron excitability and dopamine modulation in PFC

Authors: *S. C. BARRETT[1,2], J. CHANDLER[2], H. TRANTHAM-DAVIDSON[2]
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Abstract: The dopaminergic innervation of the prefrontal cortex (PFC) is highly plastic from birth through early adulthood; changes have been observed in dopamine (DA) receptor expression (Tarazi and Baldessarini, 2000) and activation (Tseng and O'Donnell, 2007). However, little is known about specific changes in D2 and D4 receptor function during this phase. Understanding how the DA system matures through development will help identify novel pharmacological targets to treat a variety of neurological disorders including schizophrenia, ADHD, and addiction. Preliminary studies indicate that there is a progressive increase in pyramidal neuron excitability and a concomitant decrease in baseline spike duration in cortical layer V in the PFC through early development (post-natal days 14-35) in male Long-Evans rats. This observation may reflect a developmental change of specific potassium channels that mediate neuronal excitation in this population of neurons. Additionally, D2 receptor stimulation in the same cellular population resulted in a progressive increase in firing rate throughout the same developmental period. Future studies will further explore the cellular mechanisms mediating these developmental changes in pyramidal neurons as well as changes in D2 and D4 receptor modulation of cortical activity. These findings will provide a detailed understanding of the developmental profile of pyramidal activity, which will contribute to our understanding of how this profile is disrupted in disorders of cognition and perhaps lead to better treatment strategies.

Disclosures: S.C. Barrett: None. **J. Chandler:** None. **H. Trantham-Davidson:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: Australian Research Council

National Health and Medical Research Council

Westmead Milennium Institute

Title: Neural field theory of calcium dependent plasticity: Prediction and application to transcranial magnetic stimulation

Authors: *P. K. FUNG[1,2], P. A. ROBINSON[2], C. KERR[2]
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Abstract: Current theoretical knowledge of system-level synaptic plasticity is limited, partly because of the relatively rare in vivo, system-level plasticity experiments. Transcranial magnetic stimulation (TMS) is a noninvasive brain stimulation technique that induces system-level plasticity, but our understanding of TMS is limited. We propose a system-level plasticity model that predicts TMS signatures, thereby partially verify our theory, and gain an understanding of TMS.

We formulate our system-level plasticity model by incorporating calcium dependent plasticity (CaDP), a physiologically realistic microscopic plasticity mechanism into a meso- to macroscopic neural field theory, and explore system-level CaDP. We explore how large-scale neural activity leads to calcium influx, which induces system-level plasticity. In particular, we model the cortical plasticity response due to different TMS protocols, and match them with experimental results. This theory predicts various existing TMS experimental signatures, plus additional predictions subject to experimental verification. Predictions of this model includes the system-level, time-asymmetric plasticity window induced by paired associative stimulation, and the frequency dependence of TMS: that low frequency TMS induces depression, whereas high frequency TMS induces potentiation.

Our work thus allows understanding and promises future optimization of TMS. The verification of our work also provides partial verification of the neural field theory and calcium dependent plasticity. Therefore, this work is of interest to both theorists and experimentalists in the mechanism of synaptic plasticity and the scientific and medical aspects of TMS.

Disclosures: P.K. Fung: Research Grant; Westmead Millenium Institute, Australian Research Council, National Health and Medical Research Council. **P.A. Robinson:** None. **C. Kerr:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

Location: Hall F-J

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Program#/Poster#:846.08/C33

Topic: B.08. Synaptic Plasticity

Title: Diabetes onset influences hippocampal synaptic plasticity in streptozotocin-treated rats

Authors: *S. SASAKI-HAMADA, H. SACAI, J.-I. OKA
Pharmacol., Tokyo Univ. of Sci., Chiba, Japan

Abstract: Children with type 1 diabetes mellitus (DM) are at risk of developing cognitive difficulties. Although when the diabetes begins influences these cognitive difficulties, synaptic properties related to the age of onset remain unknown. Here we showed that synaptic plasticity including long-term potentiation (LTP) or long-term depression (LTD), and excitatory synaptic transmission at Schaffer collateral-CA1 (SC-CA1) synapses in hippocampal slices were affected by age of onset in rats with streptozotocin-induced diabetes (STZ-rats), compared with age-matched control rats. In young adult-onset STZ-rats, LTP was impaired and the ratio of AMPA receptor-mediated EPSCs relative to NMDA receptor-mediated EPSCs (the AMPA/NMDA ratio) decreased, whereas LTD was impaired and both AMPA receptor-mediated and NMDA receptor-mediated EPSCs increased in juvenile-onset STZ-rats. These results suggest that the pathophysiology of diabetes-induced cognitive difficulties varies with the age of diabetes onset.

Disclosures: S. Sasaki-Hamada: None. **H. Sacai:** None. **J. Oka:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

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Program#/Poster#:846.09/C34

Topic: B.08. Synaptic Plasticity

Support: MURST - PRIN 2010

Title: Neural plasticity in injured spinal cord

Authors: *M. GULISANO[1], R. PARENTI[2], R. GULINO[2]
[1]Lab. Mol Biol / Dept Physiol Sci., [2]Dept BioMedical Sci. - Physiological Section, Univ. Catania, Catania, Italy

Abstract: Sonic hedgehog and Noggin are morphogenetic factors involved in neural induction and ventralization of the neural tube, but recent findings suggest that they could participate in regeneration and functional recovery after injury. Here, in order to verify if these mechanisms could occur in the spinal cord and involve synaptic plasticity, we measured the expression levels of Sonic hedgehog, Noggin, Choline acetyltransferase, Synapsin-I, and Glutamate receptor subunits (GluR1, GluR2, GluR4), in a motoneuron-depleted mouse spinal lesion model obtained by intramuscular injection of Cholera toxin-B saporin. The lesion caused differential expression changes of the analyzed proteins. Moreover, motor performance was found correlated with Sonic hedgehog and Noggin expression in lesioned animals. The results also suggest that Sonic hedgehog could collaborate in modulating synaptic plasticity. Together, these findings confirm that the injured mammalian spinal cord has intrinsic potential for repair and that some proteins classically involved in development, such as Sonic hedgehog and Noggin could have important roles in regeneration and functional restoration, by mechanisms including synaptic plasticity.

Disclosures: M. Gulisano: None. **R. Parenti:** None. **R. Gulino:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

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Program#/Poster#:846.10/C35

Topic: B.08. Synaptic Plasticity

Support: Muscular Dystrophy Association 20267

Title: Postsynaptic Dystrophin regulates the neurotransmitter release via a Dystrobrevin, Rho-GTPase and CaMKII-dependent pathway

Authors: *L. G. FRADKIN[1], S. POTIKANOND[2], G. S. K. PILGRAM[2], A. W. M. DE JONG[2], J. N. NOORDERMEER[2]
[2]Mol. Cell Biol., [1]Leiden Univ. Med. Cen, Leiden, Netherlands

Abstract: Synaptic function is regulated by homeostatic mechanisms which maintain functional parameters, such as the levels of neurotransmitter release, at their wild type set points. Although significant progress has been made in identifying presynaptic homeostatic pathway members, little is known about the postsynaptic pathways involved or how the set point is maintained in

vivo. Here, we show that Dystrobrevin is delocalized from the Drosophila neuromuscular junction in the absence of Dystrophin, leading to an abnormally high set point of neurotransmitter release. Postsynaptic relocalization of Dystrobrevin in the Dystrophin mutant background restores the normal release level. Genetic epistasis experiments indicate that the pathway's chief target is the Rho-GTPase CDC42. Supporting this, postsynaptic expression of constitutively-active CDC42 alone recapitulates the Dystrobrevin mutant phenotype. Finally, we show that this pathway likely acts to regulate postsynaptic CaMKII activity. Taken together, our results outline a novel postsynaptic pathway that regulates the homeostatic neurotransmitter release set point.

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Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

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Program#/Poster#:846.11/C36

Topic: B.08. Synaptic Plasticity

Support: NINDS Grant PO1 NS-39546

Title: Glutamatergic synaptic plasticity mediates rapid escape reflex transformation during morphallactic regeneration in the annelid worm Lumbriculus variegatus.

Authors: *Z. R. LYBRAND[1], M. J. ZORAN[1], V. G. MARTINEZ-ACOSTA[2]
[1]Biol., Texas A&M Univ., College Station, TX; [2]Biol., Univ. of the Incarnate Word, San Antonio, TX

Abstract: Neural plasticity is the process by which anatomical (structural) and physiological (functional) changes in the nervous system of an organism lead to alterations in behavior. Neural morphallaxis, a rare form of neural plasticity, involves the reorganization of the animal's nervous system during segmental regeneration following injury. In Lumbriculus variegatus, an aquatic annelid worm, neural morphallaxis is characterized by synaptic changes in the neural circuits that underlie escape reflexes. Sensory-to-giant interneuron connections that mediate tail withdrawal in posterior segments transform such that sensory inputs activate head withdrawal as segments take on a more anterior position. During this transformation, neurotransmission at the sensory-to-giant interneuron synapse rapidly becomes functional suggesting that these sensory synapses are functionally silent prior to neural morphallaxis. Here we have used neurobiotin (NB), a fluorescent neural tracer dye that passes readily through gap junctions at electrical synapses, to

label the medial giant fiber (MGF) within the ventral nerve cord (VNC). A total of 9 coupled cells, including a bilateral pair of intermediate fibers, were identified. Transmission electron microscopy analysis confirmed synaptic input onto these intermediate fibers. Serial sections identified giant fiber collaterals, projections through the myelin-like sheath surrounding the MGF, with an abundance of motor output synapses, but no identifiable synaptic inputs. Intracellular current-clamp recordings of the MGF demonstrated that CNQX, an AMPA receptor antagonist, abolished all evoked MGF responses, however AP5, an NMDA receptor antagonist, showed no effect. Immunohistochemistry of the VNC showed that GluR5-7, an AMPA/kainate receptor monoclonal antibody, labeled glutamate receptors along the periaxonal regions of the MGF and the intermediate fibers. GluR5-7 was also colocalized with the monoclonal antibody Lan3-2, a molecular marker for neural morphallactic plasticity. These results suggest that the MGF pathway includes an electrically coupled network of cells. Neural morphallactic plasticity of sensory input to this coupled network is via glutamatergic synapses onto the MGF and intermediate fibers, which function as a bilateral pair of sensory interneurons. Therefore, neural morphallaxis is mediated by the rapid unsilencing of functionally effective glutamatergic synapses that are likely pervasive along the VNC.

Disclosures: Z.R. Lybrand: None. **M.J. Zoran:** None. **V.G. Martinez-Acosta:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant T32-GM007315

Title: Nogo receptor signaling antagonizes growth promoting pathways to restrict synaptic plasticity

Authors: *K. T. BALDWIN, Y. A. MIRONOVA, R. J. GIGER
Cell and Developmental Biol., Univ. of Michigan, Ann Arbor, MI

Abstract: Proper nervous system function critically depends on the proper assembly, maintenance, and activity-dependent modification of neuronal circuits. Although a great deal is known about extracellular molecules and signaling pathways that regulate axonal growth and pathfinding during embryonic nervous system development, comparatively little is known about the mechanisms that control synaptic structure and function in the juvenile and adult mammalian brain and spinal cord. Mounting evidence suggests that there is a close overlap of molecular

players that limit neuronal sprouting in the injured central nervous system (CNS), and molecules that restrict neuronal plasticity in the healthy (uninjured) CNS. Recent studies from our lab revealed that the growth inhibitory proteins Nogo/RTN4 and oligodendrocyte myelin glycoprotein (OMgp) suppress activity-dependent synaptic plasticity in a Nogo receptor 1 (NgR1)-dependent manner. The signaling mechanisms employed by NgR1 to regulate synaptic structure and synaptic transmission, however, have not yet been defined. Mammalian target of rapamycin complex 1 (mTORC1) promotes protein synthesis through phosphorylation of its direct substrates, p70 S6 kinase (S6K) and eIF4E-binding proteins (4EBP). BDNF elicited activation of mTORC1 is essential for protein synthesis-dependent forms of synaptic plasticity, including late-long term potentiation (L-LTP). Here we show that Nogo/NgR1 signaling negatively regulates mTORC1 activity through its interaction with signaling pathways upstream of mTORC1 activation. In primary neurons, increasing Nogo signaling causes a decrease in expression of several proteins important for activity-dependent regulation of synaptic strength, including NMDA and AMPA receptor subunits. Conversely, blocking Nogo/NgR1 signaling increases expression of these proteins, suggesting Nogo/NgR1 signaling plays a key role in regulating the composition of the synaptic proteome. Studies with Nogo receptor mutant mice indicate that signaling pathways upstream of mTORC1 are regulated by NgR1 ligands *in vivo*, and contribute to defects in synaptic transmission. Ongoing studies are exploring the role of Nogo receptors in hippocampus-dependent learning paradigms.

Disclosures: K.T. Baldwin: None. **Y.A. Mironova:** None. **R.J. Giger:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:846.13/C38

Topic: B.08. Synaptic Plasticity

Title: Dynamic regulation of synaptic maturation state by voltage-gated A-type K+ channels in CA1 hippocampal pyramidal neurons

Authors: *E. KIM[1], D. HOFFMAN[2]
[1]NIH, Huntington, WV; [2]NIH, Bethesda, MD

Abstract: Neuronal activity is thought to play a critical role in the formation and modification of neural circuits during brain development while the disruption of normal neuronal activity and synaptic plasticity has been implicated in several neurological disorders. In hippocampal CA1 pyramidal dendrites, A-type voltage-gated K+ currents, formed primarily by Kv4.2 subunits,

control excitability. Here we used Kv4.2 knock-out (Kv4.2-KO) mice along with acute in vivo expression of Kv4.2 or its dominant negative pore mutant to examine the role of Kv4.2 expression in the development of CA1 synapses. We found that Kv4.2 expression induces synaptic maturation in juvenile WT mice and rescues developmentally delayed synapses in adult Kv4.2-KO mice. In addition, we show that NMDAR subunit composition can be reverted back to juvenile forms in WT adult synapses by functionally down-regulating Kv4.2 levels. We also found that abnormally abundant silent synapses in recordings from adult Kv4.2-KO mice could be remedied by Kv4.2 expression. These results suggest that Kv4.2 regulation of excitability determines synaptic synaptic maturation state.

Disclosures: E. Kim: None. **D. Hoffman:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:846.14/C39

Topic: B.08. Synaptic Plasticity

Title: Dissociation of collapsin response mediator protein-2 by phosphorylation from hippocampal cholinergic neurostimulating peptide precursor in hippocampus

Authors: M. MIZUNO[1], D. KATO[1], T. KANAMORI[1], T. TOYODA[1], T. SUZUKI[2], *K. OJIKA[1], N. MATSUKAWA[1]
[1]Nagoya City Univ. Med. Sch., Nagoya, Japan; [2]Shinshu Univ. Med. Sch., Matsumoto, Japan

Abstract: Hippocampal cholinergic neurostimulating peptide (HCNP) was purified as a peptide inducing the synthesis of cholineacetyltransferase in medial septal nucleus in vitro and in vivo. HCNP precursor protein (HCNP-pp) was also reported as a multifunctional protein, participating in signal pathways. We also reported that over-expression of HCNP-pp in hippocampus increased the amount of collapsin response mediator protein (CRMP)-2 with the inhibition of phosphorylation, and co-localized with total CRMP-2 at pre-synaptic terminal in hippocampus. Furthermore, it is known that CRMP-2 may dissociate from tau protein by sequential phosphorylation.
To clarify whether the phosphorylation in CRMP-2 involves in co-localization and/or the binding activity with HCNP-pp, we investigated the co-localization in immunohistochemistry and immunoprecipitation. Here, we demonstrated that HCNP-pp mainly co-localized with the non-phosphorylated CRMP-2 at pre-synaptic terminals in the hippocampus, while the phosphorylated CRMP-2 primarily presented at post-synaptic terminals. In addition, CRMP-2 by

phosphorylation may dissociate from HCNP-pp in the hippocampus. These findings suggest that HCNP-pp, in association with the non-phosphorylated CRMP-2, plays an important role in pre-synaptic function in the mature hippocampus.

Disclosures: M. Mizuno: None. **D. Kato:** None. **T. Kanamori:** None. **T. Toyoda:** None. **T. Suzuki:** None. **K. Ojika:** None. **N. Matsukawa:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

Location: Hall F-J

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Program#/Poster#:846.15/C40

Topic: B.08. Synaptic Plasticity

Support: CIHR Postdoctoral Fellowship to HBF

NARSAD Young Investigator Grant to HBF

Title: Non-classical targets of cAMP contribute to presynaptic plasticity in the hippocampus

Authors: *H. B. FERNANDES[1], S. KRANIOTIS[1], J. J. MARSHALL[1], S. RIORDAN[2], A. CONTRACTOR[1]
[1]Physiol., [2]Cell and Mol. Biol., Northwestern Univ., Chicago, IL

Abstract: cAMP signaling has been implicated in the mechanisms of distinct forms of long term synaptic plasticity, including those expressed in the postsynaptic spine and those expressed in the presynaptic terminal. Classically it has been thought that the actions of cAMP are through the activation of protein kinase A (PKA), resulting in phosphorylation of downstream substrates, and an increase in synaptic release of glutamate. Recently a new downstream target of cAMP has been identified which links to an alternate PKA- independent signaling pathway. This target is the cAMP guanine-nucleotide exchange factor (cAMP-GEF) Epac, which can regulate small GTPases such as Rap1. One of the members of this family, Epac2, is enriched in the brain with expression particularly high in the dentate gyrus and CA3 regions of the hippocampus. Neurotransmission at the mossy fiber synapse formed between the dentate granule neurons and the hippocampal CA3 pyramidal neurons has several characteristic features. Mossy fiber synapses display marked short-term plasticity and a robust long-term potentiation (LTP) that is expressed as a persistent increase in release probability. Elevating cAMP with forskolin induces a chemical LTP of mossy fiber synapses, which occludes further activity-induced LTP. Because of the enriched expression of Epac2 in the hippocampus we investigated whether this non-classical target of cAMP plays a role in mossy fiber plasticity. We first performed tissue

fractionation to obtain samples enriched with the large mossy fiber synaptosomes. Immunoblot analysis revealed that Epac2 was expressed at high levels in the mossy fiber boutons. To determine the functional consequence of ablation of Epac2 we performed whole-cell electrophysiological recordings from CA3 neurons in hippocampal slices obtained from Epac2 knockout ($Epac2^{-/-}$) or wild-type ($Epac2^{+/+}$) littermate mice. We found that basal synaptic transmission and short-term plasticity were not altered in $Epac2^{-/-}$ mice. Paired pulse ratios of mossy fiber EPSCs at an interstimulus interval of 40ms were 2.6 ± 0.16 (n = 20) in $Epac2^{+/+}$ recordings and 2.5 ± 0.14 (n = 23) in $Epac2^{-/-}$ recordings ($p > 0.05$). Similarly frequency facilitation at 1 Hz was not different between the two genotypes ($Epac2^{+/+}$: 2.9 ± 0.43, n = 14; $Epac2^{-/-}$: 2.5 ± 0.21, n = 16, $p > 0.05$). However, we found a significant genotype effect on tetanically induced LTP in $Epac2^{-/-}$ mice. The magnitude of LTP in $Epac2^{+/+}$ recordings 25-30 minutes after induction was 195 ± 21.5 % (n =8) whereas in interleaved recordings from $Epac2^{-/-}$ the magnitude of LTP was 133 ± 11.4 % (n =11)($p < 0.05$). These data provide evidence of a significant role for Epac2 in mossy fiber LTP.

Disclosures: H.B. Fernandes: None. **S. Kraniotis:** None. **J.J. Marshall:** None. **S. Riordan:** None. **A. Contractor:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:846.16/C41

Topic: B.08. Synaptic Plasticity

Support: NSF GRFP

Title: Hebbian mechanisms underlying the learning of Markov sequence probabilities

Authors: *K. BOUCHARD[1], S. GANGULI[3], M. BRAINARD[2]
[1]Neurosurg. and Physiol., [2]Physiol., UCSF, San Francisco, CA; [3]Applied Physics, Stanford, Palo Alto, CA

Abstract: The majority of natural sensory and motor events do not occur in isolation, but are embedded in the context of sequences with rich probabilistic structure. For example, the learned song of a Bengalese finch exhibits probabilistic transitions in the sequencing of song syllables. Further, we have previously shown that the conditional probability of song sequences is encoded in auditory responses of vocal control area HVC. Despite the ubiquity and generality of such learned probabilistic sequences, little is known about the mechanisms that develop synaptic weights that are proportional to the experienced statistics of probabilistic sequences.

Here, we investigate the conditions under which Hebbian synaptic plasticity shapes unstructured networks to reflect the conditional probability of sequential events.
Our basic approach is to focus on the degree to which the conditional probability of sequential events that are imposed on a network through training stimuli become encoded in the synaptic weights of recurrent neural networks of rate based units. For simplicity, we equate each node in our network with an elemental behavioral action and ask whether Hebbian plasticity can learn to connect nodes of the network with synaptic weights that reflect the transition probabilities between the corresponding actions.
We show through analytics and simulations that Hebbian synaptic plasticity with pre-synaptic competition develops synaptic weights proportional to the conditional forward transition probabilities [P(s(t+1)|s(t))] present in the input sequence. In contrast, post-synaptic competition develops synaptic weights proportional to the conditional backward probabilities [P(s(t-1)|s(t))] of the same input sequence. We demonstrate that to stably but flexibly reflect the conditional probability of a neuron's inputs and outputs, local Hebbian plasticity should approximately balance the magnitude of synaptic depression relative to potentiation (a competitive force) with the weight dependence of synaptic change (a homogenizing force). These forces interact to control both the rate at which structure emerges and the entropy of the final distribution of synaptic weights. Thus the relative balance of these forces induces a prior over learnable transition distributions. Together, these results demonstrate a remarkably simple correspondence between biophysics and probabilistic sequence learning: the site of synaptic competition dictates the temporal flow of the learned probabilistic structure and the balance between competitive and homogenizing forces dictates a prior expectation of the randomness of the sequence to be learned.

Disclosures: K. Bouchard: None. **S. Ganguli:** None. **M. Brainard:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:846.17/C42

Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS062771 (CH)

Title: Muscarinic control of presynaptic long-term potentiation at parallel fiber-Purkinje cell synapses

Authors: L. RINALDO[1], *C. HANSEL[2]
[1]Univ. of Chicago, Chicago, IL; [2]Neurobio., Univ. Chicago, CHICAGO, IL

Abstract: The activation of muscarinic acetylcholine receptors is known to modulate synaptic plasticity by promoting the production and release of cannabinoids in certain brain areas. In the cerebellar cortex it has been shown that cannabinoid signaling can block the induction of presynaptic long-term potentiation (PF-LTP) at parallel fiber-Purkinje cell (PF-PC) synapses. Herein, we demonstrate that the muscarinic agonist oxotremorine-m (oxo-m; 7 μM) blocks the induction of PF-LTP in a cannabinoid-dependent manner when applied during the tetanization period. Under control conditions, 120 parallel fiber stimuli delivered at 8 Hz resulted in LTP of parallel fiber EPSC's (123.9 ± 8.8%; n = 12; t = 31-35 min after; p0.05). PF-LTP was restored when the cannabinoid receptor antagonist AM251 (3 μM) was present in the bath with oxo-m (117.6 ± 5.6%; n = 7; p<0.05), indicating that muscarinic receptor activation triggers cannabinoid release in the cerebellar cortex. There are five known isoforms of muscarinic acetylcholine receptors (M1-5), of which M1, M3, and M5 are G_q-coupled receptors that exert their actions through phospholipase C (PLC), a key element in the enzymatic pathway responsible for cannabinoid production. As the effect of oxo-m on PF-LTP was abrogated by the PLC inhibitor U-73122 (122.6 ± 6.6%; n = 7; p<0.05), it is likely that one of these receptor subtypes is mediating this effect. Finally, oxo-m did not block the induction of PF-LTP when the guanosine nucleotide analogue GDP-β-S was added to the recording pipette (133.5 ± 7.9%; n = 7; p<0.05), indicating that cannabinoid production results from activating muscarinic receptors located on Purkinje cells. To compare the effects of muscarinic activation on plasticity expressed post-synaptically versus pre-synaptically, we applied oxo-m during the tetanization protocol for a post-synaptically expressed form of LTP. We found that in some cases the agonist converted the LTP to an LTD (Control: 113.1 ± 4.3%; n = 4; p<0.05; Oxo-m: 91.1 ± 8.2%; n = 6; p>0.05). Preliminary data suggests that this effect may be PKC-dependent, as it was somewhat reversed when the PKC inhibitor chelerythine (10 μM) was present in the bath throughout the recording (98.1 ± 8.3%; n = 4; p = 0.82). These results suggest that muscarinic receptors in the cerebellar cortex can control both pre- and postsynaptically expressed forms of plasticity, possibly through PLC-dependent pathways.

Disclosures: L. Rinaldo: None. **C. Hansel:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:846.18/C43

Topic: B.08. Synaptic Plasticity

Title: Evidence for the activation of presynaptically silent cortical synapses using a fluorescent probe

Authors: *T. HIKIMA, G. ARBUTHNOTT
Brain Mechanisms for Behaviour Unit, Okinawa Inst. of Sci. and Technol. Grad. Univ., Onna-Son, Okinawa, Japan

Abstract: There are several modes of changing synaptic strength and one of them is activating silent synapses. Activation of silent synapses is thought to provide reserve capacity for new neuron network processing or storage. There is ample evidence for postsynaptically silent synapses with only NMDA receptors present (e.g., Ashby & Issac, 2011 Neuron70, 510-521) but the variation of presynaptic release probability has been much less studied. We focused on adenlyl cyclase (AC) and protein kinase A (PKA) enhancement of synaptic release. We have used a method to measure exocytosis directly at presynaptic terminals in which a fluorescent pH reporter (Synaptophysin-pHluorin 2x, SypH2x) is transfected into neurons to allow us to image synaptic vesicle release. We applied this optical method to terminals of cortical neurons and measured the SypHx2 fluorescence from individual presynaptic terminals in response to electrical stimulation. Forskolin (FSK) was used as the AC/PKA activator and the SypH2x fluorescence response to stimulation was compared before and after FSK treatment. Stimulation with 5 pulses at 20Hz resulted in an increase of fluorescence on average across many terminals. The FSK treatment (10µM) enhanced the peak SypH2x fluorescence amplitude significantly. Considering synapses change their strength by jumping between discrete states, we define an active terminal as one whose fluorescence became more than twice the noise after stimulation, and a silent synapse, as one whose fluorescence remained less than twice the noise after stimulation. In this way we counted the number of terminals actively releasing transmitter. FSK treatment increased the number of such active terminals. After pretreatment of kt5720 (200nM), PKA antagonist, FSK treatment had no effect. Our result suggests that AC/PKA activation enhances the number of actively releasing cortical presynaptic terminals which is likely to increase overall synaptic efficacy.
We are grateful for the gift of SypH 2x from Dr Yongling Zhu

Disclosures: T. Hikima: None. **G. Arbuthnott:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:846.19/C44

Topic: B.08. Synaptic Plasticity

Support: R01 MH081935

Title: Synaptotagmin-12 is a PKA target required for presynaptic mossy fiber LTP

Authors: *T. J. YOUNTS[1], Y. J. KAESER-WOO[2], T. C. SUDHOF[3], P. E. CASTILLO[1]
[1]Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., BRONX, NY; [2]Picower Inst. for Learning and Memory, MIT, Cambridge, MA; [3]Howard Hughes Med. Inst. and Dept of Mol. and Cell. Physiol., Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Synaptotagmins (Syts) are membrane bound proteins, some of which are essential for neurotransmitter exocytosis. Sixteen isoforms of Syts have been identified. While key physiological roles have been ascribed to Ca2+-binding Syts1, 2, 7, 9 and 10, very little is known about other Syts that do not bind Ca2+, including Syt12. Syt12 is expressed in many brain regions, like the hippocampus, and is localized to synaptic vesicles. In addition, Syt12 can be phosphorylated in a cAMP-dependent manner at serine 97 (S97) in vitro, suggesting Syt12 could be modulated by PKA signaling in vivo. PKA can modulate transmitter release and is required for many forms of presynaptically expressed long-term synaptic plasticity, which are thought to be important for learning and memory. To determine the contribution of endogenous Syt12 to synaptic transmission and plasticity as well as a possible role for S97 in PKA-dependent signaling, we generated Syt12 knock-out and S97A knock-in mice. Synaptic transmission and plasticity were investigated using hippocampal slice electrophysiology. Results indicate that Syt12 and S97 of Syt12 are not required for basal excitatory and inhibitory synaptic transmission or short-term plasticity, as well as endocannabinoid-mediated long-term depression of inhibition. However, classical presynaptic mossy-fiber LTP and pharmacological potentiation of the cAMP/PKA-pathway at this synapse were significantly compromised in Syt12 knock-out and S97A knock-in mice. Taken together, these results identify a single amino acid in Syt12 as a PKA target required for long-term modulation of neurotransmitter release at a specific hippocampal synapse. Moreover, we reveal for the first time an endogenous role for Syt12 protein in brain.

Disclosures: T.J. Younts: None. **Y.J. Kaeser-Woo:** None. **T.C. Sudhof:** None. **P.E. Castillo:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:846.20/C45

Topic: B.08. Synaptic Plasticity

Title: Over-expression of hippocampal cholinergic neurostimulating peptide precursor protein enhances hippocampal synaptic plasticity

Authors: D. KATO, M. MIZUNO, T. KANAMORI, T. TOYODA, K. OJIKA, *N. MATSUKAWA
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Abstract: Hippocampal cholinergic neurostimulating peptide (HCNP) induces the synthesis of acetylcholine in medial septal nucleus *in vitro* and *in vivo*. HCNP precursor protein (HCNP-pp) is a multifunctional protein that participates in a number of signaling pathways, including Mitogen-activated Protein Kinase (MAPK)/extracellular signal and G-protein-coupled receptor kinase 2.
We recently demonstrated that the amount of collapsin response mediator protein-2 (CRMP-2) is increased in the hippocampus of HCNP-pp transgenic mice (Tg) and that HCNP-pp, in association with CRMP-2, might plays an important role in presynaptic function in the hippocampus by regulating synaptophysin expression.
To clarify the function of HCNP-pp in the neural activity and its effects on synaptic plasticity, we examined basal synaptic transmission and long-term potentiation (LTP).
Here, we demonstrate that basal synaptic transmission is slightly increased and LTP induction is greatly facilitated in HCNP-pp Tg mice as compared to wild-type littermates.
These findings suggest that HCNP-pp affects synaptic efficacy in the hippocampus.

Disclosures: D. Kato: None. **M. Mizuno:** None. **T. Kanamori:** None. **T. Toyoda:** None. **K. Ojika:** None. **N. Matsukawa:** None.

Poster

846. Synaptic Plasticity: Presynaptic and Other Mechanisms II

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Topic: B.08. Synaptic Plasticity

Support: MRC Grant

Title: Investigating the role of glutamate receptors in hippocampal, mossy fibre LTP *in vivo*

Authors: *J. WALLIS, D. E. JANE, G. L. COLLINGRIDGE, Z. A. BORTOLOTTO
Univ. of Bristol, Bristol, United Kingdom

Abstract: Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system, with glutamate receptors well documented for their roles in multiple forms of synaptic plasticity. NMDA receptors are integral for the induction of numerous, classical forms of long-term potentiation (LTP) and long-term depression. The hippocampal mossy fibres demonstrate an unsual form of NMDA receptor independent LTP. Both kainate receptors (KARs) and metabotrobic glutamate (mGlu) receptors have been implicated in this MF-LTP but their roles remain controversial. Recently our group has shown that antagonism of either GluK1-containing kainate receptors (with ACET or LY383885) or group I mGlu receptors (using a combination of 2-Methyl-6-(phenylethynyl) pyridine hydrochloride (MPEP) and LY367385) can inhibit MF-LTP (Dargan et al, 2009; Nistico et al., 2011). However, the sensitivity of MF-LTP to GluK1 antagonists is highly dependent on such factors as the divalent cation concentration (Lauri et al, 2003) and the slice orientation (Sherwood et al, 2012). As a result of the variability associated with in vitro approaches we sought to assess the roles of these receptors under more defined physiological conditions, by investigating the effects of GluK1 and mGlu receptor antagonists in vivo.

We studied the roles of KARs and mGlu receptors in the induction of MF-LTP, in anaesthetised rats. MF field EPSPs were evoked by stimulation of the granule cells, in dentate gyrus and were recorded in stratum lucidum of the CA3 region of the hippocampus. Drug application was made via an intrahippocampal cannula attached to the recording electrode. Ketamine (10mg/kg I.V.) was applied 10 minutes prior to tetanisation. LTP was induced by two tetani (2 x 100 Hz, 1 s). (2S,2'R,3'R)-2-(2',3'-Dicarboxycyclopropyl) glycine (DCG-IV) (2µl of 10µM I.H.) was applied after a 90 min and induced substantial depression of evoked synaptic transmission.

The present findings showed that a range of UoB synthesised, GluK1 targeting antagonists did not block the induction of MF-LTP. Furthermore mGlu receptor antagonism with (RS)-α-Methyl-4-carboxyphenylglycine (MCPG) demonstrated only limited affect on MF-LTP induction. However, co-application of MCPG and KAR antagonists at multiple concentrations significantly impaired MF-LTP. Further investigation into the mGlu receptor subtypes responsible, suggested that the effects are likely mediated via group I mGlu receptors.

These data suggest that both group I mGluRs and KARs are stimulated during, but the activation of only one type is required for, the induction of MF-LTP in vivo.

Disclosures: J. Wallis: None. **D.E. Jane:** None. **G.L. Collingridge:** None. **Z.A. Bortolotto:** None.

Poster

847. Intrinsic Membrane Properties: Neural Oscillation

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

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Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant NH46742

Title: Neuronal homeostasis: Inside the black box.

Authors: *T. O'LEARY, E. MARDER
Volen Ctr. For Complex Systems, Brandeis Univ., Waltham, MA

Abstract: Individual neurons develop characteristic electrical properties that remain stable over the lifetime of an organism despite various physiological perturbations including ion channel turnover, changes in morphology and variation in afferent input [1]. The electrical properties of neurons are controlled by cellular processes that govern ion channel regulation and these processes are thought to be activity-dependent and homeostatic in nature. Although a considerable amount of experimental evidence supports the existence and characterizes the properties of such homeostatic regulation, the details of the underlying mechanisms are unknown [2,3]. As a result, numerous conceptual models appear to be compatible with existing experimental data when implemented in simulations [4], but it is not presently clear how these models relate to one another, nor whether they constrain possible behaviour in a manner that permits experimental falsification [5]. For example, certain models (proportional controllers) predict incomplete compensation, whilst others (integral controllers) may exhibit undershoot and overshoot, or 'ringing'. We examine the abstract form of several existing models of activity-dependent ion channel regulation and show how each makes distinct, testable predictions that have not been fully investigated in an experimental setting. We relate these abstract controller models to the generic biochemical processes they correspond to in a living cell, such as ion channel insertion and calcium-dependent gene expression. Finally, we assess the sensitivity of the models to random fluctuations that are present in all biochemical systems and find that this sensitivity places constraints on the controller type. Together, our results may help eliminate candidate models of homeostatic regulation therefore constrain the form of the biological signalling pathways that the models describe.
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2. Davis, G.W. Homeostatic control of neural activity: from phenomenology to molecular design. Annu Rev Neurosci 29, 307-323 (2006).
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4. Marder, E. & Prinz, A.A. Modeling stability in neuron and network function: the role of activity in homeostasis. Bioessays 24, 1145-1154 (2002).
5. O'Leary, T. & Wyllie, D.J. Neuronal homeostasis: time for a change? J Physiol 589, 4811-4826 (2011).

Disclosures: T. O'Leary: None. **E. Marder:** None.

Poster

847. Intrinsic Membrane Properties: Neural Oscillation

Location: Hall F-J

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Program#/Poster#:847.02/C48

Topic: B.10. Intrinsic Membrane Properties

Support: Research Council of Norway

Title: Differences in intrinsic properties between dorsal and ventral CA1 pyramidal neurons

Authors: *M. MAROSI, C. HÖNIGSPERGER, J. F. STORM
Dept. of Physiol., Univ. of Oslo, Oslo, Norway

Abstract: The hippocampus shows differences in neuronal connectivity and molecular markers along the dorso-ventral axis. Behavioral and other studies indicate that the dorsal part of the hippocampus performs mainly cognitive functions, whereas the ventral is involved in emotions and stress, and place fields are larger for ventral CA1 cells. In this study we examine whether there are differences in the intrinsic electrophysiological properties of dorsal and ventral CA1 pyramidal neurons. Using somatic whole-cell patch-clamp recordings from CA1 pyramidal neurons (n=78), we tested their ability to generate subthreshold membrane potential oscillations (MPO), to respond selectively to oscillating current injections at different frequencies (resonance), and to integrate postsynaptic potentials. We also compared resting membrane potential, input resistance, spike afterpotentials, and repetitive firing evoked by depolarizing current steps. To study resonance at subthreshold potentials, we applied a frequency-modulated sinusoidal current whose frequency increased linearly with time. Our results shows that the majority of dorsal CA1 pyramidal neurons (n=22/30) have a prominent resonance peak (frequency: 2.5±0.3Hz) compared to the ventral side where only few cells (n=7/30) showed detectable resonance, and the resonance frequency was lower (0.8±0.2Hz). We also observed a significant difference in frequency of subthreshold MPOs and in the synaptic potential integration at depolarized potential. MPOs in CA1 pyramidal neurons are probably generated by interplay between persistent Na^+ current and a slowly activating K^+ current, and it has been shown that subthreshold theta resonance in these cells is generated by Kv7/M-type K^+ channels and persistent Na^+ current. The Kv7 channels also regulate excitability, spike afterpotentials, and spike frequency adaptation. We tested the effects of the selective Kv7 channel blocker XE991, as well as muscarine, which also inhibits Kv7/M channels. Application of XE991 and muscarine lowered the spike threshold and eliminated MPOs and the theta resonance, and thus had stronger effects in dorsal CA1 pyramidal cells where these Kv7/M-channel-dependent features were most prominent. XE991 also increased the synaptic integration in the dorsal CA1 pyramidal cells. Our

findings indicate that the biophysical properties of CA1 pyramidal cells differ along the dorso-ventral axis, involving Kv7-dependent mechanisms, including theta resonance, subthreshold synaptic integration and membrane potential oscillations. These dorso-ventral differences may be related to cognitive/spatial vs. emotion-related functions.

Disclosures: M. Marosi: None. **C. Hönigsperger:** None. **J.F. Storm:** None.

Poster

847. Intrinsic Membrane Properties: Neural Oscillation

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Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant R01 MH085074

DFG Grant BR2916/2-1

Title: Spike phase locking in CA1 pyramidal neurons depends on background conductance and firing rate

Authors: *T. BROICHER[1], P. MALERBA[2], A. D. DORVAL[2], A. BORISYUK[3], F. R. FERNANDEZ[2], J. A. WHITE[2]
[1]Salt Lake City, UT; [2]Brain Inst., Salt Lake City, UT; [3]Dept. of Mathematics, Univ. of Utah, Salt Lake City, UT

Abstract: Oscillatory activity in neuronal networks correlates with different behavioral states throughout the nervous system, and the frequency-response characteristics of individual neurons are believed to be critical for network oscillations.
Recent in vivo studies suggest that neurons experience periods of high membrane conductance, and that action potentials are often driven by membrane-potential fluctuations in the living animal.
To investigate the frequency-response characteristics of CA1 pyramidal neurons in the presence of high conductance and voltage fluctuations, we performed dynamic-clamp experiments in rat hippocampal brain slices.
We stimulated neurons with Poisson-process-driven inputs with sinusoidal modulation, and determined action potential phase-locking profiles with respect to background conductance and firing rate.
Modulations covered a wide range of physiological frequencies, encompassing the delta, theta, beta, and gamma bands.

We found that background conductance and firing rate qualitatively change the phase locking of CA1 pyramidal neurons: across conditions, we observed low-pass and band-pass profiles, the latter centered at either theta or beta-gamma ranges.
Three factors are important in shaping phase-locking profiles in response to changes in firing rate or membrane conductance.
Phase locking to low modulation frequencies is dominated by the degree of spike-rate adaptation.
Intrinsic frequency preferences in the spike-generating mechanism contribute to mid-frequency phase locking.
Responses to high modulation frequencies are dominated by the action potential cutoff frequency of the postsynaptic membrane.
Together, these mechanisms may provide pyramidal cells with the ability to adjust their frequency preference in response to the global changes in activity associated with different behavioral states.

Disclosures: T. Broicher: None. **P. Malerba:** None. **A.D. Dorval:** None. **A. Borisyuk:** None. **F.R. Fernandez:** None. **J.A. White:** None.

Poster

847. Intrinsic Membrane Properties: Neural Oscillation

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:847.04/C50

Topic: B.10. Intrinsic Membrane Properties

Support: Carver Foundation Grant 11-38870

UIUC ICR Start-up Fund

Title: Novel molecular players of homeostatic intrinsic plasticity

Authors: *K. LEE, S. ROYSTON, M. VEST, D. LEY, H. CHUNG
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Abstract: Homeostatic plasticity of intrinsic excitability (Intrinsic plasticity) encompass the regulatory mechanisms by which neurons maintain a physiologic balance between neuronal excitation and inhibition, and is thought to play a large role in the pathogenesis of epilepsy and pain disorders. However, the molecular basis of this important plasticity is unknown. Here we show that prolonged blacked of neuronal activity by tetrodotoxin (TTX, voltage-gated sodium channel antagonist) leads to a compensatory increase in intrinsic excitability in dissociated hippocampal cultures neurons. Conversely, prolonged enhancement of neuronal activity via

bicuculline (BC, GABAA receptor antagonist) administration results in a homeostatic decrease in intrinsic excitability. To identify novel gene transcripts whose expression is mediated by global activity changes, we performed cDNA microarray analysis following induction of homeostatic intrinsic plasticity. Genes involved "K^+ ion transport" were significantly overrepresented, the majority of which encodes K^+ ion channels that critically function to dampen neuronal excitability. TTX treatment repressed 11 out of 12 K^+ channel genes, whereas BC treatment induced 4 out of 7 K^+ channel genes. In particular, a significant bidirectional change was found for genes responsible for A-type potassium currents that serve as a critical brake for repetitive firing of action potentials. We have verified activity-dependent changes in K^+ channel gene expression using quantitative real-time PCR. Furthermore, the observed TTX and BC-induced changes in excitability correlate with induce changes in the protein expression and current density of these K^+ channels. Most importantly, the observed TTX-induced homeostatic increase in excitability is mimicked by inhibition of NMDA receptors but not L-type voltage gated calcium channels. Together, our findings suggest that activity-dependent expression of K^+ channels and their associated proteins via NMDA receptor-dependent signaling may contribute to the stabilization of hippocampal neuronal excitability by serving as key proteins in the molecular mechanisms governing homeostatic intrinsic plasticity.

Disclosures: K. Lee: None. **S. Royston:** None. **M. Vest:** None. **D. Ley:** None. **H. Chung:** None.

Poster

847. Intrinsic Membrane Properties: Neural Oscillation

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Program#/Poster#:847.05/C51

Topic: B.10. Intrinsic Membrane Properties

Support: NIH NS061097

Title: Adaptation induced by slow inactivation of Na channels underlies depolarization block in a model dopamine neuron

Authors: *K. QIAN[1,2], M. A. HUERTAS[1], C. C. CANAVIER[1]
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Abstract: Dopamine neurons pace spontaneously in vitro but go into depolarization block (DB) at applied current levels that are not sufficient to elicit the high frequency firing observed during behaviourally relevant bursts in vivo. Thus simple depolarization is not sufficient for burst signaling in vivo. Also, induction of DB in dopamine neurons has been correlated with the

efficacy of antipsychotic drugs, and antipsychotics have been shown to rapidly induce depolarization block in a developmental rat model of schizophrenia. When entering DB during an applied current step, full amplitude spikes are produced until spike failure occurs at potentials near the original spike threshold.
In order to capture the essential features of depolarization block, we started with a one compartment model of a dopamine neuron with a sodium current rigorously fitted to recent published experimental data, and a single composite potassium current. A fast-slow analysis was used to reduce the model to two variables, and as the stimulus current was increased the model entered DB in manner inconsistent with experimental observations. The maximum hyperpolarization after an action potential decreased and the membrane potential hung up at a depolarized potential relative to the initial spike threshold. Adding a second component of sodium channel inactivation that inactivates sharply above -50 mV but recovers slowly at hyperpolarized potentials allowed the model to enter DB in a manner consistent with experimental observations. Bifurcation analyses showed that the mechanism underlying the two types of transition into depolarization block are distinct. Preliminary results indicate that a slow activation of a potassium current cannot be responsible for a transition from pacemaking to DB consistent with experimental observations. Some of these results have been reproduced in a morphologically realistic, multicompartmental model of a dopamine neuron. Preliminary modeling results show that activation of an NMDA conductance can delay the entry into depolarization block, consistent with a role for that current in circumventing the frequency limitation during bursts in vivo.

Disclosures: K. Qian: None. **M.A. Huertas:** None. **C.C. Canavier:** None.

Poster

847. Intrinsic Membrane Properties: Neural Oscillation

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Topic: B.10. Intrinsic Membrane Properties

Support: Gatsby Charitable Foundation

Title: A model of carbachol dependent, self-terminating persistent activity in the rat somatosensory cortex

Authors: *S. ZIBMAN[1], H. SOMPOLINSKY[2,3], Y. YAROM[2]
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Abstract: Muscarinic activity has long been known to play a role in the functions of the somatosensory cortex (Sarter and Bruno, 1997). Additional studies have shown somatosensory neurons to display persistent activity during behavioral tasks (Fuster, 2004). Together these findings make the somatosensory cortex an attractive candidate to find single neuron muscarinic dependent persistent activity.

Using whole cell recordings accompanied by pharmacological agents, we explored persistent activity in the rat somatosensory cortex. In the presence of 20 μM carbachol, a one second burst of action potentials at 30Hz was followed by a slow after-depolarizing potential (ADP) often associated with seconds long burst of spikes. The distribution of spiking duration is multimodal with a maximum duration surpassing one minute. Using a hybrid clamp protocol, we measured a peak inward current of 15pA occurring at -60 mV with a decay of 4 seconds. The addition of 50 μM flufenamic acid (FFA), a specific blocker of calcium dependent nonspecific cation current (I_{CAN}), prevents the appearance of persistent activity, thus indicating the involvement of I_{CAN}. The fact that spiking exceeds the length of non-spiking ADPs indicates that the spiking is a regenerative process and not merely the result of the I_{CAN} raising the voltage above threshold. Yet, suppressing the spiking response for up to 30 seconds using hyperpolarizing pulses did not prevent a subsequent renewal of the spiking response. This indicates that there is also a component which is not dependent on the spiking. Based on the observation that emptying internal calcium stores with 2 μM thapsigargin reduces the inward current, we suggest that this component relates to the inositol 1,4,5-triphosphate (IP_3)-induced Ca^{2+} release from internal stores.

We present here a model that successfully reproduces these experimental findings. Persistent activity results from balancing two calcium sensitive currents, I_{CAN} and a potassium current with spike induced calcium influx. Calcium is also released from internal stores and is maintained by calcium's up regulation of IP_3 production during the early part of the response, thus providing a temporary independence of the calcium levels from the spiking. Once internal calcium release returns to base levels, persistent activity becomes sensitive to the firing rate of the neuron. Intrinsic noise can disrupt the firing rate and by extension the influx of calcium. We show that the presence of this noise explains the multimodal distribution of the spiking duration.

Disclosures: S. Zibman: None. **H. Sompolinsky:** None. **Y. Yarom:** None.

Poster

847. Intrinsic Membrane Properties: Neural Oscillation

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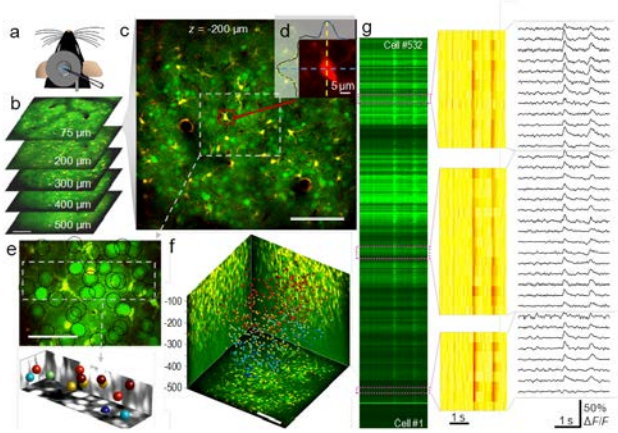
Marie Curie and EMBO fellowship to D.H

Friedrich Miescher Institute funds

Title: Three-dimensional two-photon imaging of dendritic and network activity with millimeter z-scanning range and sub-millisecond temporal resolution in *in vitro* and *in vivo*

Authors: *B. ROZSA[1,2], A. KASZÁS[1,3], P. MAÁK[4], G. SZALAY[1], B. CHIOVINI[1], M. VERESS[4], D. HILLIER[5], D. PÁLFI[1,2], K. SPITZER[1,6], B. ROSKA[5], G. KATONA[1]
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Abstract: The understanding of brain computations requires methods that read out neural activity on different spatial and temporal scales. Although following signal propagation and integration across a neuron and recording the concerted activity of hundreds of neurons pose distinct challenges, a single recording system performing both operations is desirable. Here we present a three-dimensional (3D), high-resolution, acousto-optic two-photon microscope with continuous 3D trajectory and random-access scanning modes reaching a millimeter scan range (up to 1,000 x 1,000 x 2,000 μm^3 in transparent sample), adaptable to different spatial scales. The subcellular scale is demonstrated by presenting 3D optical recordings of action potential back propagation in hundred locations and dendritic Ca^{2+} spike forward propagation in long dendritic segments situating in *in vitro*, at near-microsecond temporal resolution. Here, the high temporal resolution of mean transients made overcoming Nyquist's sampling theory possible by using a method adopted from oscilloscope technology. Finally, the network scale is shown by volumetric random-access Ca^{2+} imaging of spontaneous and visual stimulation-evoked activity from hundreds of neurons in the visual cortex *in vivo*.



Figure, High-speed 3D calcium imaging of spontaneous neuronal network activity of 532 automatically detected cells in vivo

Disclosures: **B. Rozsa:** Ownership Interest; Femtonics. **A. Kaszás:** None. **P. Maák:** None. **G. Szalay:** Employment; Femtonics. **B. Chiovini:** None. **M. Veress:** None. **D. Hillier:** None. **D. Pálfi:** Employment; Femtonics. **K. Spitzer:** None. **B. Roska:** None. **G. Katona:** Employment; Femtonics. Ownership Interest; Femtonics.

Poster

847. Intrinsic Membrane Properties: Neural Oscillation

Location: Hall F-J

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Program#/Poster#:847.08/C54

Topic: B.10. Intrinsic Membrane Properties

Support: Wellcome Trust

Title: The impact of early life stress on *in vitro* gamma oscillations

Authors: *K. NEWLING, G. RENTESI, M. KAISER, M. O. CUNNINGHAM
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Abstract: Early life adversity in the form of abuse, neglect or parental loss during early development is associated with an increased risk of many neuropsychological disorders in adulthood. These include depression, post-traumatic-stress disorder, panic disorder, attention deficit disorder (for review see 1) as well as psychosis [2] and schizophrenia [3]. Stress exposure in early life is also linked to deficits in cognitive function, including impaired spatial learning and memory [4].

A well-established rodent model of early life stress is 24 hour maternal deprivation at postnatal day 9[5]. Maternally deprived (MD) adults display a range of behavioural impairments, many of which are commonly described in neuropsychiatric disease [6]. MD rodents show alterations in the stress response system, hippocampal morphology and specific NMDA receptor expression. However, little information exists concerning the impact of early life stress on neuronal function, including the ability of cortical networks to generate persistent rhythmic activity of cognitive relevance.

Gamma oscillations (30-80Hz) are crucial for the coordination of neural activity across many brain areas simultaneously [7] and are involved in many cognitive functions [8, 9]. We examined these oscillations in MD adult male Wistar and control rats, which remained with the dam until weaning at postnatal day 21.

Extracellular local field potential (LFP) recordings were taken in LII/III of the medial entorhinal cortex (mEC), and the CA3 subfield of the hippocampus (HPC), in combined EC-hippocampal brain slices (450µm) prepared from MD and control adult rats. Gamma oscillations were induced by bath application of kainate (2-500nM). Results will potentially reveal a difference in gamma frequency and power between MD and control groups, and we hypothesize that disruptions of cortical interneuronal function underlie any deficiencies. Pharmacology and post-hoc immunocytochemistry will be used to examine any differences in parvalbumin positive interneuron number and connectivity.

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Disclosures: K. Newling: None. **G. Rentesi:** None. **M. Kaiser:** None. **M.O. Cunningham:** None.

Poster

847. Intrinsic Membrane Properties: Neural Oscillation

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Program#/Poster#:847.09/C55

Topic: B.10. Intrinsic Membrane Properties

Title: Mixed lattice models of charge distribution, interactions and transport in neural tissue

Authors: *S. PAJEVIC
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Abstract: In equilibrium, the intracellular space is at a negative potential relative to the extracellular space as a result of active transport of ions across the lipid bilayer membrane and the balance of concentration and electrical gradients. In the first part we study the charge distribution at the membrane interface and within tight extracellular spaces, in the simplest form described by the diffuse electrical double layer model. Here we extend this model to planar interfaces containing an ordered lattice of cylindrical channels, representing the transmembrane protein channels often organized into a long-range lattice order. As a particular example we use hexagonal lattice of gap junctions, and study the closed and open state (without or with current) configurations. The lattice constant (~80 A) and the radii of the approximating cylinders (~20 A) are comparable to the Debye or Bjerrum lengths, hence the structure of the lattice will be reflected in the distribution of the charges. This equilibrium model of charge distribution applies also to the non-excitable tissues, such as liver or pancreas. In the second part we focus on the excitable tissues, in our particular case the neural tissue abundant in gap junctions, and propose a non-equilibrium dynamical model. The model focuses on oscillations and transitions in non-diffusive transport and conductivity, to be contrasted with the intracellular diffusive transport that is usually emphasized in the case of gap junctions. Structurally the model contains a mixed hierarchy of lattices, suggesting that the lattices can possess different symmetries at different levels of the hierarchy (or that at particular level the lattice itself is mixed), but also that the interactions and the dynamics of collective modes at different levels can potentially be treated in a mixed fashion, the ones at higher levels treated classically. In the case of gap junctions we presently exclude the symmetries at finer scales, for example the hexagonal arrangements of connexins in a connexon, or helical or other symmetries of atoms within connexin, and focus only on two levels. The first is a honeycomb lattice of ionic traps that approximates the above derived distribution, and the second is a quasi-periodic lattice of potential wells, an idealization of the interdigitated character of the extracellular and intracellular domains which can be extended to planar or volumetric forms. In neural tissue, consisting of neurons and glial cells, this "brain lattice" model provides an interaction dependent relationship between the spatio-temporal pattern of neuronal activations and the pattern of collective modes, as the system’s relevant degrees of freedom.

Disclosures: S. Pajevic: None.

Poster

847. Intrinsic Membrane Properties: Neural Oscillation

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Topic: B.10. Intrinsic Membrane Properties

Support: the Korea Institute of Oriental Medicine (K12272)

Title: Differentation with primo vessel and smooth muscle in action potential

Authors: *Y. RYU, S. CHO, S. LEE, S. LEE, K. CHOI, S. YEON, S. CHOI
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Abstract: Recently, certain researchers have become interested in Primo-vascular system (the scientific name for Bonghan system) and have tried to investigate Primo-vascular system by finding Primo-node (Bonghan corpuscle) and Primo-vessels (Bonghan ducts) . It was too difficult to study the functions of Primo-vessels directly because of their small size. Therefore, more research based on electrophysiology was needed, for example, action potential measurements. It is well known that tissues have different action potentials depending on their structure and function. Thus, We studied the action potential of Primo-vessels in rats to determine the electrophysiological characteristics of these structures
The study presented herein was a comparison of the action potentials generated by Primo-vessels with the pacemaker potential, made by smooth muscle from the small intestine. We also introduced a mathematical analysis method, a normalized Fourier transform that is more useful for viewing the waveform and phase than the power spectrum from a general Fourier transform. We introduced a mathematical analysis method, a normalized Fourier transform that displays the sine and cosine components separately, to compare the action potentials of Primo-vessels with those for the smooth muscle. We found that Primo-vessels generated two types of action potential pulses that differed from those of smooth muscle: 1) Type I pulse had rapid depolarizing and repolarizing phases, and 2) Type II pulse had a rapid depolarizing phase and a gradually slowing repolarizing phase.
The sharp top and larger amplitude of the pulses generated by the Primo-vessels could distinguish them from the pulses generated by the smooth muscle; thus, it is possible to assume that the Primo-vessels perform a different function from the smooth muscle. For confirmation of this hypothesis, further study is needed regarding the physiological mechanism responsible for generate these pulses and regarding which ion channels are used.

Disclosures: Y. Ryu: None. **S. Cho:** None. **S. Lee:** None. **S. Lee:** None. **K. Choi:** None. **S. Yeon:** None. **S. Choi:** None.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

Location: Hall F-J

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Topic: B.11. Glia-Neuron Interactions

Support: FAPERJ

Pronex

CNPq

Title: Cultured mice Müller glia cells differentiate dopaminergic phenotype and recovers locomotor deficits in hemiparkinsonian mice

Authors: *B. STUTZ[1,2], F. CONCEICAO[3], L. SANTOS[1], D. CADILHE[3], J. HOUZEL[3], P. GARDINO[2], R. REIS[2], S. REHEN[3], F. DE MELLO[1]
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Abstract: Müller cells constitute the main glial cell type in the retina and span the tissue from the inner to the outer limiting membranes. Several functions have been attributed to these cells including structural and nutritional roles as well removal of ions and neurotransmitters from the extracellular space. The potential of Müller cells to actively participate in cellular communication within the nervous system has been recently uncovered. Moreover, it has been suggested that this type of cell can undergo de-differentiation process upon specific signals and become a progenitor-like cell. Also, it has been shown that Müller cells can generate neurons under appropriate conditions. The knowledge on controlling this particular differentiation could be extremely useful as potential therapy against neurodegenerative disorders. In the present work, we evaluate the capability of Müller cells to be used as a source of neuronal cells and evaluate the functional dopaminergic differentiation of Müller glia into dopamine releasing cells. We have shown that Müller cells in culture (n=3) not only express dopaminergic markers as the dopamine transporter (DAT), tyrosine hydroxylase (TH), dopa decarboxylase (DDC) and the transcription factor for dopaminergic differentiation Nurr1 but they are also able to produce and release dopamine in vitro (n=5), reaching values of 50 ± 10nmoles or 65 ± 8nmoles, when treated either with 10µM forskolin (FK) or 10nM PACAP38, respectively. TH activity assay confirms increase in TH activity in these cells after treatment with FK or PACAP38. Moreover, injection of functional dopaminergic differentiated Müller cells in mouse model of Parkinson's disease obtained with stereotactic injection of 6-OH-DA in the striatum clearly reduced the typical apomorphine-induced rotational behavior presented by the animals (n=7).

Disclosures: B. Stutz: None. **F. Conceicao:** None. **L. Santos:** None. **D. Cadilhe:** None. **J. Houzel:** None. **P. Gardino:** None. **R. Reis:** None. **S. Rehen:** None. **F. de Mello:** None.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

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ALF Gothenburg

STENA Foundation

EU project EduGlia (237956)

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NanoNet COST Action

AFA Insurance

Title: Behavioral and inflammatory responses to photothrombotic lesions in mice deficient for astrocyte intermediate filaments

Authors: *M. J. PORRITT, Y. DEPABLO, U. WILHELMSSON, M. NILSSON, M. PEKNA, M. PEKNY
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Abstract: Astrocytes are important for normal CNS maintenance and homeostasis. After brain ischemia, astrocytes become reactive and limit further tissue loss. Hallmarks of reactive gliosis are cellular hypertrophy of astrocyte processes and the upregulation of astrocyte intermediate filament proteins glial fibrillary acidic protein (GFAP) and vimentin. Reactive astrocytes also change the expression of many other genes. We previously demonstrated that following focal brain ischemia, the infarct is increased in mice deficient in both GFAP and vimentin (*GFAP*$^{-/-}$ *Vim*$^{-/-}$), but not in *GFAP*$^{-/-}$ or *Vim*$^{-/-}$ mice, and proposed that astrocyte intermediate filaments are important for neuroprotection in ischemic stroke. Here we studied the role of astrocyte intermediate filaments in the brain tissue response and behavioral recovery after focal cortical photothrombotic lesions. Ischemic lesion volumes between *GFAP*$^{-/-}$*Vim*$^{-/-}$ and wild-type (WT)

mice did not differ at 1 or 3 days post ischemia. Gender did not affect lesion volume. The number of neutrophils within the infarct 3 days after ischemia was higher in *GFAP*$^{-/-}$*Vim*$^{-/-}$ males mice than in WT males (p<0.018) and this difference was more pronounced within the infarct core (p<0.008) than the peri-infarct area. *GFAP*$^{-/-}$*Vim*$^{-/-}$ males showed lower number of microglia both in the peri-infarct and infarct core compared to WT males (p<0.045 and p<0.022, respectively). Proteomics analysis of the infarcted cortex 1, 2 and 3 days after ischemia suggests that components of the inflammatory system are upregulated in *GFAP*$^{-/-}$*Vim*$^{-/-}$ mice. ELISA quantification of the third complement component (C3) in the infarcted cortex showed that 1 day after ischemia, *GFAP*$^{-/-}$*Vim*$^{-/-}$ mice had substantially higher levels of C3 protein than WT mice (p<0.05). One day after ischemia, the neurological deficit was larger in *GFAP*$^{-/-}$*Vim*$^{-/-}$ than WT mice. Remarkably, 3 days after ischemia *GFAP*$^{-/-}$*Vim*$^{-/-}$ mice showed the same motor function as WT mice. We show that mice deficient in intermediate filament proteins GFAP and vimentin have reduced capacity to limit the lesion-induced loss of function in the very acute phase after ischemia, possibly due to altered modulation of the inflammatory response, but they reach remarkable functional recovery shortly thereafter.

Disclosures: M.J. Porritt: None. **Y. dePablo:** None. **U. Wilhelmsson:** None. **M. Nilsson:** None. **M. Pekna:** None. **M. Pekny:** None.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

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Topic: B.11. Glia-Neuron Interactions

Support: NIH 5R21NS072575

Title: Using *Drosophila* to model fluid volume regulation of the nervous system

Authors: W. M. LEISERSON, N. GONG, *H. S. KESHISHIAN
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Abstract: The nervous systems of humans and many animals are isolated from the rest of the organism by blood-brain and blood-nerve barriers. Membrane proteins, including pumps, transporters, and channels, permit the tight regulation of the extracellular ionic environment. The nervous system is, as a consequence, sensitive to osmotic differences between it and the rest of the organism, and the ability to regulate both ion composition and fluid volume likely co-evolved with the barrier. In humans, loss of fluid regulation, such as occurs following stroke or TBI, can have severe clinical consequences. We have identified two components of a molecular system in

Drosophila, conserved in humans, that regulates extracellular volume in the nervous system: the Na-K-Cl cotransporter, Ncc69, an ortholog of human NKCC1; and the kinase, Fray, an ortholog of human SPAK. Ncc69 and Fray function in the barrier glial cells of larval nerves. Knockdown mutations in either of the genes cause a neuropathy, where fluid accumulates within the nerves, resulting in dramatically increased nerve diameters. Mutations in either gene can be rescued by its corresponding mammalian ortholog, demonstrating a conserved molecular mechanism. Like Ncc69, human NKCC1 is found in barrier cells of the nervous system, and has been implicated in volume regulation. To find other molecular components that function to regulate the volume of the nervous system, we have undertaken a candidate RNAi screen, knocking down gene expression in the barrier glial cells, looking for swollen nerves. We identified Wnk, whose mammalian homologs regulate volume by influencing SPAK and NKCC1. Increased nerve widths were also observed when knocking down either of two K2P (two-pore-domain potassium channel) genes or the gene encoding the alpha subunit of the Na/K ATPase. Mutants bearing Wnk alleles have a nerve phenotype resembling that of fray and Ncc69, with nerve bulges that are nearly 10 times the width of normal nerves. TEM of the RNAi knockdown of Task6, one of the K2Ps, revealed fluid accumulation in the nerves, similar to Ncc69 and fray. In addition, the barrier glia in Task6 knockdowns had an unusual number of membrane foldings, in which the septate junctions appeared disorganized. Our results show that aspects of the molecular mechanisms that regulate the fluid volume of the nervous system are highly conserved. The Drosophila molecular and genetic toolkit provides a means, therefore, to gain further insights into fluid regulation in vivo, revealing mechanisms that are relevant to fluid regulation in the human nervous system.

Disclosures: W.M. Leiserson: None. **N. Gong:** None. **H.S. Keshishian:** None.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

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Program#/Poster#:848.04/C60

Topic: B.11. Glia-Neuron Interactions

Title: IKKβ inhibits adult neurogenesis in the hippocampal dentate gyrus in mice

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Abstract: Hippocampal dentate granule neurons are sustainably generated from adult neural stem cells (NSCs) in the mammalian brain. The local niche and environment regulate the fate specification of adult NSCs. Toll-like receptors (*TLRs*) have been known to play an important role in adult neurogenesis in the hippocampus. However, the role of *IKKβ*, a downstream molecule of *TLRs*, is not understood in adult neurogenesis. To investigate the role of *IKKβ* in adult neurogenesis, we used tamoxifen inducible GFAP-CreERT2/$IKK\beta^{F/F}$ mice (*IKKβ* conditional knock down mice) in which the *IKKβ* gene is specifically ablated in GFAP- positive neural stem cells. Our results may be summarized as follows.
1. Total *IKKβ* expression in hippocampus and *IKKβ* in Nestin-GFAP positive cells (radial stem cells) were decreased around 50% in GFAP-CreERT2/ $IKK\beta^{F/F}$ mice.
2. NeuroD1 and BDNF expression were increased around 17 and 1.5 times in respectively in *IKKβ* - ablated adult neural stem cells.
3. Ablation of *IKKβ* increased proliferation and differentiation (immature and mature) of adult neural stem cells in the SGZ.
4. Ablation of *IKKβ* increased cell survival of neural stem cells.
5. There's no significant differences in glial differentiation between *IKKβ* deficient mice and vehicle treated mice.
These data suggest that *IKKβ* inhibits adult neurogenesis from the hippocampal dentate gyrus through decreasing proliferation, survival and differentiation of the neural stem cells.

Disclosures: K. Seong: None. **M. Baek:** None. **S. Choi:** None. **J. Jung:** None. **W. Kim:** None. **M. Kim:** Research Grant; Department of Oral Physiology, School of Dentistry, Chonnam National University.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:848.05/C61

Topic: B.11. Glia-Neuron Interactions

Support: 1P30NS069324-02

Title: Epigenetic regulation of Kir4.1 following traumatic injury in the developing brain

Authors: S. E. NWAOBI, *M. L. OLSEN
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Abstract: Traumatic brain injury (TBI) is the leading cause of death and disability in young children in the United States. Once thought to be more resilient to head trauma, new data

suggests pediatric patients are more susceptible than their adult counterparts to secondary injury processes including excitotoxicity, apoptosis, cerebral swelling and vascular damage.
Astrocytes play a critical role in maintaining homeostatic extracellular potassium, $[K^+]_e$, levels within the CNS. Regulation of $[K^+]_e$ is largely mediated by Kir4.1, which is an inwardly rectifying potassium channel. Kir4.1 sets the hyperpolarized resting membrane potential in 'mature' astrocytes; this serves as a source of energy, driving several cellular processes including glutamate uptake. The importance of Kir4.1 in K^+ regulation in the CNS is exemplified in animal models where knocking down or pharmacological inhibition of this channel results in seizures, ataxia and premature death. **Notably, mutations in Kir4.1 in humans are linked to developmental abnormalities characterized by early onset seizures, ataxia and epilepsy.**
Following injury, there are persistent alterations in the biophysical membrane properties of astrocytes, including depolarized resting membrane potentials, reduced Kir4.1 mediated currents and altered extracellular potassium dynamics, suggesting an overall dysregulation of potassium. In human patients, acute increases in $[K^+]_e$ contribute to lesion expansion and edema. Importantly, brain edema is twice as likely to occur in children as adults following TBI and is a major risk factor for death and neurologic impairment. Chronic elevations of $[K^+]_e$ are implicated in neuronal hyperexcitability, seizures and injury induced epilepsy.
We propose that loss of Kir4.1 is a key component in these pathological sequelae and may impact recovery and brain maturation, particularly in the highly plastic, developing pediatric brain. We have developed a pediatric model of TBI to study changes in Kir4.1. By use of lateral fluid percussion injury on postnatal day 14 rats, we demonstrate astrogliosis accompanied by a decrease in Kir4.1 protein following injury.
Despite the importance of Kir4.1 protein for normal brain function, little is known regarding its regulation in the developing brain or after injury. We further propose that K^+ homeostasis is dysregulated following injury due to epigenetic repression of Kir4.1. We hypothesize that post-injury decreases in Kir4.1 are mediated by DNA methylation, providing a potential drug-modifiable mechanism that can be therapeutically targeted to minimize effects of dysregulated K^+.

Disclosures: S.E. Nwaobi: None. **M.L. Olsen:** None.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:848.06/C62

Topic: B.11. Glia-Neuron Interactions

Support: NIH

Title: Knockout of type 2 IP3 receptor in astrocytes enhances cell proliferation in the subgranular zone in dentate gyrus of hippocampus in mice

Authors: W. CHANG[1], H. LI[2], N. ZHANG[1], *S. DING[3]
[1]Univ. of Missouri, Columbia, MO; [2]Universitry of Missouri, Columbia, MO; [3]Univ. of Missouri, COLUMBIA, MO

Abstract: Neurogenesis (birth of neurons) is a process by which neurons are generated from neural progenitor cells. It mainly occurs in two regions of brain, one is the sub-ventricular zone (SVZ) and the other is the subgranular zone (SGZ) in dentate gyrus of hippocampus. Astrocytes release calcium from intracellular stores in response to the activation of different types of Gq-linked G-protein-coupled receptors (GPCRs) through the binding of inositol 1, 4, 5-trisphosphate (IP_3) to its receptor (IP3R). Astrocyte Ca^{2+} has been deemed necessary and sufficient to trigger the release of glio-transmitters, such as ATP and glutamate, to modulate neuronal activity. Several lines of evidence suggest that IP_3R type 2 (IP_3R2) is the primary IP_3R expressed in astrocytes. In this study, we use IP_3R2 knockout mice (KO) mice to determine whether IP_3R2 affects neuronal proliferation in SGZ. We injected Brdu in the KO and WT mice consecutively for 6 days and scarified mice one day after the last injection of Brdu. Then we used immunostaining to study the cell proliferation and lineage commitment of Brdu labeled cells Our results show that IP_3R2 KO mice from age one to 3 month have a larger number of Brdu-labeled cells in SGZ as compared with age-matched wild type (WT) mice. To study the lineage commitment of the Brdu labeled cell, we performed double staining of Brdu/DCX, Brdu/NeuN, Brdu/GFAP. We find IP_3R2 KO mice have higher percentage of Brdu/DCX, Brdu/GFAP double labeled cells, but lower NeuN/Brdu double labeled cell. Furthermore, we studied survival of Brdu labeled cells. As IP_3R2 is mainly expressed in astrocytes, our study suggests that astrocytes may play an important role in adult neurogenesis through IP_3R-mediated calcium signaling pathway.

Disclosures: W. Chang: None. **H. Li:** None. **N. Zhang:** None. **S. Ding:** None.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:848.07/C63

Topic: B.11. Glia-Neuron Interactions

Title: Effect of epoxomicin administration in a epilepsy model (amigdaloid-kindling) in rat.

Authors: *M. H. CERÓN, SR, E. GONZALEZ, J. FRANCO, J. MARTINEZ, M. RUBIO, V. CUSTODIO, C. PAZ
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Abstract: Epilepsy is a chronic disease characterized by repetitive crisis caused by an excessive discharge of the neuronal cells and it is well described that many etiologies can cause it. Amigdaloid-kindling has been proposed for it study. It has been demonstrated experimentally that astrocytosis occurs in different cerebral structures due to recurrent seizures, but little is known about the role of this phenomenon in epilepsy. Because it has been described that epoxomicin
administration inhibits the astrocyte response, in this work we wanted to assess its effect in amigdaloid-kindled rats as well as in the changes in electrographic activity and conductual states of this model. In order to achieve our objectives we implanted in wistar male rats both an amigdala-electrode for the kindling induction and a cannula through which we administered intra-ventricular epoxomicin in increasing doses (50, 126, 312 µM). After seven days of post-operative recovery the rats were daily stimulated until they reached the conductual state 5 described by Racine (1972) corresponding by tonic-clonic generalized seizures. Once this state was observed, the rats were injected with epoxomicin. Our results showed an epoxomicin dose-dependent increase in the afterdischarge duration compared with the control and sham group and we also found that the higher dose group (312 µM) showed ataxia, lethargy, spontaneous seizures and death. Therefore we can conclude that there is a dose-related response in the increasing duration of the afterdischarge, supporting the role of the astrocytes as a epileptic modulators, and that high-dose epoxomicin intra-ventricular administration can be the lethal dosage in rats.

Disclosures: M.H. Cerón: None. **E. Gonzalez:** None. **J. Franco:** None. **J. Martinez:** None. **M. Rubio:** None. **V. Custodio:** None. **C. Paz:** None.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:848.08/C64

Topic: B.11. Glia-Neuron Interactions

Title: SNARE-dependent vesicular release of neuroactive molecules from astrocytes modulates the structure of the node of Ranvier

Authors: H. LEE, H. WAKE, P. R. LEE, *R. FIELDS
NICHD, NIH, BETHESDA, MD

Abstract: The node of Ranvier, which increases conduction velocity of action potentials, is a key element of information processing in the brain. Astrocytes, which are a major glial cell in the central nervous system, surround nodes to maintain appropriate ion concentration. However, regulation of nodal development and organization by astrocytes and the consequences for information processing remain unresolved. Recently, it has been shown that astrocytes modulate neuronal activity by releasing neurotransmitters at neuronal synapses. We have utilized transgenic mice that express an inducible dominant negative SNARE domain in astrocytes (Courtesy of Ken D. McCarthy) to determine whether release of synaptic vesicles from astrocytes affects formation of the node of Ranvier. These mice enable us to impair SNARE dependent vesicular release in astrocytes in early postnatal life when nodes of Ranvier are being formed. Astrocytic specific expression of the transgene resulted in an enlargement of the nodal gap at nodes of Ranvier. This finding was observed in optic nerves and corpus callosum in the brain. G-ratio was increased in the optic nerve of transgenic mice, indicating that myelin thickness was reduced. Our results suggest that SNARE-dependent vesicular release from astrocytes modulates nodal formation and myelination. This could alter saltatory conduction along myelinated axons and greatly affecting information processing in the brain.

Disclosures: H. Lee: None. **H. Wake:** None. **P.R. Lee:** None. **R. Fields:** None.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:848.09/C65

Topic: B.11. Glia-Neuron Interactions

Support: NIH Grant MH086522

Title: Chondroitin sulfate proteoglycan-positive glial cells in the human amygdala and entorhinal cortex: Anatomical distribution in relationship to axonal pathways

Authors: A. SANTOS, A. BOYER-BOITEAU, H. PANTAZOPOULOS, M. MARKOTA, *S. BERRETTA
Translational Neurosci. Lab., Mrc3- McLean Hosp., Belmont, MA

Abstract: Recent results show that a distinct subpopulation of glial fibrillary acidic protein (GFAP)-positive glial cells in the human temporal lobe strongly expresses chondroitin sulfate

proteoglycans (CSPGs). CSPGs are main components of the extracellular matrix, known to regulate developmental and adult neural functions including axonal outgrowth and plasticity. In the healthy human brain, CSPG-positive glial cells have a distinct, highly segregated, and interindividually consistent, pattern of anatomical distribution within restricted subregions of the amygdala and entorhinal cortex. In subjects with schizophrenia, CSPG-positive glial cells were found to be markedly increased in these regions, raising the possibility that these cells may play a role in the pathogenesis of this disorder. Characterization of CSPG-positive glial cells in the human brain is thus necessary to the understanding of the pathophysiological significance of these abnormalities. With this study we tested the hypothesis that the distinct distribution of CSPG-positive glial cells in the human amygdala and entorhinal cortex may be linked to that of incoming axonal pathways. To test this hypothesis, distinct fiber markers, i.e. myelin (Black Gold II; 2',3'-cyclic nucleotide 3' phosphodiesterase (CNPase)) and p75 (a putative marker for cholinergic fibers) were combined with CSPG labeling in medial temporal lobe sections from human subjects (n=3). Myelin-positive fibers were abundant within the amygdala and the entorhinal cortex. CSPG-positive glial cells were located within myelin-rich areas surrounding the central nucleus of the amygdala and the ventral portion of the cortical nucleus. In the entorhinal cortex, myelinated fibers course in bundles running perpendicular to the pial surface but become parallel to it when they reach layer II. CSPG-positive glial cells were highly enriched within dense portions of these parallel coursing myelinated fibers. Putatively cholinergic, p75-labeled fibers form bundles coursing dorsoventrally within the lateral portion of the amygdala, and then disperse along the ventral amygdala border. CSPG-positive glial cells were strongly associated with p75-positive fiber bundles. Together, these results support the hypothesis that the location of CSPG-positive glial cells within the human amygdala and entorhinal cortex may be linked to the distribution of distinct incoming pathways. This spatial relationship is consistent with a key role of CSPGs in axonal guidance and raises the possibility that CSPG-positive glial cells may contribute to a disruption of neural connectivity in schizophrenia.

Disclosures: A. Santos: None. **A. Boyer-Boiteau:** None. **H. Pantazopoulos:** None. **M. Markota:** None. **S. Berretta:** None.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

Location: Hall F-J

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Topic: B.11. Glia-Neuron Interactions

Support: NIMH K01MH087845

NARSAD Young Investigator Award 2009

NIMH R01MH067715-01A1

Title: Transcriptional profiling of astrocyte-specific mRNAs in conditional Fgfr1 mutants

Authors: *K. M. SMITH, R. R. H. DEEGAN, F. M. VACCARINO
Yale Univ., NEW HAVEN, CT

Abstract: Fibroblast growth factors (Fgfs) and several of their receptors (Fgfr) are expressed by astrocytes and astrocytic stem cell lineages of the developing and adult CNS. We previously showed that conditional inactivation of Fgfr1 in neuronal and astrocyte progenitors of the dorsal telencephalon results in locomotor hyperactivity. However, it was not known whether this effect was due to loss of Fgfr1 in glia or neurons. To address this question, we have performed tamoxifen-inducible inactivation of Fgfr1 in postnatal astrocytes (Postnatal day 14-17, 60 mg/kg i.p.) using the hGFAP-CreERT2 (GCE) transgene. Loss of Fgfr1 in postnatal astrocytes results in locomotor hyperactivity, as was seen with loss of Fgfr1 in glial and neural progenitors. Loss of Fgfr1 in postnatal astrocytes also resulted in decreased anxiety on the elevated plus maze test in both embryonic and postnatal mutants. No differences in learning were observed on a modified one-day morris water maze test. In order to identify molecular targets for the actions of Fgfr1 in astrocytes, we have crossed the ALDH1-L10a GFP BAC into the embryonic Fgfr1 knockout line in order to perform mRNA BACTRAP experiments. Transcripts from the cortex and hippocampus of control and Fgfr1 knockout mice were hybridized to Illumina bead array mouse whole genome expression arrays. Ingenuity pathway analysis (IPA) was performed on the expression array data to examine which biological pathways are disrupted by loss of Fgfr1. In the cortex, IPA analysis identified oxidative phosphorylation and mitochondrial dysfunction as top canonical pathways that are significantly altered in Fgfr1 mutants. In the hippocampus, glucocorticoid receptor signaling, NF-κB signaling, and PTEN signaling were identified as top canonical pathways that were significantly altered.

Disclosures: K.M. Smith: None. **R.R.H. Deegan:** None. **F.M. Vaccarino:** None.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:848.11/C67

Topic: B.11. Glia-Neuron Interactions

Support: National MS Society #RG4019

Title: Endothelin-1 regulates Jagged/Notch signaling to inhibit remyelination

Authors: *T. R. HAMMOND[1,2], A. GADEA[3], A. AGUIRRE[4], V. GALLO[3,2]
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Abstract: Multiple sclerosis (MS) results in demyelinated lesions throughout the central nervous system. In response to demyelination, oligodendrocyte progenitor cells (OPCs) repopulate the lesion and begin to differentiate into mature myelinating oligodendrocytes (OLs). Some lesions, however, fail to remyelinate for unknown reasons. The Jagged1/Notch1 pathway has been previously shown to attenuate OPC development in demyelinated lesions. Here we show that the neuropeptide, endothelin-1 (ET-1), induces an upregulation of Jagged1 expression in cultured astrocytes, which is blocked by pan-endothelin-receptor (ET-R) antagonist PD142,893. We also show that ET-1 pre-treated astrocytes inhibit OPC differentiation in a co-culture system. To investigate the effects in vivo, we used focal injections of lysolecithin (LPC) into the mouse corpus callosum/cingulum to induce focal demyelination. In LPC-injected mice we found that ET-1 levels are upregulated near the lesion, and that ET-1 is expressed by astrocytes at high levels at 3 and 7 days post lesion (dpl). In Western blot analysis of micro-dissected tissue we saw increases in ET-1 at 3 dpl, and a peak in Jagged1 expression at 7 dpl. We also saw increases in Notch intracellular cleaved domain (NICD) expression at 7dpl. Next, we investigated cell specific activation of Notch1 in demyelinated lesions using a transgenic Notch reporter mouse (TNR). We found that Notch1 signaling is activated in remyelinating lesions around 7 dpl, and that Notch1 is mainly activated in oligodendrocyte lineage cells (CC1+ and NG2+). To determine if ET-1 directly influenced Notch1 activation, we infused the ET-R pan-antagonist PD142,893 into the lesion following demyelination and found a decrease in the number of EGFP+ TNR cells. Finally, longer term infusion of ET-R antagonists led to increased numbers of mature oligodendrocytes and increased levels of myelin proteins. Altogether these data show that ET-1 regulates Jagged1 expression in astrocytes, and that this leads to activation of Notch1 in demyelinated lesions. Activation of Notch1 in demyelinated lesions inhibits OPC differentiation and Notch is activated throughout the oligodendrocyte lineage, not just in OPCs. These data show that ET-1 is a major factor that modulates Notch1 signaling during remyelination to inhibit differentiation of oligodendrocytes, and to prevent remyelination. Supported by MS society grant #RG4019.

Disclosures: T.R. Hammond: None. **A. Gadea:** None. **A. Aguirre:** None. **V. Gallo:** None.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:848.12/C68

Topic: B.11. Glia-Neuron Interactions

Support: NIMH Grant MH67121

Title: EphrinB1/EphB receptor signaling in the adult mouse hippocampus following severe traumatic brain injury

Authors: *A. M. NIKOLAKOPOULOU[1], A. OBENAUS[2], I. M. ETHELL[1]
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Abstract: Molecular and cellular mechanisms of neuron-glial interactions that regulate brain repair following traumatic brain injury (TBI) have attracted the attention of many investigators. Recent studies have identified an up-regulation of many genes and molecules in reactive astrocytes that may promote or hinder regeneration of brain circuits after brain injury. Here, we focus our studies on the involvement of ephrinB1/EphB2 receptor signaling in reactive synaptogenesis and brain repair after severe TBI. EphrinB1 acts as a ligand for the EphB receptors activating intracellular signaling pathways which participate in cell adhesion, axon guidance, stem cell proliferation and synaptogenesis. We tested ephrinB1 expression by immunohistochemistry at 1, 3 and 7 days post severe TBI in a controlled cortical impact model. We observed a significant increase in ephrinB1 expression in hippocampal astrocytes. EphrinB1 upregulation was noted in reactive astrocytes 3 days after TBI in the hippocampus including the stratum radiatum and the stratum lacunosum-moleculare, whilst the hilus of the dentate gyrus showed a moderate increase in the number of GFAP positive astrocytes and ephrinB1-expressing cells. Seven days post-injury, we also observed additional upregulation in ephrinB1 expression in astrocytes as well as non-glial cells within the hilus. Surprisingly, the contralateral hippocampus also exhibited a significant increase in ephrinB1 expression in astrocytes at synaptic sites one day post TBI that peaked at 3 days after injury to return to normal levels at seven days after TBI. Interestingly, we see no astrocytic expression of ephrinB1 in the cortex, suggesting that astrocytic upregulation of ephrinB1 is specific to the hippocampus. We have also examined ephrinB1 mRNA expression following the TBI and we are currently investigating the effects of the ephrinB1 up-regulation on EphB receptor signaling in neurons. Furthermore, we are testing the effects of specific ephrinB1 ablation in astrocytes on reactive synaptogenesis and regeneration following TBI in the hippocampus of conditional ephrinB1 knockout mice. Our studies demonstrate that reactive astrogliosis in the adult hippocampus is followed by an early upregulation of ephrinB1 after TBI in regions of ongoing synaptogenesis and neurogenesis/gliogenesis. Interestingly, the non-injured hippocampus is also affected showing an immediate response to injury by increasing microglia activation, astrogliosis and ephrinB1 expression, suggesting that ephrinB1 may play a neuroprotective role in the brain by affecting neuron-glial interactions.

Disclosures: A.M. Nikolakopoulou: None. **A. Obenaus:** None. **I.M. Ethell:** None.

Poster

848. Glia-Neuron Interactions: In vivo approaches II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:848.13/C69

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: CNPq

FAPESP

CAPES

Title: Neuroinflammatory profile and astrocytic morphology in chronic cyclosporine treated rats.

Authors: M. M. CARARO[1], D. G. SOUZA[2], D. Z. ANDREOTTI[1], L. RODRIGUES[2], L. S. LIMA[1], M. ACHAVAL[3], L. V. PORTELA[2], D. O. SOUZA[2], *C. SCAVONE[1], A. E. BÖHMER[1]
[1]Pharmacol., Univ. of Sao Paulo, Sao Paulo, Brazil; [2]Biochem., [3]Morphological Sci., Univ. of Rio Grande do Sul, Porto Alegre, Brazil

Abstract: Cyclosporine (CsA) dependent neurotoxicity occurs in up to 60% of transplant patients and the clinical predisposition and mechanisms of CsA-induced neurotoxicity remain controversial and poorly understood. Cerebrovascular complications are most frequent in transplanted patients treated with CsA and we already showed that chronic CsA treatment altered biochemical parameters related to cardiovascular and cerebrovascular risk factors. Ischemia process, excitotoxicity and oxidative stress activated microglial cells and astrocytes which in turn react secreting cytokines and chemokines. This process results in neuronal cell death and increased injury to the ischemic area. Since inflammatory signaling is involved in all stages of schemic cascade, the present work investigated the release of cytokines and chemokines related to ischemic brain injury and the astrocytes morphology of the hippocampus of rats submitted to a chronic CsA treatment. Adult male Wistar rats were divided in control group (received vehicle - corn oil), and treated group received 15 mg/kg, by daily gastric gavage during 8 weeks. Cytokines and chemokines were measured on hippocampus, striatum and cortex and astrocytic cell bodies and processes were identified in the CA1 *stratum radiatum*. CsA treated rats showed increase expression of RANTES, MCP1, Gro/KC, IL-1β and IL-12. Quantitative results showed that the number of astrocytes and glial fibrillary acidic protein (GFAP) density were not affected by CsA treatment. However CsA treatment decreased the number of fusiform astrocytes. At the same time, the astrocytes that predominate at CsA treated rats presented an intermediate shape,

between the fusiform and stellate ones. Considering the key importance of astrocytes in controlling the number and function of neuronal synapses and blood flow in the brain, they are capable to change their shapes due to cerebral insult. The increase expression of pro inflammatory cytokines and chemokines related to ischemia and stroke may give rise to chronic neuroinflammation which has been implicated in several neuropathologies.

Disclosures: M.M. Cararo: Research Grant; CNPq, FAPESP, CAPES. **D.G. Souza:** None. **D.Z. Andreotti:** None. **L. Rodrigues:** None. **L.S. Lima:** None. **M. Achaval:** None. **L.V. Portela:** None. **D.O. Souza:** None. **C. Scavone:** Research Grant; CNPq, FAPESP, CAPES. **A.E. Böhmer:** None.

Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:849.01/C70

Topic: C.01. Translational Mechanisms

Support: 1ZIAMH002768

Title: MicroRNA-338 regulates the axonal expression of multiple nuclear-encoded mitochondrial mRNAs encoding subunits of the oxidative phosphorylation machinery.

Authors: A. N. KAR[1], A. S. ASCHRAFI[1], O. NATERA-NARANJO[1], M. A. MACGIBENY[1], A. E. GIOIO[1], *B. B. KAPLAN[2]
[1]Lab. Mol Biol, NIMH/ NIH, BETHESDA, MD; [2]Lab. Mol Biol, NIMH/ NIH, BETHESDA, MD

Abstract: microRNAs (miRNAs) constitute a novel class of small, non-coding RNAs that act as post-transcriptional regulators of gene expression. Remarkably, it has been shown that these small molecules can coordinately regulate multiple genes coding for proteins with related cellular functions. Previously, we reported that brain-specific miR-338 modulates the axonal expression of Cytochrome c oxidase IV (COXIV), a nuclear-encoded mitochondrial protein that plays a rate-limiting role in the assembly of the mitochondrial enzyme complex IV of oxidative phosphorylation. Moreover, miR-338-mediated modulation of axonal COXIV mRNA levels resulted in alterations in ATP synthesis and axonal function (Aschrafi et al., 2008). Here, we report that, similar to the COXIV mRNA, the 3'UTR of ATP synthase subunit 9 (ATP5G1), a component of the mitochondrial enzyme complex V, contains a putative miRNA-338 binding site. Using Campenot-compartmentalized cultures of superior cervical ganglion neurons, we show that modulation of miR-338 levels in the axon results in alterations in both COXIV and

ATP5G1 mRNA and protein levels. Importantly, miR-338-mediated modulation of local COXIV and ATP5G1 expression has a marked effect on cytosolic and mitochondrial ROS levels in the axons as measured by 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate (H2-DCFDA) and MitoSOX-Red fluorescence intensity, respectively. Additionally, modulation of axonal miR-338 levels affects the growth of the axon in part by altering local expression of COXIV and ATP5G1. These findings point to a mechanism by which miR-338 can modulate local energy metabolism through the coordinate regulation of the expression of multiple nuclear-encoded mitochondrial mRNAs in the axon.

Disclosures: A.N. Kar: None. **A.S. Aschrafi:** None. **O. Natera-naranjo:** None. **M.A. Macgibeny:** None. **A.E. Gioio:** None. **B.B. Kaplan:** None.

Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:849.02/D1

Topic: C.01. Translational Mechanisms

Title: Differential effects of chronic mitochondrial dna depletion on schwann cells and drg neurons

Authors: V. IDIKUDA[1], A. ISAAC[2], A. BHUSHAN[1], *J. C. LAI[3]
[1]Biomed. and Pharmaceut. Sci., [2]Biomedicl and Pharmaceut. Sci., [3]Idaho State Univ., POCATELLO, ID

Abstract: Mitochondria constitute one of the most dynamic organelles in mammalian cells and play multiple physiological and pathophysological roles in all neural cell types. Because aberrations in mitochondrial DNA have been implicated in neurodegenerative diseases, we have developed chronic mitochondrial DNA (mtDNA) depletion models in vitro employing dorsal root ganglion (DRG) neurons and Schwann cells to facilitate elucidation of mitochondrial dynamics in various peripheral neuropathies. Our models allow us to investigate the hypothesis that neurons and Schwann cells are differentially susceptible to mtDNA depletion. Our ongoing studies have demonstrated that in both DRG neurons and Schwann cells, expression of NADH dehydrogenase subunit 6 (ND6) was inhibited sooner that of cytochrome c oxidase subunit 2 (COX2) as their mtDNA was gradually depleted. Consistently with our hypothesis, we noted that expression of both ND6 and COX2 was inhibited in Schwann cells earlier than that in DRG neurons. We have also observed biphasic changes in their expression of hexokinase 2. Thus, our

findings may have pathophysiological implications in the role(s) of mitochondrial metabolism/dynamics in Schwann cell and DRG neurons in peripheral neuropathies.

Disclosures: V. Idikuda: None. **A. Isaac:** None. **A. Bhushan:** None. **J.C. Lai:** None.

Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:849.03/D2

Topic: C.01. Translational Mechanisms

Title: Inhibition of glutamine synthesis induces glutamate dehydrogenase-dependent ammonia fixation into alanine in co-cultures of astrocytes and neurons

Authors: *S. DADSETAN[1], E. KUKOLJ[1], L. K. BAK[1], M. SØRENSEN[2,3], S. KEIDING[2,3], H. VILSTRUP[3], P. OTT[3], R. LEKE[4], A. SCHOUSBOE[1], H. S. WAAGEPETERSEN[1]
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Abstract: Hyperammonemia is a major etiological factor in the development of hepatic encephalopathy, a neuropsychiatric syndrome occurring secondary to liver failure. Brain ammonia detoxification is believed to occur primarily in astrocytes by glutamine synthesis through glutamine synthetase (GS) and it has been proposed that elevated glutamine levels observed during hyperammonemic conditions is the principal cause leading to astrocyte swelling and concomitant cerebral edema. Interestingly, administration of methionine sulfoximine (MSO), which specifically inhibits GS ameliorates astrocyte swelling and brain edema. Recent studies using neuronal and astrocytic co-cultures have indicated that ammonia may be detoxified not only by incorporation in glutamine but also by the concerted action of glutamate dehydrogenase (GDH) and alanine aminotransferase (ALAT) leading to trapping of ammonia in alanine. The aim of this study was to investigate if the GS inhibitor MSO enhances alanine synthesis by blocking the GS-dependent ammonia scavenging process. Co-cultures of neurons and astrocytes were incubated for 2.5 h with [U-13C]glucose in the absence and presence of 5 mM NH4Cl and 10 mM MSO. Furthermore, incubations with increasing concentrations of 15NH4Cl (1 - 5mM) was performed in the absence and presence of MSO in the same co-culture system. Exposure of co-cultures to [U-13C]glucose in combination with ammonia did not lead to an increased amount of alanine and glutamine intra- and extracellularly. However, 13C labeling in alanine from [U-

13C]glucose was significantly increased in the presence of ammonia and cultures exposed also to MSO exhibited a dramatic increase in content and 13C incorporation of alanine, while the same parameters were decreased for glutamine. Thus, it was demonstrated that inhibition of GS enhances the activity of the alanine synthesizing pathway in this culture system, representing an ammonia scavenging process alternative to the glutamine synthesizing pathway. These results are supported by incubation studies performed with 15NH4Cl, clearly demonstrating a dose-dependent fixation of 15NH4 into alanine together with increased 15N enrichment in glutamate. This provides direct evidence of ammonia being detoxified by the concerted action of GDH and ALAT. Our data points to the potential of GS inhibitors as novel drugs to ameliorate the astrocyte toxicity induced by hyperammonemia.

Disclosures: S. Dadsetan: None. **E. Kukolj:** None. **L.K. Bak:** None. **M. Sørensen:** None. **S. Keiding:** None. **H. Vilstrup:** None. **P. Ott:** None. **R. Leke:** None. **A. Schousboe:** None. **H.S. Waagepetersen:** None.

Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:849.04/D3

Topic: C.01. Translational Mechanisms

Support: CAPES

FAEPA

CNpq

FAPESP

Title: Systemic administration of tempol, an antioxidant agent, attenuates the increased conditioned emotional response induced by restraint-stress

Authors: *A. FEDOCE[1], S. F. LISBOA[2], L. B. M. RESSTEL[3]
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Abstract: Objectives: Stress is a risk factor for the development of affective disorders, such as anxiety and depression. The organism response to stress leads to neurochemical and behavioral changes that may be associated with oxidative stress. Recent evidences suggest that anxiolytic

drugs may have antioxidant properties. The hypothesis of the present study was that systemic administration of 4-hydroxy-2, 2, 6, 6-tetramethylpiperidine 1-oxyl oxil (Tempol), a superoxide dismutase (SOD) mimetic, reduces the consequence of a stress situation evaluating the contextual conditioned emotional responses. Methods. Male Wistar rats (230-270 g) were divided in two groups: control or restrained for 3 h (acute stress). These animals received Tempol (5 or 10 mg/kg, i.p.) 30 minutes before the restraint-stress (RS). Twenty four hours later animals were submitted to the contextual fear conditioning (3 electrical foot shocks, 0.8 mA, 2 s). After this, a polyethylene catheter was implanted in the femoral artery for cardiovascular recordings. 24h later the animals were re-exposed to the chamber, but without shock presentation, and during this period the freezing behavior and the cardiovascular activity were continuously acquired. Results: The RS increased freezing behavior during re-exposition to the aversive chamber (non-stressed vehicle: 55 ± 1.5 % vs stressed vehicle: 86 ± 2.7%; $F_{1,29}=6.4$, $P<0.02$). In addition, there was significant effect the Tempol ($F_{2,29}=100.7$, $P<0.001$) which was only observed on stressed animals ($F_{2,29}=74.4$, $P<0.001$). Both doses of Tempol attenuated freezing behavior in stressed rats (veh: 86.4±2.7%; 5 mg/Kg: 29.2 ± 1.1%; 10 mg/Kg: 22.5 ± 3.4%; $F_{2,14}=171.4$, P0.05, n=2-6/group). The RS also increased the cardiovascular response during re-exposure to the chamber (MAP: $F_{1,150}=124$, $P<0.0001$ and HR: $F_{1,150}=102$, $P<0.0001$, n=5-6/group). In addition, treatment with both dose of tempol attenuated this effect (5 mg/Kg - MAP: $F_{1,135}=118$, $P<0.0001$ and HR: $F_{1,135}=116$, $P<0.0001$and 10 mg/Kg - MAP: $F_{1,150}=154$, $P<0.0001$ and HR: $F_{1,135}=192$, $P<0.0001$, n=5-6/group).
Conclusion: The present results showed that the increase of behavioural and cardiovascular responses during re-exposure to an aversive context observed in stressed animals is attenuated by systemic administration of an antioxidant agent before the stress session. Our findings suggest that acute stressors could sensitize the animal to a subsequent aversive condition by increase oxidative state.

Disclosures: A. Fedoce: None. **S.F. Lisboa:** None. **L.B.M. Resstel:** None.

Poster

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Topic: C.01. Translational Mechanisms

Support: NIH Grant HD02528

Forrest & Sally Hoglund

Title: Effects of a high fat diet on brain metabolism in rats: An *in vivo* 1H-MRS study

Authors: *J. A. STANFORD[1], J. L. HARRIS[2], H.-W. YEH[3], I.-Y. CHOI, PhD[2], W. M. BROOKS[2]
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Abstract: Although aging is the primary risk factor for developing Alzheimer's disease (AD) and Parkinson's disease (PD), regular consumption of a high-fat diet has been shown to increase the risk for these neurodegenerative disorders. Despite this, little is known about how neuronal glucose regulation, neurotransmission and metabolism are affected by a high fat diet. While preclinical studies using invasive methods have reported increased neuronal markers of inflammation and oxidative stress following a high fat diet, non-invasive brain imaging methods allow for longitudinal studies with more translational utility. In this study we used high field, short echo time proton magnetic resonance spectroscopy (^{1}H-MRS) to quantify ~20 brain metabolites in the hippocampus and striatum of young adult rats following four months on either normal chow or a high fat diet (60% calories from fat). We evaluated markers related to inflammation, oxidative stress, edema, free fatty acids, bioenergetics and neurotransmission under fasting and fed conditions. We measured decreased total creatine and increased glutamine in both the hippocampus and striatum of high fat-fed rats under non-fasting conditions. These results indicate altered metabolic homeostasis in nuclei related to AD and PD. They also support the utility of ^{1}H-MRS in preclinical studies.

Disclosures: J.A. Stanford: None. **J.L. Harris:** None. **H. Yeh:** None. **I. Choi:** None. **W.M. Brooks:** None.

Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

Location: Hall F-J

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Topic: C.01. Translational Mechanisms

Title: Mitochondrial gene copy numbers are reduced in hippocampal pyramidal neurons from Alzheimer's disease cases

Authors: *A. C. RICE, J. P. BENNETT, Jr
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Abstract: Mitochondrial dysfunction has been implicated as a causal factor in neurodegeneration in Alzheimer's Disease (AD). Most studies use tissue homogenates, which contain multiple cell types, mainly glia. We used the Arcturus laser capture microdissection system to isolate hippocampal pyramidal neurons from AD cases and compared these to pyramidal neurons isolated from hippocampi of control cases matched for age and postmortem interval . Pyramidal neurons for capture were identified in cresyl violet stained, fresh-frozen hippocampal cryostat sections fixed briefly in ethanol. Multiplex quantitative PCR was used to amplify 4 genes in the minor and major arcs of the mitochondrial genome (12S rRNA, ND2, CO3, ND4). Mitochondrial DNA gene (mtDNA) copy numbers in extracts of captured neurons were derived from a standard curve generated by simultaneous multiplex qPCR of purified, circular human mtDNA. We found a decrease in population distribution of mtDNA gene copy numbers in AD pyramidal neurons compared to controls. Decreased mtDNA gene copy numbers likely derive from decreased mitochondrial mass and imply decreased mitochondrial respiratory function. This mitochondrial deficiency likely results from impaired mitochondrial biogenesis signaling and produces impaired respiration, which we recently reported in frontal cortex samples from the same AD cases. Our findings support the hypothesis that hippocampal pyramidal neurons from AD cases have impaired mitobiogenesis and decreased mitochondrial function, which would increase their vulnerability to early death in AD.

Disclosures: A.C. Rice: None. **J.P. Bennett:** None.

Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

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Support: Muscular Dystrophy Association Research Award (BMM)

NIH Grant R01NS33958-15 (JDR)

Title: Monocarboxylate transporter 1 (MCT1) is critical for regeneration in peripheral nerves

Authors: A. TSINGALIA[1], S. LENGACHER[2], L. PELLERIN[3], P. MAGISTRETTI[2], J. D. ROTHSTEIN[1], *B. M. MORRISON[1]
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Abstract: Axons, and particularly regenerating axons, have high metabolic demands in order to maintain critical functions such as axon transport and membrane depolarization. Though some of the required energy likely comes from extracellular glucose and ATP generated in the soma, we hypothesize that some of the energy may be supplied locally by lactate. Unlike glucose that requires glycolytic enzymes to produce pyruvate, lactate can be converted directly to pyruvate by lactate dehydrogenase and transported into mitochondria for oxidative metabolism. In order to be transported across membranes, lactate requires specific monocarboxylate transporters (MCTs), the most abundant of which in the nervous system is MCT1. If MCT1 and lactate are critical for nerve function and regeneration, we hypothesize that MCT1 heterozygote null mice, which appear phenotypically normal despite having approximately 40% MCT1 as compared to wild-type littermate mice, will have reduced capacity for regeneration following nerve injury. To investigate this, adult MCT1 heterozygote null mice or wild-type mice underwent unilateral sciatic nerve crush in the proximal thigh. We found that regeneration of the sciatic nerve, as measured by recovery of compound muscle action potentials (CMAP) in the lateral plantar muscles following proximal sciatic nerve stimulation or quantification of axons in distal sural and tibial nerves, was delayed from a median of 21 days in wild-type mice to greater than 38 days in MCT1 heterozygote null mice. In fact, half of the MCT1 heterozygote null mice had no recovery of CMAP by the endpoint of the study at 42 days, while all of the wild-type mice had recovered. In addition, the maximal amplitude of CMAP recovery in MCT1 heterozygote mull mice was reduced from a mean of 3mV to 0.5mV. Our experiments show that lactate supplied through MCT1 is necessary for nerve regeneration. Experiments are underway to determine the mechanism by which axon regeneration is reduced and whether loss of MCT1 prevents nerve regrowth directly due to reduced energy supply to axons or indirectly by dysfunction of perineurial cells that normally express MCT1.

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Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

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Support: PRONEX-CNPq/FAPERGS

FINEP/IBN-Net

Title: Kinetic studies on the inhibition of creatine kinase activity by organotellurium compound in cerebral cortex of rats

Authors: *R. B. DE ANDRADE, SR[1], T. GEMELLI[1], D. ROJAS[1], R. GUERRA[2], C. FUNCHAL[2], C. WANNMACHER[1]
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Abstract: Tellurium (Te) is used in the industry of rubber, metal and of electronic equipments. Te affects a great number of neural processes, such as modulation of the glutamatergic system, memory and myelinization. Few studies have evaluated the interference of tellurium on critical enzyme activities of energy metabolism. Considering that the exposition to Te brings risks for humans and for environment, the objective of the present study was to investigate the kinetic of the in vitro inhibition of creatine kinase (CK) activity caused by a new Te compound, 3-butyl-1-phenyl-2-(phenyltelluro)oct-en-1-one in cerebral cortex of 30-day-old Wistar rats. Rats (n=3-5) were killed by decapitation and the cerebral cortex was dissected and homogenized in TRIS-sucrose buffer. In the kinetic assay, concentrations were: 2; 3 and 6 mM of ADP / 1; 2; 3 mM of phosfocreatine with 30 and 5 min of pre-incubation in absence (controls) or presence at 5 µM or 20 µM of organotellurium and 1 mM of reduced glutathione (GSH). First, competition experiments between organotellurium and the enzyme substrates ADP or phosphocreatine with preincubation of 30 minutes were performed. The Lineweaver-Burk plot was compatible with non-competitive inhibition. This type of inhibition is defined because Km values and Ki values remains approximately constant independently of the concentration of the organotellurium for the two substrates and for the two isoenzymes from mitochondria and cytosolic fractions. Next, the two subcellular fractions were pre-incubated for 5 min with the two concentrations of the organotellurium. The Linweaver-Burk plot was similar for phosphocreatine as substrate, but suggested mixed inhibition, competitive and non-competitive for ADP as substrate in the two subcellular fractions. This type of inhibition is defined because Km varies according the concentration of the organotellurium, as well the Ki. In order to better understand the mixed type of inhibition, we added reduced glutathione (GSH), a known protector of sulfhydryl groups, to the pre-incubation of 5 min. The Lineweaver-Burk plot did not change for phosphocreatine as substrate, but was of the competitive type for ADP as substrate. The higher variation of the values of Km and Ki are compatible with this type of inhibition. These results suggest that the new Te compound inhibits the activity of the CK by two mechanisms: competition with ADP and oxidation of critical groups for the functioning of the enzyme.

Disclosures: R.B. De Andrade: None. **T. Gemelli:** None. **D. Rojas:** None. **R. Guerra:** None. **C. Funchal:** None. **C. Wannmacher:** None.

Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

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Program#/Poster#:849.09/D8

Topic: C.01. Translational Mechanisms

Title: Genetic manipulation of mTOR signaling in mouse cerebral cortex

Authors: *H. KASSAI[1], Y. OHNE[1], K. NAKAO[1], T. MAEDA[2], A. AIBA[1]
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Abstract: Mammalian target of rapamycin (mTOR) is an evolutionarily conserved protein kinase, and functions as two distinct complexes, termed mTORC1 and mTORC2. mTOR is activated by amino acids or growth factor stimuli, and plays an important role in many cellular processes such as cell growth and autophagy. In many human cancers, mutations are often found in PI3 kinase-Akt pathway, and TSC1 and 2 are genes responsible for tuberous sclerosis, which causes abnormalities in nervous system, such as brain tumor, epilepsy and autism. Since all these mutations are associated with chronic activation of mTOR pathway, we focused on addressing mTOR functions in these diseases by establishing animal models of chronically activated mTOR pathway. In this study, we used a constitutively active mutant of mTOR, which retains its kinase activity even under starvation conditions, and established two lines of transgenic (Tg) mice which are different in temporal patterns of active mTOR expression; embryonic and postnatal expression (hereafter referred as embryonic-mTOR Tg and postnatal-mTOR Tg mice, respectively). In embryonic-mTOR Tg mice, active mTOR is expressed in dorsal telencephalon from embryonic day 10. These mice are viable and fertile, but cerebral cortex of the Tg mouse is much smaller than that of the control in adulthood. Reduced cortical size in Tg mice was observed as early as embryonic day 12, and cortical progenitor cells progressively underwent apoptosis at this stage. Therefore, embryonic activation of mTOR pathway leads to the depletion of cortical neurons by neuronal progenitor cell death. Next, we established postnatal-mTOR Tg mice, in which active mTOR is expressed in the forebrain after postnatal 7. Postnatal mTOR Tg mice displayed severe growth retardation, having a smaller body size compared to the control. We detected numerous cytoplasmic inclusions in many neurons in postnatal-mTOR Tg cortex. Accumulation of cytoplasmic inclusions was associated with activation of microglial cells. Therefore, postnatal activation of mTOR pathway induces neurodegenerative disease-like phenotype. In summary, mTOR signaling is important for neuronal cell survival in the cerebral cortex.

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Poster

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Topic: C.01. Translational Mechanisms

Support: PRONEX

CAPES

CNPq

FAPERGS

Title: Histidine administration to female rats during pregnancy and lactation induces behavioral changes and alters enzymes activity of phosphoryltransfer network in cerebral cortex and hippocampus of the offspring

Authors: *D. B. ROJAS, R. DE ANDRADE, T. GEMELLI, V. ANDRADE, L. OLIVEIRA, A. CAMPOS, C. WANNMACHER
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Abstract: Histidinemia is a common inborn error of metabolism of amino acids caused by deficiency of histidase activity in liver and skin with consequent accumulation of histidine (His) in plasma and tissues. It is usually considered harmless to the affected patients and their offspring, but some patients and children born from histidinemic mothers have mild neurological disorders. Recent studies have shown that His induces oxidative stress and affects processes of learning / memory. Thus, the aim of this study was to investigate the effect of His load to female rats during pregnancy and lactation on some behavioral parameters in the open-field task and some enzymes of the phosphoryltransfer network in cerebral cortex and hippocampus of the offspring at 21 and 60 days of age. Fourteen 60-day-old Wistar female rats received L-His (0.5 mg/g of body weight) or 0.85% saline administered subcutaneously in the course of pregnancy and lactation. Pyruvate kinase, cytosolic and mitochondrial creatine kinase activities decreased in cerebral cortex and in hippocampus of treated offspring at 21 days of age and this pattern remained at 60 days of age. On the other hand, adenylate kinase activity was reduced in the cerebral cortex and in hippocampus of the offspring at 21 days of age, whereas the activity was increased in the two tissues at 60 days of age. Behavioral results showed that His decreased distance traveled, number of rearings and crossings in the male offspring at 30-day-old, but at 60-day-old the results did not differ from the control group. Our results suggest that administration of His to female rats in the course of pregnancy and lactation may impair phosphoryltransfer network in the cerebral cortex and hippocampus of the offspring and reduce

creatine and pyruvate content, two important antioxidants and neuroprotectors. Oxidative stress caused by L-histidine is a possible mechanism for the decreased enzymatic activity. Moreover, behavioral alterations indicate a decreased exploratory and locomotory activity in the offspring, which can be due to alteration of energy homeostasis and its consequences to brain functioning. Considering that histidinemia is usually a benign condition and little attention has been given to maternal histidinemia, it seems important to perform more studies in the children born from histidinemic mothers. It is possible that histidinemia, usually a benign condition for children exposed to high postnatal levels of histidine, may cause alterations if the brain is exposed to high levels during intrauterine development and breastfeeding.

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Poster

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Totman Medical Research Trust

Title: Inversion of neurovascular coupling after subarachnoid hemorrhage

Authors: M. KOIDE[1], A. D. BONEV[1], M. T. NELSON[1,2], *G. C. WELLMAN[1]
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Abstract: Aneurysmal subarachnoid hemorrhage (SAH) is associated with high rates of morbidity and mortality. The cellular events contributing to SAH-induced ischemic neuronal damage, a major cause of poor outcome, are still elusive. Here we examined the impact of SAH on neurovascular coupling (NVC) in brain slices from control and SAH model rats. Brain slices were loaded with the fluorescent Ca^{2+} indicator dye, fluo-4, and astroctyic endfoot Ca^{2+} concentration and adjoining parenchymal arteriolar diameter were simultaneously measured using two-photon and infrared-differential interference contrast (IR-DIC) microscopy. As

anticipated, neuronal activation by electrical field stimulation (EFS) caused an increase in endfoot Ca^{2+} followed by arteriolar dilation in brain slices from control rats. Remarkably, EFS caused a similar increase in astrocyte endfeet Ca^{2+} but induced vasoconstriction rather than vasodilation in brain slices from day 4 SAH animals. Similarly, Ca^{2+} uncaging in astrocyte endfeet caused vasodilation in control brain slices and vasoconstriction in brain slices from SAH rats. Paxilline, a blocker of large-conductance Ca^{2+}-activated K^+ (BK) channels, greatly diminished both EFS-induced vasodilation and vasoconstriction in brain slices from control and SAH rats, respectively. Interestingly, we also observed an increase in the magnitude of spontaneous astrocytic Ca^{2+} oscillations in brain slices from SAH animals. The peak amplitude of spontaneous astrocytic Ca^{2+} oscillations in brain slices after SAH was ~490 nM compared to ~ 320 nM in brain slices from control animals. Our data are consistent with a model in which SAH increases the amplitude of spontaneous astrocytic Ca^{2+} oscillations leading to increased activity of endfoot BK channels and elevation of basal extracellular K^+ in the restricted perivascular space between astrocytic endfeet and parenchymal arteriolar myocytes. This SAH-induced elevation in basal perivascular K^+ combined with further K^+ efflux stimulated by neuronal activity elevates K^+ above the dilation/constriction threshold, switching the polarity of arteriolar responses from vasodilation to vasoconstriction. This inversion of NVC may contribute to decreased cerebral blood flow and the development of delayed ischemic neuronal deficits following SAH.

Disclosures: M. Koide: None. **A.D. Bonev:** None. **M.T. Nelson:** None. **G.C. Wellman:** None.

Poster

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Totman medical research trust

Title: Picomolar pituitary adenylate cyclase activating polypeptide (PACAP) potently dilates middle meningeal artery: Potential role in migraine headache

Authors: A. SYED[1], M. KOIDE[1], *V. MAY[2], G. WELLMAN[1]
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Abstract: Migraine is a debilitating neurological disorder characterized by unilateral pulsating headache that is often accompanied by aura, nausea, photophobia, phonophobia and other neurological symptoms. The underlying mechanism of pain in migraine is still poorly understood. Among several proposed mechanisms, vasodilation of the dural vasculature, specifically the middle meningeal artery (MMA), has been shown to coincide with migraine headache. Neuropeptides, including calcitonin gene-related peptide, contained within the trigeminal sensory and sphenopalatine postganglionic parasympathetic fibers innervating these vessels have been implicated in the process. Recently pituitary adenylate cyclase activating polypeptide (PACAP), a neuropeptide present in trigeminal sensory fibers, has been linked to migraine pain. PACAP is a neurotransmitter and neurotrophic peptide in the secretin, glucagon and vasoactive intestinal peptide (VIP) superfamily and shares some G protein coupled receptor selectivity with VIP. PACAP binds potently and specifically to the PAC1 receptor which may be coupled to multiple intracellular signaling cascades; by contrast, both PACAP and VIP bind with near equal affinity to VPAC1 and VPAC2 receptors coupled principally to adenylyl cyclase. To date the ability of PACAP to relax isolated unpressurized MMA segments pre-contracted with exogenous vasoconstrictors is controversial. The objective of the present study was to examine the ability of PACAP to induce dilation of pressurized rat MMAs ex-vivo. Isolated segments of rat MMA were cannulated on glass micropipette and pressurized to an intravascular pressure of 40 mmHg. At 40 mmHg, MMAs developed intrinsic myogenic tone, representing ~40% decrease in diameter. Under these conditions, PACAP potently dilated MMAs with an IC_{50} of ~1 pM. In contrast, VIP was found to be ~1000-fold less potent (IC_{50} ~1 nM). The PAC1 receptor antagonist, PACAP(6-38), abolished MMA dilation caused by picomolar concentrations of PACAP. Cerebral arteries isolated from the brain surface were ~1000-fold less sensitive to PACAP than MMAs. RT-PCR analysis of the cerebral arteries exhibited expression of all 3 PACAP receptor subtypes (PAC1, VPAC1 and VPAC2), however MMAs demonstrated the presence of PAC1 and VPAC2 receptors only. Further, MMAs expressed multiple splice variants of the PAC1 receptor subtype. In summary, our data demonstrate that PACAP is a potent dilator of pressurized MMA ex vivo. MMA dilation in response to picomolar concentrations of PACAP appear to be mediated via activation of the PAC1 receptor. PACAP-induced MMA dilation may play a role in the etiology of migraine headache.

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Poster

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Title: Broad therapeutic window for ONO-2506 in preventing subarachnoid hemorrhage (SAH)-associated cerebrovascular and neurologic dysfunction

Authors: H.-L. XU, C. PAISANSATHAN, F. VETRI, J. WATCHAROTAYANGUL, *D. A. PELLIGRINO
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Abstract: We recently observed a substantial loss of pial arteriolar dilating function, as well as impaired neurologic function, at 48 h post-SAH (anterior cerebral artery suture perforation model). Those changes were largely prevented by acute application (30 min post-SAH) of either ONO-2506 (selective S100B inhibitor) or soluble RAGE [decoy peptide for RAGE (receptor for advanced glycation end-products)], indicating that S100B/RAGE engagement plays an important role in the pathogenesis of SAH-induced cerebrovascular dysfunction. In this study, we examined whether delaying inhibitor treatments until 6h post-SAH conferred a similar benefit toward recovering neurologic and pial arteriolar dilating function measured at 48h. We anticipated an extended therapeutic window for ONO-2506 treatment, since the rise in S100B levels in cisternal CSF does not reach its peak until 2 days post-SAH. Rats were randomized into three groups: (1) sham surgical controls; (2) vehicle-treated SAH controls; and (3) ONO-2506-treated SAH rats. Rats in group 3 received 10 mg/kg ONO-2506 ip at 6 h post-SAH, followed by same dosage every 24 h; while animals in groups 1 and 2 were treated with normal saline. Using a closed cranial window system, we measured dilator responses at 48 h after SAH. We observed diminished pial arteriolar responses to hypercapnia, sciatic nerve stimulation (SNS), and topically-applied adenosine (ADO), acetylcholine (ACh), and S-nitroso-N-acetyl penicillamine (SNAP). Treatment with ONO-2506 restored most of the impaired pial arteriolar dilating responses, except the response to SNS. Additionally, a significant improvement in neurologic outcome scores (the lower the score, the greater the impairment) was revealed in ONO-2506-treated animals (neuroscore = 16.67±1.25 vs 11.29±2.66 in vehicle-treated rats). In summary, the results suggest that (1) the S100B inhibitor, ONO-2506, exerts its protective effect toward SAH-related neuropathy by preventing losses in cerebral vascular dilating function; and (2) ONO-2506 treatment is associated with a wide therapeutic window (up to 6 h post-SAH).
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Poster

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NIH Grant NCRR P20 RR16435

Title: Subarachnoid hemorrhage (SAH) induces delayed, but not acute, inversion of neurovascular coupling in rat cortical brain slices

Authors: *A. C. PAPPAS[1,2], M. KOIDE[2], G. C. WELLMAN[2]
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Abstract:
Pathological changes within the intracerebral microcirculation contributing to the development of delayed neurological deficits following aneurysmal SAH are not well understood. Physiologically, neurovascular coupling (NVC) represents the matching of increased local blood flow to focal increases in neuronal activity. However, we have recently observed inversion of the NVC response from vasodilation to vasoconstriction in brain slices from rats 4 days (d) after the initiation of experimental SAH. The objective of the present study was to determine whether inversion of NVC represents an acute or delayed effect of subarachnoid blood. Experimental SAH was induced in anesthetized adult male rats by injecting 0.5 mL of whole autologous blood into the cisterna magna on two consecutive days. Cortical brain slices were obtained 4 hours (hr), 2 d, 4 d, 7 d and 14 d following the induction of SAH, and from un-operated control animals. Brain slices were loaded with the Ca^{2+} indicator dye, Fluo4-AM. Astrocytic endfoot Ca^{2+} and arteriolar diameter were recorded simultaneously using combined multi-photon and infrared differential interference contrast microscopy. Electrical field stimulation (EFS) was used to evoke neuronal activity. As expected, EFS caused an increase in endfoot Ca^{2+} that preceeded arteriolar dilation in brain slices from control animals. A similar increase in endfoot Ca^{2+} and arteriolar dilation was observed in brain slices 4 hr after induction of SAH. However, brain slices from later SAH time points exhibited EFS-induced increases in endfoot Ca^{2+}, followed by vasoconstriction rather than vasodilation. Our data demonstrate that SAH-induced inversion of NVC does not occur immediately (within the first 4 hours) despite the observed presence of red blood cells in the cortical perivascular space. Further, we found that the delayed inversion of

NVC can last up to 14 d after SAH. These data suggest that the mere presence of extravascular blood is insufficient to directly invert the NVC response. Rather, SAH-induced inversion of NVC is likely due to time-dependent changes within the brain parenchyma following exposure to blood. SAH-induced inversion of NVC may contribute to the development of delayed neurological deficits that frequently occur in patients in the days to weeks following cerebral aneurysm rupture.

Disclosures: A.C. Pappas: None. **M. Koide:** None. **G.C. Wellman:** None.

Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:849.16/D14

Topic: C.01. Translational Mechanisms

Title: Compromise of spinal cord microvasculature in cervical spondylotic myelopathy

Authors: *S. K. KARADIMAS[1,2], W. YU[1], E. MOON[1], M. FEHLINGS[1,2]
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Abstract: Introduction:
Cervical spondylotic myelopathy (CSM) is the most common cause of spinal cord dysfunction in adults, however little is known about the molecular mechanisms of the disease. Currently little is known regarding the fate of blood vessels and the integrity of the BSCB during chronic cervical spinal cord compression. We hypothesize that progressive compression of cervical spinal cord in CSM compromises the spinal cord microvasculature.
Material and Methods: In the present study we used two different experimental approaches. *Human CSM:* We examined the extravascular expression of fibronectin, as a measure of BSCB permeability and counted the number of Von Willebrand Factor (VWF) positive vessels in post-mortem human spinal cord tissues derived from 8 CSM patients and ten control cases. *Rat CSM:* Complementary studies were conducted in CSM rats (compression group) and in sham operated rats. At 9 weeks post-surgery MRI acquired from the animals of the compression group before and after T1-gadolinium diethylene triamine pentaacetic acid (Gd-DTPA) administration to evaluate *in vivo* the BSCB permeability. Animals were sacrificed at 10 weeks post-surgery. Prior to sacrifice 100 µl of 4% Evans Blue (EB) was injected. The chronic BSCB breakdown reflected by the leakage of fluorescent EB was evaluated rostral and caudal to and at compression

epicenter. Finally, to assess the number of mature vessels in the cervical spinal cord, quantitative confocal immunohistochemistry will be performed on sections labeled with RECA-1 and laminin.
Results: *Human CSM:* Significant increase in number of fibronectin positive vessels and fibronectin positive cells of the
parenchyma in human CSM patients but not in non-CSM controls, suggesting chronic BSCB permeability. Increased von Willebrand factor expression was also seen in human CSM patients, suggesting endothelial cell dysfunction. *Rat CSM:* At 10 weeks post-surgery, the overall EB and fibronectin intensity in the total spinal cord section area of the compression group was found to be statistically significant increased compared to sham operated group ($p=0.008$ and $p=0.021$). Moreover, we
found that the ratio of the relative signal Gd-DTPA intensity between the compression level (C6) and 3 levels above(C3) was 1.5±0.11. Finally, statistical significant decreased number of RECA-1 and laminin positive blood vessels was found at the compression group compared to sham operated group ($p<0.009$ and $p=0.012$).
Conclusion: In conclusion, we report for first time novel evidence indicating chronic BSCB disruption and vascular damage under the chronic progressive compression of the cervical spinal cord in CSM.

Disclosures: S.K. Karadimas: None. **W. Yu:** None. **E. Moon:** None. **M. Fehlings:** None.

Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

Location: Hall F-J

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Program#/Poster#:849.17/D15

Topic: C.01. Translational Mechanisms

Title: FKBP51 shapes stress response and signaling pathways involving protein kinases and autophagy

Authors: N. C. GASSEN, J. ZSCHOCKE, J. HARTMANN, L. KOLLMANNSBERGER, *F. HOLSBOER, M. V. SCHMIDT, T. REIN
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Abstract: Gene association studies linked FK506-binding protein (FKBP) 51 to psychiatric diseases like major depression and also suggested a connection of FKBP51 with the actions of antidepressants. Furthermore, FKBP51 protein levels have been correlated with the efficacy of antidepressants. While FKBP51 is firmly established as a potent inhibitor of the glucocorticoid

receptor (GR), we here set out to identify novel molecular networks beyond FKBP51's action on GR and to test the relevance of these networks for the actions of antidepressants. We investigated the influence of FKBP51 on protein kinases including Akt; Akt is a key regulator of the PI3K/Akt/mTOR pathway and is thought to be modulated by several antidepressants. We report a specific interaction of FKBP51 and its close homologue FKBP52 with Akt1 and 2, but not Akt 3. FKBP51 recruited the phosphatases PHLPP1 and 2 to Akt1 and 2 leading to dephosphorylation of the latter and thus inactivation of downstream signalling as assessed by phosphorylation of Foxo3a. FKBP52 did not bind to PHLPP1 and 2, and therefore did not affect Foxo3a phosphorylation status as well as other substrates of Akt. FKBP51 also affected targets downstream of the Akt pathway, as exemplified for its impact on autophagy: Analysis of LC3B distribution revealed that ectopically expressed FKBP51 induced redistribution of LC3 into autophagosomal vesicles in rat cortical astrocytes. Furthermore, the levels of beclin1 and LC3B-II were increased. Virtually all of these FKBP51 actions were shared by antidepressants; importantly, the effects of antidepressants were greatly enhanced by increasing FKBP51. Altogether, we put forward the hypothesis that FKBP51 is a crucial determinant of antidepressants' action on Akt/mTOR signaling and downstream physiological processes such as autophagy.

Disclosures: N.C. Gassen: None. **J. Zschocke:** None. **J. Hartmann:** None. **L. Kollmannsberger:** None. **F. Holsboer:** None. **M.V. Schmidt:** None. **T. Rein:** None.

Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

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Topic: C.01. Translational Mechanisms

Support: MDA Grant 110856

NIH Grant NS033958-17

P2ALS

Title: Uncovering roles of monocarboxylate transporter-1 in the cerebellum

Authors: *Y. LEE[1], L. CHAKRAVARTI[1], I. SHATS[1], S. VIDENSKY[1], L. JIN[1], L. PELLERIN[2], P. J. MAGISTRETTI[3], J. D. ROTHSTEIN[1]
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Abstract: The cerebellum, as a highly energetic structure, may be particularly susceptible to alterations in metabolic coupling between neurons and glia during development and in disease. Although glucose is considered the primary energy substrate for neurons, neurons can also utilize lactate released by neighboring glia through monocarboxylate transporters (MCTs). MCT-1, one of 14 members of the *SLC16A* family, is a predominant isoform expressed in the nervous system. However, the expression pattern and function of MCT-1 in the cerebellum are still poorly understood.From the analysis of BAC MCT-1 tdTomato reporter mice, we found that oligodendroyctes in the white matter of the cerebellum strongly express MCT-1. In addition, tdTomato expression is detectable in mature, differentiated Purkinje neurons. Migrating progenitors in the external germinal layer strongly express MCT-1 but do not express the glucose transporter found in differentiated neurons, suggesting a metabolic switch during cerebellar development. MCT-1 heterozygous knockout mice display prominent axonopathy in the granular cell layer as well as white matter, suggesting impaired metabolic support of axons by myelinating oligodendroglia via MCT-1. These mice also show mild Purkinje neuron degeneration as evidenced by immunohistochemical analysis, electron microscopy analysis, and behavior tests. Degeneration may be caused by damage of Purkinje axons due to lack of myelin support or loss of MCT-1 in Purkinje neurons. Strikingly, ectopic overexpression of MCT-1 in Bergmann glia and astrocytes also leads to severe Purkinje neuron degeneration and abnormal dendritic morphology as well as hindlimb clasping, suggesting an alteration of metabolic coupling between Purkinje neurons and Bergmann glia. Since glutamate excitotoxicity is thought to contribute to non-cell autonomous cerebellar neurodegeneration in some forms of ataxia, such as spinocerebellar ataxia type 7 (SCA7), and MCT-1 expression is also altered in amyotrophic lateral sclerosis, we investigated whether MCT-1 knockout or ectopic overexpression in Bergmann glia affected glial glutamate transporter expression. qRT-PCR analysis showed marked reduction (~40%) in glutamate aspartate transporter (GLAST) mRNA in the cerebellum in both MCT-1 heterozygous knockout mice and overexpressor mice as compared to control, suggesting that excitotoxicity along with altered metabolite shuttling may contribute to cerebellar degeneration. Taken together, these results indicate that MCT-1 expression in the cerebellum is essential for Purkinje cell integrity and survival as well as axonal homeostasis.

Disclosures: Y. Lee: None. **L. Chakravarti:** None. **I. Shats:** None. **S. Videnskiy:** None. **L. Jin:** None. **L. Pellerin:** None. **P.J. Magistretti:** None. **J.D. Rothstein:** None.

Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

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Topic: C.01. Translational Mechanisms

Support: BMBF 01ES0812

Title: Neuregulin 1 type I ameliorates axonal loss in a mouse model for Charcot-Marie-Tooth-Disease 1A

Authors: *M. W. SEREDA, R. STASSART, R. FLEDRICH, K.-A. NAVE
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Abstract: Charcot-Marie-Tooth Disease 1 A is the most common inherited neuropathy caused by a duplication of the peripheral myelin protein of 22kDa (PMP22). CMT1A is a slowly progressive disease, characterized by demyelination, axonal loss and muscle atrophie. A mouse model for CMT1A harbouring additional copies of the Pmp22 gene displays the typical features of the disease such as axonal loss and impaired motor function. Neuregulin1 belongs to a family of transmembrane and secreted growth factors subdivided into three classes according to their extracellular domain. Neuregulin 1 isoforms are essential for survival and proliferation of Schwann cells and Neuregulin 1 type III has been shown to regulate myelin sheath thickness in the peripheral nervous system. In this study, we investigated the role of different Neuregulin 1 isoforms in a mouse model for CMT1A. Neuregulin 1 type III mRNA was found to be downregulated in DRG neurons of Pmp22 transgenic mice, whereas Neuregulin 1 type I mRNA expression levels were increased in DRG neurons. At the age of four month, Pmp22 transgenic mice displayed axonal loss in the sciatic nerve. Crossbreeding of Pmp22 overexpressing mice with Neuregulin 1 type I transgenic animals caused axonal preservation. Moreover, Neuregulin 1 type I Pmp22 double transgenic mice displayed an increase in the compound muscle action potential (CMAP) compared to single Pmp22 transgenic mice. Unexpectedly, Neuregulin 1 type III Pmp22 double transgenic mice showed no axonal preservation.
Analysis of an acute nerve injury model revealed that mice overexpressing the type I isoform of Neuregulin 1 displayed an improved axonal regeneration compared to wildtype controls. Along with the enhanced axonal regeneration, Neuregulin 1 type I transgenic animals depict an improvement in phenotype and CMAP after nerve crush. We conclude, that Neuregulin 1 type I is beneficial for axonal preservation and regeneration in the peripheral nervous system.

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Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

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Topic: C.01. Translational Mechanisms

Support: Dana Foundation

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Title: Effect of hyperglycemia on white matter microstructural integrity in youth with T1DM

Authors: *J. V. ANTENOR-DORSEY[1], E. MEYER[2], J. RUTLIN[1], D. PERANTIE[1], N. H. WHITE[2], A. ARBELAEZ[2], J. SHIMONY[3], T. HERSHEY[1]
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Abstract: Hyperglycemia is hypothesized to affect white matter integrity in the developing brain. Although decreased white and gray matter volumes have been reported in youth with type 1 diabetes (T1DM), the effects of hyperglycemia on white matter microstructural integrity have not been quantitatively assessed during development. Based on previous volumetric findings, we hypothesized that white matter microstructural integrity in the superior parietal lobule and hippocampus would be particularly sensitive to any negative effects of hyperglycemia exposure in youth with T1DM. Diffusion tensor images from a retrospective study of T1DM youth (n=73; mean$\pm$SD age=16.8$\pm$2.9) and their non-diabetic sibling controls (n=30; mean+SD age=15.9$\pm$3.4) were analyzed using two complimentary approaches, region-of-interest and voxel-wise tract-based spatial statistics (TBSS), to quantify white matter microstructural integrity in both *a priori* regions and across the brain. Degree of lifetime exposure to hyperglycemia (lifetime median HbA1c weighted for T1DM duration) and the number of severe hyperglycemia and severe hypoglycemia episodes were estimated from review of patients' medical records, HbA1c levels and parent/youth interviews. Univariate analyses controlling for the effects of age and gender showed that the T1DM group had lower fractional anisotropy (FA) in the superior parietal lobule ($F(1,99)=2.84$, $p=0.007$), but not the hippocampus. The T1DM group also had reduced mean diffusivity in the thalamus ($F(1,99)=22.81$, $p=0.002$) compared to controls. A history of three or more severe hyperglycemic episodes (3+ SHyper) was associated with reduced FA in the superior parietal lobule ($F(5,97)=2.71$, $p=0.008$; 3+ SHyper vs. controls, $p=0.009$) and increased diffusivity in the hippocampus $F(5,97)=2.67$, $p=0.03$; 3+ SHhyper vs. 1-2 SHyper, $p=0.038$). In the TBSS analyses, the T1DM group had lower axial diffusivity across multiple white matter tracks compared to controls. The T1DM group with no prior history of severe hypoglycemia also had reduced mean and axial diffusivity compared to controls. Blood glucose at the time of scan was not different across severe hyperglycemic and hypoglycemic subgroups. These results add microstructural integrity of white matter to alterations observed in the brains of youth with T1DM and highlight the vulnerability of the superior parietal lobule and hippocampus to

glycemic extremes during brain development. Longitudinal analyses will be necessary to determine how these alterations change with age or additional glycemic exposure.

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Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

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Topic: C.01. Translational Mechanisms

Support: NICHD Grant Number: HD053692

BGSU Distinguished Teaching Professor Award, Department of Biological Sciences

Title: The effect of polychlorinated biphenyl exposure on behavioral development and thyroid status in Sprague-Dawley rat pups: Gestation vs. lactation.

Authors: *D. E. MANKIN[1], E. N. DOVER[1], Z. M. WANG[1], H. C. CROMWELL[2], L. A. MESERVE[1]
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Abstract: Polychlorinated biphenyls (PCBs) are a group of persistent environmental contaminants that are known endocrine disrupting compounds and have been found in placental tissue and breast milk. Incorporation of PCB into the diet of pregnant rats has been shown to alter maternal care as well as motor, sensory, and psychological functions in pups during neurological development. Some of these alterations are thought to result from the ability of PCB to affect thyroid hormone (TH) synthesis, circulation and feedback mechanisms. Proper TH levels are crucial during gestation for proper neurological development. In the present study, a cross-fostering paradigm was used to compare the effects of PCB exposure during gestational development versus exposure during lactation. 32 female Sprague-Dawley rats were used and once pregnant, either placed on a diet containing equal amounts of two PCB congeners (47/77, 25 ppm total) or left on a control diet. After birth, all dams were once again placed on control diets and each litter was either cross-fostered to a dam of the opposite dietary treatment or left with their birth dam. To test for potential developmental deficits, several neurobehavioral tests were conducted on various postnatal days (PNDs). These tests included analysis of ultrasonic vocalization (USV) production (PND 10) following isolation from the dam, conditioned odor preference (COP) on PNDs 12 - 14, open field behavior (PNDs 15 - 16), and grooming behavior

(PNDs 15 - 17). Serum thyroxine (T_4) concentrations were determined on PND 17. PCB exposure increased the number per time of isolation USVs (69%) and line crosses in the open field (122%). During COP testing, pups gestationally exposed to PCB showed an aversion to lemon scent, spending only 29% of their time near it. In contrast, controls failed to show a preference or aversion to the lemon scent. Grooming behavior was also disrupted in rats exposed to PCB, most notably on PND 16. Controls had the highest rate of completed grooming chains (42%) followed by pups that were born to a PCB dam and then cross-fostered to a control dam (27%). Pups remaining with PCB dams, and therefore exposed to PCB both during gestation and lactation, had the lowest rate of completed grooming chains (19%). PCB exposure also significantly reduced T_4 concentrations, with pups remaining with PCB dams showing the most reduction (35%), and those cross-fostered in either direction showing an intermediate reduction (15%) as compared to control pups. Taken together, these data suggest that exposure to small amounts of PCB can cause altered behavioral development regardless of whether exposure occurs during gestation only, lactation only, or both.

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Poster

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Topic: C.01. Translational Mechanisms

Support: NIMH MH077159

NIMH MH084060

NIMH MH092984

Title: Opposing actions of genetic and gonadal sex on affect regulation

Authors: *M. L. SENEY, E. SIBILLE
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Abstract: Sexual dimorphism in major depressive disorder (MDD) is well established, but the mechanism(s) underlying increased female vulnerability remain unknown. While evidence suggests an influence of adult circulating hormones (*activational hormone effects*), female MDD prevalence is higher across life stages, implying contribution by additional biological factors.

Studies suggest a developmental origin for adult mood, and notably, developmental processes also establish brain sexual dimorphism (*organizational hormone effects, X/Y genetic sex*). We hypothesize that developmental processes contribute to adult female vulnerability to MDD. We used the genetically engineered Four Core Genotype (FCG) mice, in which genetic and gonadal sex are dissociated, to independently examine effects of X/Y genetic sex, developmental hormones, and adult circulating hormones on behavior ($N \geq 12$/group; total $N = 116$). Adult mice were gonadectomized and implanted with a testosterone (T) or blank capsule. Mice were then tested for anxiety-like (elevated plus maze, open field) and anhedonia-/depressive-like (novelty suppressed feeding, sucrose preference) behaviors at baseline and after unpredictable chronic mild stress (UCMS). Dependent measures were analyzed by ANOVA with 3 main effects: developmental gonadal sex, adult hormone treatment, and genetic sex. Genetic male sex increased anxiety-like behavior (XY > XX) and decreased activity (XY < XX), but had no effect on anhedonia-/depressive-like behavior (XY = XX). Presence of testes during development had little effect on anxiety-like behavior or activity (testes = ovaries), but decreased anhedonia-/depressive-like behavior (testes < ovaries). Circulating T decreased anxiety-like behavior (blank > T) and increased activity (blank < T), but had no effect on anhedonia-/depressive-like behavior (blank = T). Consistent patterns were observed under baseline conditions and after UCMS. Genetic sex and circulating testosterone (activational) modulated anxiety-like behavior, whereas developmental gonadal sex (organizational) affected anhedonia-/depressive-like behavior. Hence, in a wild-type male, a surprising dynamic equilibrium between opposing actions of testosterone and XY genetic sex appears to regulate anxiety-like behavior, resulting in lower levels compared to females. The robust dichotomy between increased anxiety-like and reduced locomotor activities has been observed in other systems, including in primates and possibly in humans, and is thought to reflect epistatic phenomena downstream from common genes, for which our data now point to X and Y chromosome genes as potential mediators.

Disclosures: M.L. Seney: None. **E. Sibille:** None.

Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

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FELLOWSHIP CONACYT 220655

POSGRADO EN CIENCIAS BIOMEDICAS UNAM

Title: Low doses of ozone altered the regulation of the immune response in rats hippocampus.

Authors: *N. GOMEZ-CRISOSTOMO[1], E. JALPA-HERNANDEZ[2], L. MEJIA-CARMONA[2], S. RIVAS-ARANCIBIA[2]
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Abstract: The immune response in the central nervous system (CNS) has been a subject of controversy. Several researches have highlighted the role of cytokines in neurodegeratives processes. Has also been demonstrated that exposure to ozone could suppress or activate the immune response. However, many works only shown the effect of ozone on immune regulation on respiratory system and other systems, nevertheless the effect of ozone on the CNS is poorly understood. In our laboratory we have developed a murine model that produces a chronic and irreversible neurodegenerative process by chronic exposure to low doses of ozone. The aim of this work is to evaluate the inflammatory response generated by exposure to low doses of ozone in hippocampus of rats. Seventy-two Wistar male rats were randomly divided into 6 groups: 1) Control (air), 2) Ozone (O3) for 7 days, 3) O3 for 15 days, 4) O3 for 30 days, 5) O3 for 60 days and 6) O3 for 90 days. Ozone-exposed groups received a dose of 0.25 ppm for 4 hours. Two hours after treatment each group was divided into two subgroups. In the first subgroup, animals were anesthetized and perfused, the brains obtained and processed for immunohistochemistry technique. Second subgroup, animals were anesthetized and the hippocampus dissected for western blot technique for 3-Nitrotyrosine, GFAP, NFkβ, IL-10 and IL-17. Our results showed increased expression of 3-Nitrotyrosine from the 7-day and until 90 days after exposure to ozone. GFAP increases significantly after 30 days exposure to 90 days. NFkβ and IL-17 increased after 60 days to exposure of ozone. IL-10 expression is decreased from 60 to 90 days of exposure to ozone. In conclusions the chronic exposure to low doses of ozone produce oxidative stress state that lead to neurodegenerative processes and a dysregulation of the immune response.
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Poster

849. Energetics, Neurovascular, Neuroimmunolgy and Neuroendocrine

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Topic: C.01. Translational Mechanisms

Support: NIH Grant NS060881

Title: Hematopoietic factors contribute to CNS vascular permeability and microhemorrhage formation in CD8 T cell-initiated blood brain barrier disruption

Authors: *H. JOHNSON[1], Y. CHEN[2], G. SUIDAN[2], J. MCDOLE[2], A. LOHREY[2], L. HANSON[1], F. JIN[1], I. PIRKO[1], A. JOHNSON[1]
[1]Mayo Clin., Rochester, MN; [2]Univ. of Cincinnati, Cincinnati, OH

Abstract: Blood brain barrier disruption is an integral feature of numerous neurological disorders. A central question in neurological disease is the extent blood-derived factors or stromal tissue contribute to blood brain barrier disruption. Using a variation of the Theiler's murine encephalomyelitis virus (TMEV) model designed to study immune-mediated CNS vascular permeability, we report that despite having indistinguishable CD8 T cell responses and neutrophil infiltration, C57BL/6 mice exhibited increased CNS vascular permeability, microhemorrhage formation, and functional deficits compared to 129 SvIm mice. To investigate the contribution of hematopoietic cells in this model, resistant 129 SvIm mice were reconstituted with either C57BL/6 or autologous 129 SvIm bone marrow. Three-dimensional volumetric analysis of gadolinium-enhanced T1-weighted MRI scans revealed that 129 SvIm mice reconstituted with C57BL/6 bone marrow but not autologous bone marrow exhibited a profound increase in CNS vascular permeability. 129 SvIm mice reconstituted with C57BL/6 bone marrow also presented with increased microhemorrhage formation, as measured by T2*-weighted MRI, and functional deficits when compared to 129 SvIm mice receiving 129 SvIm syngeneic bone marrow transfer. We conclude that C57BL/6 susceptibility to CNS vascular permeability, microhemorrhage formation, and functional deficit is transferable with the bone marrow compartment, demonstrating that hematopoietic factors contribute to CNS vascular permeability and microhemorrhages in immune-mediated fatal blood brain barrier disruption.

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Poster

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Topic: C.01. Translational Mechanisms

Support: NIH Grant 5T32A1007495-14

Title: Attenuation of experimental autoimmune encephalomyelitis by high mobility group box 1 fusion protein

Authors: *X. PARILLON[1], L. GUO[2], S. HAN[2]
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Abstract: High Mobility Group Box 1 (HMGB1) is a ubiquitously expressed protein that has been linked to central nervous system inflammation. HMGB1 expression is elevated in the serum and tissues of patients with multiple sclerosis (MS). Currently, many of the therapeutics to treat MS target lymphocytes. However, the factors, in particular HMGB1, that regulate lymphocyte activation during MS are incompletely understood. We have generated a HMGB1 fusion protein to determine the role of HMGB1 on lymphocyte activation in the mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). EAE was induced by immunizing C57/BL6 mice with myelin oligodendrocyte glycoprotein $(MOG)_{33-55}$. Mice were injected intraperitoneally with HMGB1 fusion protein before onset of disease. Clinical scores and incidence were determined. MOG specific IgM antibody levels, which is involved in remyelination, was analyzed. IL-17 expression, a pro-inflammatory cytokine linked to EAE pathogenesis was evaluated. HMGB1 fusion protein treated mice exhibit significantly reduced clinical scores during the induction and recovery phases of disease. Reduced severity of disease was associated with a reduced frequency of CD4+T cells in the spinal cord. T cells and B cells were reactive to HMGB1 fusion protein. The CD4+T cell cytokine, IL-17 expression was reduced in the periphery of HMGB1 treated mice. MOG specific IgM antibody titers were significantly elevated compared to the control treatment group. These data indicate that HMGB1 fusion protein attenuates EAE. HMGB1 appears to be involved in regulating inflammation in EAE by negatively influencing CD4+T effector cell functions and positively regulating the maintenance of MOG specific antibody responses. This study reveals new insights into the potential affect of HMGB1 as a therapeutic to reduce inflammation in multiple sclerosis.

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Poster

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Topic: C.01. Translational Mechanisms

Support: CNPq-Brazil

FAPERGS, RS-Brazil

Title: *In vivo* effects of β-alanine on some parameters of oxidative stress and enzymes of energy metabolism in cerebral cortex and cerebellum of rats

Authors: *T. GEMELLI, SR[1], R. B. DE ANDRADE[1], D. B. ROJAS[1], N. B. FERNER[1], C. FUNCHAL[2], C. M. D. WANNMACHER[1]
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Abstract: β-alanine is derived from the degradation of uracil. In high concentrations develops a very rare disorder of pyrimidine degradation pathway, known as β-alaninemia. In this study we evaluated the in vivo acute and chonic effects of β-alanine on some parameters of oxidative stress and the activity of the enzymes creatine kinase, pyruvate kinase and adenylate kinase in cerebral cortex and cerebellum of 21-day-old rats. In the acute treatment the animals received three intraperitoneal injections of β-alanine (300 mg /Kg of body weight) and the controls received the same volume of saline solution (0.85%) at 3 hours of intervals. In the chonic treatment the animals received one intraperitoneal injection each 12 hours from 8 to 21 days of age. 2',7'-dihydrodichlorofluorescein (DCFH) oxidation and the content of thiols increased in the two tissues studied. No significant difference in the levels of glutathione (GSH) and thiobarbituric acid reactive species (TBARS) was verified. Catalase (CAT) activity was increased, whereas superoxide dismutase (SOD) activity was inhibited in the two tissues. Considering the enzymes of energy metabolism the results were similar in all enzymes, but different in the two tissues studied: β-alanine was able of inhibit the enzyme pyruvate kinase, cytosolic creatine kinase and adenylate kinase activities in cerebral cortex. However, pyruvate kinase and adenylate kinase activities were increased in the presence of β-amino acid in cerebellum. In the chronic experiment we observed increased content of thiols in the cerebral cortex, whereas no significant difference in the levels of GSH and SOD activity was verified. The activity of the antioxidant enzyme glutathione peroxidase (GPx) and TBARS were reduced. In cerebellum DCFH oxidation was increased whereas the other parameters were decreased. Therefore, the activity of the enzymes of the energy metabolism was different in the two studied tissues. The β-amino acid decreased pyruvate kinase activity and increased cytosolic creatine kinase activity, but did not change adenylate kinase activity in cerebral cortex. However, the pyruvate kinase and adenylate kinase activities decreased in the cerebellum. Finally, we found that β-alanine administration altered the activity of the antioxidant enzymes and increased the content of reactive species possibly generating oxidative stress. The oxidative stress may be a possible cause of the observed alteration in the enzymes of the energy metabolism. Taken

together, these results suggest that oxidative stress and alteration of energy metabolism may contribute, at least in part, to the neurological alterations found in patients with β-alaninemia.

Disclosures: T. Gemelli: None. **R.B. de Andrade:** None. **D.B. Rojas:** None. **N.B. Ferner:** None. **C. Funchal:** None. **C.M.D. Wannmacher:** None.

Poster

850. APP/Abeta: Cellular Models

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:850.01/D25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SSCC grant SSCC-08-011

Title: Amyloid precursor protein modulates the nerve transduction in the CNS

Authors: *C. LIU[1], Q. MA[2], S. LI[3], G. DAWE[4], Z. XIAO[5]
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Abstract: Amyloid precursor protein (APP) is best known for its involvement in the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease, and of other forms of dementia, like Down's syndrome. Although the release of component peptides of APP, including Aβ deposition, has been thought to occur predominantly at synapses, increasing evidence strongly suggests that an important component of AD is damage to axons. APP's physiological distribution and function along axons remain unknown. We report that APP null and APP overexpressing transgenic mice show minor hypo- and hyper-myelination, respectively. The length of node changes in APP KO mice. Interestingly, APP is colocalized with an ion channel in the CNS. Coexpression of APP with the ion channel in Xenopus oocytes resulted in an increase in the peak currents. The cell surface expression of these channels in the white matter of spinal cord is decreased in APP null mice. Consistently, APP deficiency significantly reduces spinal conduction velocity. Thus, APP acts along the axons and modulating the nerve transduction.

Disclosures: C. Liu: None. **Q. Ma:** None. **S. Li:** None. **G. Dawe:** None. **Z. Xiao:** None.

Poster

850. APP/Abeta: Cellular Models

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Program#/Poster#:850.02/D26

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The California Institute for Regenerative Medicine (CIRM)

Title: Aß Effects on vascular endothelial cells include tip cell formation, migration and lipid peroxidation

Authors: *T. ALKAM, D. W. ETHELL
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Abstract:
Alzheimer’s pathology
includes dense aberrant blood vessel networks between plaques in the brains of affected people and some mouse models of this disease. We recently reported that high physiological levels of monomeric amyloid-beta (Aß1-42) induce angiogenic responses in vitro and in vivo. Here we report on subsequent studies into the mechanisms responsible for angiogenic changes in Human Umbilical Vein Endothelial Cells (HUVEC) that are induced by Aß1-42. An early event in angiogenesis is the transformation of endothelial shaft cells into tip cells, which lead migration away from the shaft to form of new blood vessels. We found that Aß1-42 monomer significantly increased tip cell formation in HUVEC, but the shorter alpha-secretase pathway product, Aß17-42 (monomer), did not. Further, this effect of Aß1-42 could be blocked by several anti-oxidants, including water soluble ascorbic acid, fat-soluble d-alpha-tocopheryl, and a synthetic catalase/superoxide dismutase mimetic EUK207. Cell migration analysis, using a scratch assay, showed that Aß1-42 induced significantly more migration of HUVEC treated with Aß1-42, whereas Aß17-42 did not; and this Aß1-42 migration effect was also blocked by co-treatment with anti-oxidants. Aß1-42 has copper-binding residues and a putative lipid interaction motif that are absent in Aß17-42, so determined if lipid peroxidation correlated with the ability of Aß1-42 to induce migration and tip cell formation. As angiogenesis often requires the production of additional endothelial cells be produced, we have also explored if Aß1-42 and Aß17-42 affect cell cycle progression in HUVEC. Ongoing studies will determine extracellular and intracellular signaling components of that drive these Aß1-42-induced effects on vascular endothelial cell morphology and behavior.

Disclosures: T. Alkam: None. **D.W. Ethell:** None.

Poster

850. APP/Abeta: Cellular Models

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Program#/Poster#:850.03/D27

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS NS062184

NIGMS GM47368 (ELB)

Title: Quantitative analysis and modeling of particle transport in the living axon: Further evidence for a role of amyloid precursor protein in axonal transport.

Authors: *G. ZIOMEK[1], E. BEARER[2], M. LOEWENBERG[3]
[1]Pathology, Univ. of New Mexico Sch. of Med., Albuquerque, NM; [2]Univ. of New Mexico, Albuquerque, NM; [3]Yale, New Haven, CT

Abstract: Several different cargo-motor receptors for transport have been proposed based on their in vitro binding affinities to kinesin-1. One of these, a 15-amino acid domains from the carboxyl terminus of amyloid precursor protein has been reported to mediate motility in living systems. Here we describe an experimental assay for identification of cargo-motor receptors by their ability to drive transport of exogenous cargo in the axon. Cargo is engineered by derivatizing the surface of polystyrene fluorescent nanospheres with charged residues or with synthetic peptides derived from candidate motor receptor proteins, all designed to display a terminal COOH group. After injection into the squid giant axon, particle movements are imaged by laser-scanning confocal time-lapse microscopy. In this report, new control beads and a strategy to quantitate frequency of bead movements are evolved beyond our previous reports. The ensuing quantitative analysis of time-lapse digital sequences reveals detailed information about bead movements: instantaneous velocity, run lengths, pause frequencies and pause durations. These measurements are then used to provide parameters for a mathematical model that predicts the spatiotemporal evolution of distribution of bead cargo in the axon. The results reveal the dynamics of active intracellular transport of cargo displaying a single species of motor receptor on its surface. The significance of this data and accompanying model pertains to the role transport plays in neuronal function, connectivity, and survival, and has implications in the pathogenesis of neurological disorders such as Alzheimer’s, Huntington and Parkinson’s diseases. NINDS NS062184 and NIGMS GM47368 (ELB).

Disclosures: G. Ziomek: None. **E. Bearer:** None. **M. Loewenberg:** None.

Poster

850. APP/Abeta: Cellular Models

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:850.04/D28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RIH departmental grant

Title: mTOR pathway characterization in AD brain and a Cellular Model.

Authors: *H.-K. LEE, B. KWON, H. W. QUERFURTH
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Abstract: ___The mTOR pathway is a central controller of cell growth, proliferation and physiological processes such as protein biogenesis and autophagy. The mTOR is recently involved in maintaining glucose homeostasis. Activation of mTOR and downstream signaling is inhibited in some animal and cellular models of AD whereas it is up-regulated in others. Moreover, the mTOR complex is in turn partially regulated by Akt which has additional trophic actions on mitochondrial function and synaptic plasticity. Interference with the Insulin/PI3K/Akt signaling pathway has emerged as another important aspect of AD, however there is again disagreement between groups using various models on the direction of change.
___We used a cellular model of AD characterized by accumulation of beta-Amyloid42 in cultured neurons and compared results to brain tissue from AD patients. Levels of total and phospho-mTOR were determined by Western in native N2a and SHSY5Y cultures and after experimental expression of beta-amyloid1-42.
___mTOR inhibition showed partial blockage of intracellular beta-Amyloid induced toxicity. Inhibition of Akt and CREB under beta-amyloid expression conditions was accompanied by an increase in p-mTOR/tot-mTOR ratio and in p-4EBP1. This paradoxical shift and the Akt change were both also reversed by rapamycin. The added role of insulin resistance, while appearing to follow and exacerbate the amyloid effect, is being examined in molecular detail. Inhibition of mTORC1 and activation of mTORC2 may rescue both amyloid and non-amyloid mechanisms of insulin resistance.

Disclosures: H. Lee: None. **B. Kwon:** None. **H.W. Querfurth:** None.

Poster

850. APP/Abeta: Cellular Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Velux Foundation

Swiss National science foundation NCCR grant

Novartis Foundation grant

Hans-Ilse Breuer Stiftung

Swiss National Science Foundation grant

Title: Systems Biology of β - amyloid peptide in Alzheimer's disease

Authors: *J. BALI[1,2], B. SIEGENTHALER[1,2], C. TACKENBERG[1], G. THAKUR[1,2], S. ZURBRIGGEN[1,2], L. RAJENDRAN[1,2]
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Abstract: β- Amyloid (Aβ) peptide, causally linked to neurodegeneration in AD is produced by sequential processing of amyloid precursor protein (APP) by the β-and γ-secretases. While the production of Aβ requires only 3 core components, namely, APP, β and γ secretase, its production at the cellular and organismal context is a complex process due to the interplay of many genes, proteins and signaling networks. To elucidate the cellular signaling mechanisms that regulate the production of Aβ peptide we used gene silencing strategies using small interfering RNAs (siRNA) and performed genome wide screen of all kinases. To analyze the effect of knock down on APP processing, we developed a multiplexing high-throughput screening platform to assay levels of Aβ and sAPPβ (the two products of amyloidogenic processing of APP) within the same well of a 384 well plate. Based on Aβ, sAPPβ values we divided the hits into two groups, those regulating APP and/or β-secretase, (affecting Aβ and sAPPβ) and those regulating γ-secretase, (affecting only Aβ). We identified a large set of kinases as regulators of APP processing. We also found that APP processing is regulated by signaling networks, including Wnt signaling, chemokine receptor signaling and the MAPK pathway. This work reveals the importance of signaling networks and pathways in Aβ metabolism and the utility of functional genomics in understanding the complexity of neurodegenerative diseases.

Disclosures: J. Bali: None. **B. Siegenthaler:** None. **C. Tackenberg:** None. **G. Thakur:** None. **S. Zurbriggen:** None. **L. Rajendran:** None.

Poster

850. APP/Abeta: Cellular Models

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Program#/Poster#:850.06/D30

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

Title: Insulin signaling affects the levels of cholesterol and Aβ production in neuroblastoma 2a cells

Authors: *D. NAJEM[1], W. ZHANG[2,3]
[1]CMM, [2]Fac. of Med., Univ. of Ottawa, Ottawa, ON, Canada; [3]Natl. Res. Council of Canada, Ottawa, ON, Canada

Abstract: One of the neuro-pathological hallmarks of Alzheimer's disease (AD) is the accumulation and deposition of β-amyloid peptides (Aβ) in brain parenchyma and cerebral vessels. These peptides form oligomers and senile plaques, evoke neuroinflammation, and have been implicated in insulin signaling disruption. Also, higher levels of cholesterol are reported in AD pointing to dysergulation of cholesterol homeostasis. In this study, we hypothesize that dysregulation of insulin signaling in AD may affect the expression of the enzymes involved in cholesterol regulation/synthesis leading to alteration of cholesterol metabolism, secretase activities, APP processing and Aβ production in neural cells. To test whether insulin treatment affects cholesterol levels, cholesterol synthesis enzymes, and Aβ production, mouse neuroblastoma 2a cells transfected with human APP gene (N2a-APP) were treated with 10µg/ml insulin for 2, 6 and 12 hours. Cholesterol levels are measured in the cytosolic fraction of the cells and Aβ1-40 and Aβ1-42 levels are measured by ELISA in the medium. Western blot confirms Akt signaling was activated in cells. The cholesterol levels decreased at 2 and 6 hours and increased at 12 hours in parental neuroblastoma 2a cells with similar trends observed for N2a-APP cells. The levels of Aβ1-40 decreased at 2 and 6 hours in N2a-APP cells with similar decreasing trends for Aβ1-42 levels. Protein levels of cholesterol regulatory enzymes were investigated, including 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), and 7-dehydrocholesterol reductase (DHCR-7). There is a trend of decreased levels of both proteins at 6 and 12 hours, an increased level of HMGCR-7 at 2 hours and a trend of decreased levels of DHCR7 at 2 hours for both cell lines mimicking the results obtained in the cholesterol assay. Furthermore, there may be a redistribution of cytosolic cholesterol to the membranes at earlier time points accounting for the observed cytosolic level decrease. The trend of decrease of Aβ may be due to increased expression of insulin degrading enzyme in response to increased insulin

levels observed by western blotting at 2 and 6 hours in N2a-APP cells. Further experiments are underway to evaluate cholesterol distribution using a cell-base detection assay and fractionation assay. Future experiments will investigate mRNA levels of tested proteins by QPCR, α,/β,/γ-secretase activities by activity assays, and cholesterol levels will be measured in brains extracted from IDE/LDLr-KO mice. In conclusion, activation of insulin signaling seems to have significant effects on Akt activation, cholesterol homeostasis and Aβ metabolism in neural N2a and N2a-APP cells.

Disclosures: D. Najem: None. **W. Zhang:** None.

Poster

850. APP/Abeta: Cellular Models

Location: Hall F-J

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Program#/Poster#:850.07/D31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant DA025143

NIH Grant OD008761

AFAR New Investigator Award

Title: Synaptic homeostatic changes alter the expression and localization of amyloid precursor protein

Authors: *C. DELBOVE, R. M. LAZARENKO, X. GAO, B. EDBERG, Z. QI
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Abstract: Recent clinical data suggest that chronic change of synaptic activity is associated with the generation of soluble oligomers of beta-amyloid (A beta) and likely amyloid plaque deposition. As the production of A beta aggregates involves amyloid precursor protein (APP) expression, APP proteolysis, and A beta cleansing, the mechanism underlying homeostasis-induced amyloid development remains unknown. We are investigating this by examining the expression, distribution and processing of most APP isoforms found in the central nervous system. Using reverse transcription quantitative PCR, we found that the neuronal and glial APP variants exhibited different expression levels upon chronic elevation or suppression of synaptic activity over time. Using western blot and immunocytochemistry, we investigated the changes in APP distribution in the plasma membrane or cytosolic lipid compartments in different parts of various types of neurons. Using APP knockout and overexpression mice, we were able to

decouple APP expression and synaptic homeostasis. We found that consistently higher or lower levels of APP feed back to the homeostatic changes, especially affecting presynaptic terminals. Therefore, we concluded that APP is an indispensible regulator of synaptic homeostasis and abnormal synaptic activity can lead to pathological accumulation of APP and A beta.

Disclosures: C. Delbove: None. **R.M. Lazarenko:** None. **X. Gao:** None. **B. Edberg:** None. **Z. Qi:** None.

Poster

850. APP/Abeta: Cellular Models

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:850.08/D32

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The effect of the activation/inhibition of the canonical Wnt Pathway on APP processing

Authors: C. PARR, N. MIRZAEI, *M. SASTRE
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Abstract: Alterations in the Wnt signalling pathway may play an important role in the pathogenesis of Alzheimer’s disease (AD) because of its effects on axonal growth, on synaptogenesis and amyloid-β (Aβ) toxicity. Activation of the canonical Wnt pathway depends on inhibition of GSK3, leading to decreased phosphorylation of β-catenin, preventing its proteasomal degradation and allowing it to enter the nucleus to regulate gene transcription by complexing with TEF/LCF transcription factors. In this study we sought to determine whether activation or inhibition of the canonical Wnt pathway affected the processing of the amyloid precursor protein (APP). We have previously shown that GSK3 inhibition affects Aβ generation (Parr et al., 2012). To evaluate Wnt activation, we transfected N2a cells with either Wnt3a or β-catenin S33 (a mutant that cannot be degraded in the proteasome). On the other hand, the Wnt pathway was inhibited by transient transfection of N2a cells with two known antagonists, sFRP-1 and DKK-1. sFRP-1 inhibits the pathway by antagonizing the Wnt/Fz binding. DKK-1 inhibits the canonical Wnt pathway by binding to LRP6, the Wnt co-receptor. Activation of the canonical Wnt pathway resulted in a decrease in Aβ levels and in β-carboxy terminus fragments (CTFs). In addition, transient transfection of Wnt3a and β-catenin S33 reduced the transcription, expression and activity of BACE1. Conversely, inhibition of the Wnt pathway increased Aβ generation and elevated the amyloidogenic processing of APP. Consistent with these results, inhibition of the pathway was also found to increase the transcription, expression and activity of BACE1. In conclusion, the canonical Wnt signalling pathway seems to be involved in the regulation of

BACE1 transcription and consequently in the generation of Aβ. The specific mechanisms of this transcriptional control remain to be elucidated.

Disclosures: C. Parr: None. **N. Mirzaei:** None. **M. Sastre:** None.

Poster

850. APP/Abeta: Cellular Models

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Program#/Poster#:850.09/D33

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Swiss National Science Foundation

NCCR (National Center of Competence in Research) Neural Plasticity and Repair of the Swiss National Science Foundation

Title: Calsyntenin-1 vesicles shelter APP from proteolytic processing during anterograde axonal transport

Authors: *T.-M. DIEP[1], M. STEUBLE[1], P. SCHÄTZLE[1], A. LUDWIG[1], M. TAGAYA[2], P. SONDEREGGER[1]
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Abstract: Endocytosis of amyloid-β precursor protein (APP) is thought to represent the major source of substrate for the production of the amyloidogenic Aβ peptide by the β-secretase BACE1. The irreversible nature of proteolytic cleavage implies the existence of an efficient replenishment route for APP from its sites of synthesis to the cell surface. We recently found that APP exits the trans-Golgi network in intimate association with calsyntenin-1, a transmembrane cargo-docking protein for Kinesin-1-mediated vesicular transport. Here we used selective immunoisolation and biochemical characterization of organelles of the intracellular trafficking routes, immunocytochemistry and live-imaging of intracellular trafficking organelles, and RNAi to characterize the function of calsyntenin-1 in neuronal APP transport. We found that APP is co-transported with calsyntenin-1 along axons to the early endosomes in the central region of growth cones in a carrier that excludes the α-secretase ADAM10. Intriguingly, the calsyntenin-1/APP organelles contained BACE1, suggesting premature cleavage of APP along its anterograde path. However, we found that APP contained in calsyntenin-1/APP organelles was stable. We further analyzed vesicular trafficking of APP in cultured hippocampal neurons, in which calsyntenin-1 was reduced by RNAi. We found hitchhiking of APP with other anterograde

carriers, such as ADAM10-containing vesicles of the secretory pathway. Increased proteolytic processing of APP and Aβ secretion in these neurons suggested that the reduced capacity for calsyntenin-1-dependent APP transport resulted in the premature encounter of unprotected APP with its ectodomain proteases. In combination, our results characterize calsyntenin-1/APP organelles as a carrier for sheltered anterograde axonal transport of APP.

Disclosures: T. Diep: None. **M. Steuble:** None. **P. Schätzle:** None. **A. Ludwig:** None. **M. Tagaya:** None. **P. Sonderegger:** None.

Poster

850. APP/Abeta: Cellular Models

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Program#/Poster#:850.10/D34

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Exploration of epigenetic small molecule modulators of β-amyloid production

Authors: *H. SALAH-UDDIN, C.-H. VOLMAR, P. HALLEY, C. WAHLESTEDT
Ctr. for Therapeut. Innovation and Dept. of Psychiatry and Behavioral Sc, Univ. of Miami - Miller Sch. of Med., Miami, FL

Abstract: Alzheimer’s disease (AD) is the leading cause of senile dementia with approximately 5.4 million people afflicted with the disease in the USA alone (Alzheimer’s disease facts and figures, 2012). The amyloid cascade hypothesis links amyloid beta (Aβ) to the pathophysiology of AD, triggering plaque formation, tau hyperphosphorylation, neurodegeneration and eventually death. Aβ is the product of the sequential proteolytic cleavage of the amyloid precursor protein (APP) by the proteases β- and γ-secretases. Emerging research suggests that epigenetics may play a role in the etiology of late onset AD (LOAD), the most common form of the disease. Indeed, abnormal changes in DNA methylation and/or acetylation have been observed in AD cases and in vitro. Targeting both histone deacetylases (HDAC) and histone acetyltransferases (HAT) have thus been proposed as potential therapies against AD and small molecules that effect epigenetic targets have been shown to attenuate Aβ accumulation in AD models. In this study, we have confirmed anti-amyloidogenic effect of trichostatin A and resveratrol, in vitro. We have also identified other small molecules with possible epigenetic mechanism(s) of action that cause greater reduction of Aβ1-40 and Aβ1-42 levels. Work is under way to further decipher the mode of action of these molecules on APP and its processing. This study supports the hypothesis that targeting epigenetics pathways with small molecules may be a suitable therapeutic approach against AD.

Disclosures: H. Salah-Uddin: None. **C. Volmar:** None. **P. Halley:** None. **C. Wahlestedt:** None.

Poster

850. APP/Abeta: Cellular Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cure Alzheimer Fund

Title: Alzheimer’s disease models based on immortalized human neural progenitor cells

Authors: *Y. KIM[1,2], C. D'AVANZO[1], R. E. TANZI[1], D. M. KOVACS[1], D. KIM[1]
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Abstract: Research on Alzheimer’s disease (AD) has been hampered by lack of appropriate models to recapitulate the human nervous system. Advances in stem cell technology make it possible to generate human neurons harboring the mutations that are strongly associated with specific neurodegenerative diseases. Amyloid-β peptide (Aβ) is the key pathogenic molecule in AD. Mutations in amyloid β precursor protein (APP) and presenilin (PS) are strongly associated with early-onset AD by increasing toxic Aβ. Here, we developed human neural progenitor cells (hNPCs) genetically modified to over-produce Aβ. For these studies, we used an immortalized human neural progenitor cell line derived from the fetal brain. Lentiviral constructs were generated to overexpress AD genes, including APP with K670N/M671L and V717I familial AD (FAD) mutations (APPSL), PS1, PS1 with deltaE9 FAD mutation (PS1dE9), and BACE1. We established hNPC lines stably expressing APPSL, PS1, PS1dE9, or BACE1. hNPCs co-expressing APPSL/ PS1dE9 or APPSL/PS1dE9/BACE1 were also generated to produce more toxic Aβ species. The hNPCs were then differentiated into neuronal and glial cells under growth factor free media for 30-40 days and the expression of the transfected AD genes were confirmed by Western Blot analysis. We also measured secreted Aβ40 and 42 levels by ELISA. As expected, the hNPCs with APPSL, APPSL/PS1dE9, and APPSL/PS1dE9/BACE1 showed dramatic increases in Aβ40 (up to 12 fold) and Aβ42 (up to 13 fold) levels compared to control hNPCs. Remarkably, coexpression of APPSL and PS1dE9 increased the Aβ42/40 ratio by 5 fold, confirming a pathogenic role of PS1dE9 mutation. As a proof-of-concept experiment for future AD drug screening, we treated the APPSL cells with an experimental γ-secretase modulator and

found that this modulator significantly decreased both total Aβ levels and the Aβ42/40 ratios. Taken together, our data strongly support that the genetically modified hNPCs can serve as a rapid and robust AD models for basic research and drug screening.

Disclosures: Y. Kim: None. **C. D'Avanzo:** None. **R.E. Tanzi:** None. **D.M. Kovacs:** None. **D. Kim:** None.

Poster

850. APP/Abeta: Cellular Models

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Program#/Poster#:850.12/D36

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG16573

AG00096

AG00538

Title: Neutrophin trafficking deficts are mediated by impaired ubiquitin recycling in AD

Authors: B. L. AGUILAR, *W. W. POON, A. J. CARLOS, C. W. COTMAN
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Abstract: Utilizing a novel microfluidic culture chamber, we demonstrate that neurotrophin-mediated retrograde signaling deficits caused by Aβ is mimicked by LDN, an inhibitor of Ubiquitin-C-terminal hydrolase L1 (UCH-L1), a deubiquitinating enzyme that functions to maintain cellular ubiquitin homeostasis. We demonstrate that Aβ impairs Trk receptor internalization and retrograde signaling endosome trafficking, including p-ERK5 signaling and CREB-dependent gene expression. We also show that some of these deficits can be reversed by increasing cellular UCH-L1 levels. Our results suggest that a consequence of the impaired proteasome function is defective regulation of ubiquitin homeostasis leading to impaired Trk-mediated retrograde signaling compromising neuronal survival.

Disclosures: B.L. Aguilar: None. **W.W. Poon:** None. **A.J. Carlos:** None. **C.W. Cotman:** None.

Poster

850. APP/Abeta: Cellular Models

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Program#/Poster#:850.13/D37

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Cross-linking of cell surface APP leads to increased Aβ production in hippocampal neurons - Implications for Alzheimer's disease

Authors: *R. LEFORT, J. POZUETA, M. SHELANSKI
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Abstract: The accumulation of the β-amyloid peptide (Aβ) in Alzheimer's disease (AD) is thought to play a causative role in triggering synaptic dysfunction in neurons leading to their eventual demise through apoptosis. Aβ is produced and secreted upon sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretases. However, while Aβ levels have been shown to be increased in AD patients' brains, little is known about how the cleavage of APP and the subsequent generation of Aβ is influenced, or if the cleavage process changes over time. It has been proposed that Aβ can bind APP and promote amyloidogenic processing of APP, further enhancing Aβ production. This idea has remained controversial due to a lack of a clear mechanism and complicated by the promiscuous nature of Aβ binding. To work around this problem, we used an antibody-mediated approach to bind and cross-link cell surface APP in cultured rat primary hippocampal neurons. Here we show that cross-linking of APP is sufficient to trigger a loss of dendritic spines in neurons, while simultaneously increasing the levels of Aβ in viable neurons. We further demonstrate that this involves two independent mechanisms, with caspase-2 mediating the synaptic effects and caspase-3 regulating the increase in Aβ production. More specifically, cross-linking of APP triggers the activation of caspase-2, followed by the activation RhoA, leading dendritic spine collapse. Cross-linking of APP also activates caspase-3 which inactivates the adaptor protein GGA3, leading to the accumulation of BACE1 and Aβ. Taken together, our data suggest the occurrence of a positive pathogenic feedback loop involving Aβ and APP in affected neurons possibly allowing Aβ to spread to nearby healthy neurons.

Disclosures: R. Lefort: None. **J. Pozueta:** None. **M. Shelanski:** None.

Poster

850. APP/Abeta: Cellular Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSC98-2320-B-001-020-MY3

Title: The effect of zinc on the dimerization, processing, and trafficking of amyloid precursor protein

Authors: *W.-W. CHANG, Y.-R. CHEN
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Abstract: Alzheimer's disease (AD) is the most prevalent, age-related neurodegenerative disease that accounts for > 50% of the dementia cases. The presence of extracellular β-amyloid (Aβ) in the brain is one of the classic characteristics of AD besides the intracellular neurofibrillary tangles. In post-mortem AD brains, zinc is significantly increased within Aβ plaques which renders zinc an important player in AD etiology. Moreover, zinc has been shown to accelerate Aβ deposition. Aβ is a cleaved peptide fragment of amyloid precursor protein (APP) that is a type I transmembrane protein with a large ectodomain (contains two major regions, E1 and E2), a single transmembrane region, and a short intracellular region. APP can form dimers in which three regions have been reported to promote its dimerization. They are the E1 domain, E2 domain ,and the G*XXX*G motif near the luminal face of the transmembrane region. APP dimerization is able to modulate APP processing and Aβ production. Since APP contains zinc binding motifs in its amino-terminal domain and Aβ region and previous study has shown that zinc can induce dimerization of $APP^{170-188}$ peptide without knowing the effect on full-length APP, we would like to elucidate the mechanism of zinc effect toward APP dimerization, processing, and trafficking using cellular assays. In this study, by the strategy of bimolecular fluorescence complementation and chemical cross-linking, we demonstrated that zinc indeed induced APP dimerization in human kidney cells, HEK293T and human neuroblastoma, SH-SY5Y cells. Besides, zinc enhanced Aβ production that is likely resulting from APP dimerization. Further, we investigated the zinc binding motifs and observed the effect on APP trafficking. Overall, our results help to elucidate the roles of APP and zinc in AD pathogenesis and facilitate the development of future therapeutic strategies.

Disclosures: W. Chang: None. **Y. Chen:** None.

Poster

850. APP/Abeta: Cellular Models

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:850.15/D39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSC98-2320-B-001-020-MY3

Title: Influence of ganglioside biosynthesis in Alzheimer's amyloid precursor protein (APP)-expressing cells

Authors: *T.-W. LIN, Y.-R. CHEN
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Abstract: Alzheimer's disease (AD) is the most common dementia in the elderly. Dementia describes significant loss of certain mental functions such as memory, attention, and abstract thinking. The primary neuropathologic hallmark in AD is senileamyloid plaques comprising of amyloid fibrils. Plaque formation is believed to result from increased production or decreased clearance of the amyloid-β beta (Aβ) peptide, which is generated fromby subsequent, proteolytic cleavages of a type I membrane protein larger precursor protein known as amyloid precursor protein (APP) via β- and γ-secretases. Several strong indications point towards an important role of gangliosides in AD pathogenesis. Gangliosides are sialic acid-containing glycosphingolipids (GSLs) that are expressed in the outer leaflet of the plasma membrane of all vertebrate cells and enriched in the central nervous system. Gangliosides play are involved important roles in a variety of cellular functions, including serving as antigens, receptors for bacterial toxins, mediators of cell adhesion, and mediators/modulators of signal transduction. In this study, we have generatedcreated stable cell lines for different APP isoforms stable lines including APP770, APP751, APP695 in human neuroblastoma SH-SY5Y and human embryonic kidney cells HEK293T cells. By using flowcytometry analysis, Wwe found ganglioside contents were much more abundant in SH-SY5Y than HEK293T. In addition, we observed decrease of GD3 and GD1a in all overexpressed APP770/751/695 HEK293T stable lines. However, the changes of other gangliosides examined (including GM1, GM2, GD3, GD2 and GD1a) levels awere presented differently in APP isoformisoform expressing SH-SY5Y stablecell lines. Furthermore, we used thin layer chromotagraphychromatography (TLC) to confirm thise result and used reverse transcription to detect different expressing levels of the glycotransferase levels. Our results Through the cell surface ganglioside examination, we suggest molecular mechanisms for the changes of gangliosides biosynthesis in AD.

Disclosures: T. Lin: None. **Y. Chen:** None.

Poster

850. APP/Abeta: Cellular Models

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:850.16/D40

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Mitochondrial dysfunction in a human neuronal cell model of early Alzheimer`s disease

Authors: *C. SCHILLER[1], K. LEUNER[2], W. E. MUELLER[1]
[1]Pharmakologie für Naturwissenschaftler, Goethe Univ. Frankfurt, Frankfurt Am Main, Germany; [2]Dept. of Mol. and Clin. Pharm., Univ. of Erlangen, Erlangen, Germany

Abstract: The important role of mitochondrial dysfunction in Alzheimer's disease (AD) is widely acknowledged. Besides brain aging, mitochondrial dysfunction is a consequence of high amyloid beta (Aß) and hyperphosphorylated tau levels, two major histopathological hallmarks of AD.
In previous studies we were able to show that HEK and PC12 cells, which are stably transfected with DNA constructs for wildtype human amyloid precursor gene (APPwt) exhibit mitochondrial dysfunction compared to the corresponding vector cells (Co). Because human embryonic kidney cells are a peripheral cell line and phaeochromocytoma cells12 are neuronal, but originate from the rat, we now focus on SY5Y cells as a human neuronal cell line. When transfected again with the same construct, these cells have Aß levels approximately 1.5 fold higher than the control levels. Thus, SY5Y-APPwt cells represent an appropriate human cell model for the early stages of AD.
In order to investigate mitochondrial function of the transfected cells relative to vector controls, we measured ATP levels, mitochondrial membrane potential, and mitochondrial length. Both ATP and mitochondrial membrane potential are significantly reduced in APPwt cells underlying the assumption of mitochondrial dysfunction representing a very early event in the development of AD. Using confocal microscopy analysis, we were also able to demonstrate that the mitochondrial morphology is clearly different between the two cell types. In comparison to APPwt cells control cells possess much more tubular and elongated mitochondria, suggesting that already low Aß levels lead to enhanced fragmentation.
To further characterize the neuronal function of these human neuroblastoma cells we have determined neurite outgrowth in both cell types in the presence of BDNF. Interestingly, APPwt cells show significantly longer neurites under basal conditions, indicating that very low Aß levels can lead to a compensatory increase of neurite outgrowth, at least in the beginning.
In summary, our findings highlight the impairment of mitochondrial function as consequence of only slightly elevated amyloid beta levels as well as a compensatory improvement of other parameters.

Disclosures: C. Schiller: None. **K. Leuner:** None. **W.E. Mueller:** None.

Poster

850. APP/Abeta: Cellular Models

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:850.17/D41

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Society

MRC

Title: Epicatechin inhibits beta-secretase mediated APP processing and reduces amyloid beta pathology in APP/PS1 transgenic mice

Authors: *C. J. COX[1], F. CHOUDHRY[2], D. R. HOWLETT[2], P. T. FRANCIS[2], R. J. WILLIAMS[1]
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Abstract: Mounting epidemiological evidence suggests that a flavonoid-rich diet can inhibit the development of dementia (Williams and Spencer, 2012). In relation to Alzheimer's Disease (AD) some flavonoid-rich extracts have been shown to exert beneficial effects on amyloid beta (Aβ) pathology, although the mechanism remains uncertain. The aim of this study was to identify single, isolated flavonoid compounds that were able to modulate Aβ pathology at physiologically relevant concentrations. Development of a cell-based reporter gene luciferase assay in primary neurons has enabled βγ-secretase-mediated APP processing to be studied in cells with functional synapses. Primary cultured embryonic mouse cortical neurons (5 DIV) were transfected with APP_{695}Gal4, pFR-luciferase and TK-Renilla, with or without Fe65, using lipofectamine 2K and activity assessed by luminescence. Basal luciferase expression was potentiated 10 fold following co-transfection with the adaptor protein Fe65. Mutagenesis of APP_{695}Gal4 to generate the Swedish mutant (KM/NL) APP_{695}SweGal4 caused a 2 fold increase in expression demonstrating sensitivity to pathological conditions. Transfected neurons were treated with select flavonoids (100nM and 10 μM, 24 h) to identify novel secretase modulators. Epigallocatechin (EGC) and (-)-epicatechin (EC) were taken forward as lead compounds and showed potent inhibition of luciferase expression with IC50s of 18.6 nM and 20.5 nM respectively. Despite similar potencies, the kinetics of inhibition differed; EGC was effective at 24h whilst EC was effective at 6h. Due to the established bioavailability of EC it was selected for administration to APP/PS1 mice via drinking water (10mg /day) for 21 days and Aβ pathology assessed. EC supplemented mice showed decreased Aβ plaque density and evidence of reduced soluble Aβ in cortex. Collectively this study supports the development of EC and other proanthocyanidins (Wang et al., 2012) for therapeutic use in AD.
Project supported by MRC and Alzheimer's Society.
Wang, J., Ferruzzi, M.G., Ho, L., Blount, J., Janle, E.M., Gong, B., Pan, Y., Gowda, G.A., Raftery, D., Arrieta-Cruz, I., *et al.* (2012). Brain-Targeted Proanthocyanidin Metabolites for

Alzheimer's Disease Treatment. J Neurosci *32*, 5144-5150.
Williams, R.J., and Spencer, J.P. (2012). Flavonoids, cognition, and dementia: Actions, mechanisms, and potential therapeutic utility for Alzheimer disease. Free Radic Biol Med *52*, 35-45.

Disclosures: C.J. Cox: None. **F. Choudhry:** None. **D.R. Howlett:** None. **P.T. Francis:** None. **R.J. Williams:** None.

Poster

850. APP/Abeta: Cellular Models

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:850.18/D42

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Public Grant BFU2010-20396, from Innovation and Science Ministry (Spain)

Title: DNA-damage response as a possible mechanism underlying common pathways involved in the effects of beta-amiloid overexpression, distress, and cancer.

Authors: *S. ORTEGA MARTINEZ[1], J. SERRANO[1], A.-P. FERNÁNDEZ[1], S. GONZALO[2], R. MARTÍNEZ-MURILLO[1], J.-L. TREJO[1]
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Abstract: Objective: We have addressed the putative interaction between beta-amyloid overexpression and cancer signalling in brain stem cells focused on the DNA-damage response, both in basal conditions and after administration of stress hormones.
Materials and methods: For this goal we used 3 astrocytomas cells lines [CT2A (control), APP-PS1 L.1 and APP-PS1 L.3 (cell lines of astrocytoma obtained from mice APP/PS1)] and both neural stem cells from the brain of control mices and neural stem cells from the brain of APP-PS1 mices.
To cause DNA damage, cells were irradiated with 5 Gray (Gy) for all experiments. We evaluated this DNA damage by immunofluorescence p53 binding protein 1 (53BP1) foci as indicator of Non Homologous End-Joining (NHEJ) pathway and γH2AX foci as indicator of double strand breaks (DSBs). To set DNA repair kinetic we based on Neutral comet assay.
Results: We have found that APP/PS1 cell lines studied show a basal DNA damage weighed up by the presence of γ-H2AX, and these cells show a marker corresponding by oxidative DNA damage [8-hydroxy-2′-deoxyguanosine (8OHdG)], which is also present in neural stem cells from the brain of APP/PS1 mice.
It worths to take into account the results obtained after administration of dexamethasone (50μM)

on the different cell lines. The response consisted of an exacerbation of the DNA-damage signalling and repair compared to the controls treated with vehicle, especially at the longer time periods analyzed, although no differences were found between cell lines. Furthermore studies realized by Neutral comet assay show that all astrocytomas cell lines impair non-homologous end joining pathway when we treated with dexamethasone before irradiation. Interestingly, this result is completely different when assayed in neural stem cells from normal brains, where preincubation with dexamethasone before irradiation decreased number of cells displaying foci. On the other hand, neural stem cells from APP-PS1 brain show a different signal. Basal DNA damage is the most important characteristic in this cells which have the highest level found corresponding with γ-H2AX foci, but dexamethasone treatment decrease levels of positive cells foci when cells are not irradiated.
Conclusion: The deleterious effects of beta-amyloid overexpression and the cancer signalling can result additive when appear together in the same cell, at least as observed for the DNA-damage response and repair system. Similarly, the different responses of DNA-damage to high levels of stress hormones may also depend on the distinct combination of beta-amyloid expression level and cancer signalling, as demonstrated by the different effects in the distinct cell lineage.

Disclosures: S. Ortega Martinez: None. **J. Serrano:** None. **A. Fernández:** None. **S. Gonzalo:** None. **R. Martínez-Murillo:** None. **J. Trejo:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.01/D43

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: A grant of the Korea Healthcare Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (No.A101482)

Title: Spatial memory facilitation by electrical stimulation of the medial septum in rats

Authors: *D. JEONG[1], J. LEE[1], S. LEE[2], S. KIM[2], J. CHANG[1]
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Abstract: Recently, deep brain stimulation has been used to treat various neurological disorders. Some studies support that DBS can be a strategy to treat Alzheimer’s disease. The aim of this

study was to evaluate the effect of electrical stimulation in the medial septum using rat model mimicking basal forebrain cholinergic deficits of Alzheimer's disease.
Four experimental groups were composed of normal, lesion, lesion + implantation and lesion + stimulation. 192 IgG-saporin (Selective cholinergic toxin, 8ul of 0.63ug/ul) were bilaterally injected into the lateral ventricle. Electrode was stereotactically implanted into the left medial septum (AP +0.6, ML 0.16, DV -6). Stimulation parameters are 50Hz, 120us pulse width and 1 volt. One week after implantation, Stimulation started for 2 weeks. Two weeks after surgery, water maze was performed for 1 week and rats were sacrificed immediately after behavioral test. Features were verified by immunochemistry and AChE assay.
During the training trials, latencies of lesion and implantation significantly increased in day3 and day4. In contrast, latency of stimulation group had no differences as compared to normal group but it decreased significantly when compared to lesion group in day4. In the probe test, lesion group had decreases in time in target quadrant, time in platform zone and the number of platform crossing. Although they did not perform as normal group, stimulation group showed tendency of recovery. IHC and AChE assay are ongoing.
Spatial memory is associated with hippocampus. We had expected activation of hippocampus by stimulation of the medial septum. We confirmed that stimulation of the medial septum facilitates acquisition and recall of spatial memory. Currently we are studying the effects of medial septal stimulation on the hippocampus.

Disclosures: D. Jeong: None. **J. Lee:** None. **S. Lee:** None. **S. Kim:** None. **J. Chang:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: American Health Assistance Foundation

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Cure Alzheimer's Fund

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Title: Group II mGluR antagonist reduces brain Aβ, stimulates hippocampal neurogenesis, and reverses transgene-induced behavioral deficits in APP E693Q transgenic mice

Authors: *S. KIM[1,2], J. W. STEELE[1,2,3], S. W. LEE[6], D. CLEMENSON[6], C. GLABE[7], M. E. EHRLICH[1,4], F. H. GAGE[6], S. GANDY[1,2,5,8]
[1]Neurol., [2]Alzheimer's Dis. Res. Ctr., [3]Grad. Sch. of Biol. Sci., [4]Pediatrics, [5]Psychiatry, Mount Sinai Sch. of Med., New York, NY; [6]Lab. of Genet., The Salk Inst. for Biol. Studies, La Jolla, CA; [7]Mol. Biol. and Biochem., Univ. of California, Irvine, Irvine, CA; [8]James J Peters VA Med. Ctr., Bronx, NY

Abstract: A key event in the pathogenesis of Alzheimer's disease (AD) is accumulation of neurotoxic amyloid beta (Aβ) oligomers in the brain, formation of which is dependent on the concentration of monomeric Aβ42 peptides. We reported that activation of Group II metabotropic glutamate receptors (Gp II mGluR: mGluR 2, mGluR3) triggers Aβ42 production at the synapse in vitro, which is selectively suppressed by antagonist pretreatment (Kim et al., J Neurosci, 2010). We thus hypothesized that chronic suppression of Gp II mGluR signaling might slow disease progression by reducing the accumulation of Aβ oligomers. Notably, Gp II mGluR antagonists stimulate hippocampal neurogenesis, enhance cognition, and provide antidepressant-like/anxiolytic effects in wildtype rodents. Combined with the synaptic Aβ42-reducing activity, Gp II mGluR antagonists appear to possess a number of properties that might be useful in the prevention or treatment of AD. Pilot studies were performed in "oligomer-only" mice (APP E693Q; Gandy et al., Ann Neurol, 2010) and wildtype littermates (7-10 months old), both of which were treated with either BCI-838 (oral prodrug of BCI-632, a Gp II mGluR antagonist, 5 mg/kg p.o.) or vehicle for 3 months. Reduction in anxiety was observed in BCI-838 treated oligomer-only mice in the elevated plus maze. Chronic administration of BCI-838 corrected transgene-induced learning and memory deficits in oligomer-only mice in both contextual fear conditioning and novel object recognition tasks. Chronic inhibition of Gp II mGluR activity reduced the levels of prefibrillar oligomeric Aβ in the cortex and Aβ42 levels in the hippocampi of APP E693Q mice when compared to vehicle-treated APP E693Q mice. Notably, chronic BCI-838 treatment stimulated hippocampal progenitor cell proliferation, which resulted in a significantly increased number of surviving newborn neurons in APP E693Q mice. We propose that Gp II mGluR antagonists are promising compounds for the prevention or treatment of AD because of their unique combination of synaptic Aβ42- and Aβ oligomer-lowering activity combined with pro-cognitive, antidepressant, anxiolytic, and pro-neurogenic activities. [S.H. Kim and J.W. Steele contributed equally.]

Disclosures: S. Kim: None. **J.W. Steele:** None. **S.W. Lee:** None. **D. Clemenson:** None. **C. Glabe:** None. **M.E. Ehrlich:** None. **F.H. Gage:** Consultant/Advisory Board; BrainCells Inc. **S. Gandy:** Research Grant; Amicus Pharmaceuticals. Consultant/Advisory Board; Pfizer-Janssen Alzheimer's Immunotherapy Alliance.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.03/D45

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG034606

Title: A novel drug candidate for Alzheimer’s disease increases the number of functional synapses in the CA1 region of the hippocampus, in the absence of enhanced LTP

Authors: *A. L. MEGILL[1], A. KIRKWOOD[1], H.-S. HOE[2], H.-K. LEE[1]
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease characterized by a loss of synaptic contacts in both the neocortex and hippocampus, causing a decrease in synaptic transmission and cognitive decline. While AD is usually characterized by accumulation of amyloid plaques (comprised of aggregated Aβ peptides), loss or alteration of dendritic spines might be one of the first signs of AD pathology, preceding plaque deposition. An ethylene glycol derivative of the benzothiazole aniline (BTA) class of compounds, BTA-EG4, is a novel drug candidate for AD, which was identified by its ability to bind Aβ. Current therapies in AD are symptomatic only, but BTA-EG4 may be able to prevent the progression of AD pathology by potentially decreasing pathologic functions of Aβ. Our collaborators at Georgetown University found that BTA-EG4 decreases Aβ production, increases the density of dendritic spines in CA1, and improves spatial learning and memory. Here we examined whether the structural and behavioral changes are associated with functional changes at synapses. To do this, we measured synaptic transmission and plasticity in the CA1 region of the hippocampus after low (15 mg/kg) and high (30 mg/kg) dose injections of BTA-EG4 in C57BL/6 mice. We found that BTA-EG4 increases the frequency of AMPA receptor-mediated miniature excitatory postsynaptic currents (mEPSCs), which suggests that the increased spine density reflects increases in the number of functional excitatory synapses. However, there was no change in the amplitude of mEPSCs suggesting that the drug does not alter the strength of individual synapses. In addition, there was no change in the input-output function of synapses following BTA- EG4 treatment, which suggests normal basal synaptic transmission. Furthermore, we did not observe an increase in the magnitude of LTP induced at the Schaffer collateral (SC) input nor at the temporo-ammonic (TA) input to CA1. To the contrary, there was a significant reduction in the magnitude of LTP with certain induction protocols. This suggests that the improved learning and memory is not due to enhancement of LTP at synapses. Interestingly, BTA-EG4 decreased the summation of

synaptic responses during all LTP induction protocols suggesting that there may be enhancement of downstream signaling to sustain LTP. Indeed, our collaborators observed that BTA-EG4 increased Ras activity, which is necessary for its ability to increase dendritic spine density. Collectively, our results suggest that BTA-EG4 may benefit cognitive function by increasing the number of functional synapses, which may provide a larger capacity to store information, in the absence of enhanced LTP.

Disclosures: A.L. Megill: None. **A. Kirkwood:** None. **H. Hoe:** None. **H. Lee:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01-AG031859

American Health Assistance Foundation

Title: PPARγ agonism promotes the formation of a nuclear hippocampal pERK-PPARγ complex during the memory consolidation phase that mediates cognitive rescue in Tg2576 Alzheimer's mice

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Abstract: The hippocampus is requisite for the establishment and storage of new memories and is particularly vulnerable to the ravages of Alzheimer's disease (AD). Recent clinical trials suggest that treatment of early-AD patients with drugs that activate PPARγ can improve hippocampus-dependent cognitive function. PPARγ agonism via the selective agonist rosiglitazone (RTZ) rescues hippocampus-dependent cognitive deficits in the Tg2576 AD mouse model. Using qPCR and quantitative mass spectrometry, we found that RTZ-mediated cognitive rescue is accompanied by the induction of several PPRE-containing genes and PPARγ-regulated proteins, many of which are important for mitochondrial and synaptic function including components of the ERK MAPK pathway. ERK MAPK activity (pERK) is required for proper hippocampus-dependent cognitive performance.
We found that during memory consolidation Tg2576 treated with RTZ exhibit increased hippocampal PPARγ in the nucleus that associates with pERK. Furthermore, PPARγ antagonism via the irreversible, selective, full antagonist GW9662 disrupts both the pERK-PPARγ

complexes and cognitive rescue in RTZ-treated Tg2576. We further demonstrate that these novel nuclear pERK-PPARγ complexes are elevated under steady state conditions in untreated Tg2576 mice with higher cognitive function, indicating a correlation between high complex levels and high cognitive ability. Thus, in AD mice the RTZ-mediated increase in nuclear pERK-PPARγ complexes serves a compensatory role by increasing pERK activity in the nucleus during memory consolidation. Finally, in a manner similar to Tg2576 brain, a correlation was also found in human cortex between the amount of pERK-PPARγ complex and the severity of AD (MMSE score).
Supported by grants from the NIH (R01-AG031859) and the American Health Assistance Foundation.

Disclosures: J.B. Jahrling: None. **L. Denner:** None. **K.T. Dineley:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Program#/Poster#:851.05/D47

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA (AG031687)

NARSAD YI 2006/2008

Title: Phosphodiesterase-4A knockout protects against abeta42-induced memory decline

Authors: *R. HANSEN, III[1], Q. WU[2], C. WANG[2], M. CONTI[3], H.-T. ZHANG[2]
[1]Neurobio. and Anat., [2]Behavioral Med. and Psychiatry , Physiol. & Pharmacol., West Virginia Univ., Morgantown, WV; [3]Obstetrics & Gynecology, Univ. of California, San Francisco, CA

Abstract: Alzheimer’s disease (AD) is a debilitating dementia currently affecting ~ 5 million Americans resulting in the 6th leading cause of death in this country. The main pathological hallmark of AD is the accumulation of amyloid plaques in the brain which are comprised of beta-amyloid peptides 1-40 (Abeta40) and 1-42 (Abeta42). Despite years of research and numerous failed clinical trials, there are still no effective drugs to alleviate symptoms in AD such as memory loss, or underlying pathology such as plaque accumulation and neuron/synapse loss. Interestingly, AD has been shown to significantly suppress cAMP signaling resulting in decreased levels of the nootropic transcription factor CREB. Inhibition of phosphodiesterase 4 (PDE4), a family of enzymes responsible for the hydrolysis of cAMP, may provide a novel potential therapeutic in AD by raising cAMP levels. PDE4A, which is one of four subtypes

(PDE4A-D), is strongly expressed in the hippocampus which is particularly affected in AD; however, the functional role of PDE4A has not been investigated. The purpose of this study was to identify the role of PDE4A in memory loss associated with AD through the use of PDE4A knock out (KO) mice. This study was conducted by injecting Abeta42 into the hippocampus of PDE4A KO mice and wild type (WT) controls and after 2 weeks recovery, memory analyzed by the step through passive avoidance test. In addition, cAMP/phospho-CREB (pCREB) levels were also analyzed to identify molecular correlates involved. PDE4A KO significantly attenuated the memory deficits caused by Abeta42 infusions into the hippocampus as seen in passive avoidance. Also, PDE4A KO resulted in a significant increase of cAMP and pCREB levels and attenuated the down expression of pCREB caused by Abeta42 injection. In conclusion, PDE4A inhibition may represent a novel nootropic for the treatment of AD related memory loss.

Disclosures: R. Hansen: None. **Q. Wu:** None. **C. Wang:** None. **M. Conti:** None. **H. Zhang:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.06/D48

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Takeda Global Research & Development, Inc.

Title: Acute and repeat dosing with pioglitazone produces changes in resting state brain functional connectivity in awake rats

Authors: *K. E. ASIN[1], D. CRENSHAW[2], K. GOTTSCHALK[2], N. ZHANG[3], Z. LIANG[3], A. ROSES[2]
[1]NCSE, Takeda Pharma, Deerfield, IL; [2]Zinfandel Pharmaceuticals, Inc., Research Triangle Park, NC; [3]Psychiatry, Ctr. for Comparative Neuroimaging (CCNI), Univ. of Massachusetts Med. Sch., Boston, MA

Abstract: Pioglitazone HCl (PIO) is a thiazolidinedione (TZD) PPARγ receptor agonist approved for treatment of type 2 diabetes (T2D). In addition to reducing peripheral insulin resistance, evidence suggests that PIO improves or protects neuronal mitochondrial function and increases mitochondrial biogenesis. TZDs are reported to ameliorate some of the cognitive deficits seen in murine models of Alzheimer’s disease (AD) and in humans with AD, and have been proposed for the treatment or prevention of AD. This abstract reports the effects of low-dose PIO treatment on resting state functional connectivity (FC) in the brains of awake rats.

Resting state FC reflects temporal correlations in spontaneous fluctuations in hemodynamic activity across brain regions, as measured using blood oxygen level-dependent (BOLD) functional MRI.
Awake, adult male Wistar rats were acclimated to the scanning procedure for ~7 days. After acclimation, a baseline scan was performed using a 4.7 Tesla/40cm horizontal magnet and BOLD imaging 2.5-3h after dosing with citric acid (Veh). Thereafter, rats were administered Veh or PIO orally at doses estimated to provide plasma exposures equivalent to treating humans with 1.5, 3, 6 or 12 mg/day. One group of Veh- and one group of PIO-treated rats were scanned after the 2nd dosing; all rats were scanned after the last (7th) daily dose (n=5/group).
Each brain was demarcated into 57 regions of interest (ROIs). The time course of each ROI was extracted and cross-correlation coefficients between all pairs of ROIs were calculated to evaluate functional connectivity. Using a repeated measures ANOVA, it was demonstrated that 17 ROI pairs showed significant ($P<0.005$, uncorrected) changes in functional connectivity on Day 7 of treatment with PIO (all dose groups combined) compared with Veh. Greatest FC changes were seen between the midline nuclei of the dorsal thalamus and somatosensory cortex, and the lateral nuclei of the dorsal thalamus and globus pallidus. Significant changes also were seen in 2 pairs of ROIs on day 2 of dosing. A voxel-by-voxel analysis indicated increased connectivity between the CA1 region of the hippocampus as the seed and the hypothalamus and ventral thalamus after 7 days of dosing; changes in connectivity were least apparent at the highest dose.
Our results indicate that PIO at doses lower than those used to treat T2D leads to changes in FC in conscious rats within 2 days of treatment. These results provide preliminary evidence that low doses of PIO cross the blood brain barrier and change brain physiology, and support the hypothesis that this drug may be a beneficial pharmacotherapy for neurodegenerative diseases such as AD.

Disclosures: K.E. Asin: Employment; Takeda Global Research & Development Center, Inc. **D. Crenshaw:** Other; Contractor to Zinfandel Pharmaceuticals, Inc. **K. Gottschalk:** Other; Contractor for Zinfandel Pharmaceuticals, Inc.. **N. Zhang:** None. **Z. Liang:** None. **A. Roses:** Consultant/Advisory Board; Zinfandel Pharmaceuticals, Inc..

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

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Program#/Poster#:851.07/D49

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ARUK funding

DEL Funding

Title: DAla2-GIP reduces amyloid plaque load, oxidative stress and inflammation in APP/PS1 transgenic mice.

Authors: *A. DUFFY[1,2], C. HÖLSCHER[2]
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Abstract: Alzheimer's disease (AD) is a neurological disorder characterized by formation of senile plaques and neurofibrillary tangles with an associated decline in cognitive function. Substantial evidence also shows the significant role of inflammation and oxidative stress in the pathogenesis of AD. The exact cause of the disease remains unknown, but recently T2DM has been identified as a risk factor. Glucose-dependent insulinotropic polypeptide (GIP), an incretin hormone released from intestinal K cells, has been shown to regulate insulin secretion in diabetic patients and is produced in neurons. It has recently been shown that the long lasting incretin hormone analogue DAla2GIP has growth factor properties and is successful in improving synaptic plasticity, cell proliferation and neurogenesis. This study investigates the effect of DAla2GIP on amyloid plaques, oxidative stress and inflammation in the APP/PS1 mouse model of AD.

12 & 19month old male APP/PS1 mice were injected with DAla2GIP (25 nmol/Kg)/saline once daily for 35 days. Immunohistochemistry was performed for GFAP, a marker for astrocytes, and 8-oxoguanine, a marker for oxidative stress, in the cortex and CA1 & CA3 regions of the hippocampus. Beta amyloid plaque load was measured using a specific antibody.

There were significant reductions of beta amyloid plaques and oxidative stress in the cortex of 12month old mice when treated with DAla2GIP compared to saline, $P<0.02$ & $P<0.01$ respectively. In 19month old mice there were again significant reductions of beta amyloid plaques and oxidative stress as well as in GFAP positive astrocytes, $P<0.001$ beta amyloid & 8-oxoguanine, $P<0.01$ GFAP. Similar results were found in CA1 & CA3 of the hippocampus. In CA1 at 12 & 19months, there were significant reductions in the amount of oxidative stress in drug treated mice compared to saline, $P<0.01$. Again in the CA3 region there were reductions in both age groups after DAla2GIP injections, $P<0.01$ 12months, $P<0.05$ 19months. There were significantly less GFAP positive astrocytes in CA1 at 12 & 19months, $P<0.01$ & $P<0.05$ after DAla2GIP treatment compared to saline. In the CA3 region, GFAP positive astrocyte levels were also significantly lower after drug treatment, $P<0.01$ & $P<0.05$. Data for 6 month old mice will be available on poster at conference, along with data analyzing the effect of a double knockout of GIPR and GLP-1R on learning and memory.

This study shows that the long lasting GIP receptor agonist DAla2GIP significantly reduces the hallmarks of AD and has potential to be developed as a therapeutic treatment. Interestingly, the drug has effect in both the moderate and later more severe stages of the disease.

Disclosures: A. Duffy: None. **C. Hölscher:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.08/D50

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF Grant 2011-0030928

NRF Grant 2012-5003338

NRF Grant 2011-0003839

NRF Grant 2011-0030775

Title: HDAC inhibition improves abnormal neuronal functions in Alzheimer’s disease model Tg6799 mice

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Abstract: The AD model Tg6799 transgenic mice overexpress mutant human APP (695) with the Swedish (K670N, M671L), Florida (I716L) and London (V717I) mutations and mutant human PS1 (M146L and L286V). To determine the effects of histone deacetylase (HDAC) inhibition in AD, valproic acid (VPA) were administered in Tg6799 mice. Three different age groups of 1, 5, and 10 months were tested for pre-symptomatic, early symptomatic, and late symptomatic stages. The blood and brain samples were isolated from Tg6799 mice for RT-PCR and Western blot analysis. The mRNA levels of IL-1β and NF-kB increased in plasma of Tg6799 mice at pre-symptomatic stage. In the hippocampus of Tg6799 mice, the mRNA levels of IL-1β and APOE increased at 10 months of age, compared to littermate control. VPA decreased the mRNA levels of IL-1ß and APOE in the hippocampus of Tg6799 mice at 10 months of age. In addition, VPA altered NGF expression levels in hippocampus of Tg6799 mice at 5 and 10 months of age. Moreover, VPA decreased the escape latency of Tg6799 mice in Morris Water Maze assessment at 5 months and 10 months of age. These data suggest that inhibition of histone deacetylation can improve neuronal function through trophic regulation in AD model.

Disclosures: H. Noh: None. **H. Seo:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.09/D51

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Disease Association Grant #IIRG-08-89927

Glenn Foundation for Medical Research

Title: Evaluating two novel calorie restriction mimetics for treating alzheimer's disease

Authors: P. J. PISTELL[1], K. MANAYE[2], T. UTSUKI[3], J. DOWDEN[3], B. RAY[5], D. LAHIRI[5], *D. K. INGRAM[4]
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Abstract:
We aim to assess the efficacy of candidate calorie restriction (CR) mimetics as possible treatments for Alzheimer's disease (AD) characterized by excessive brain amyloid-beta (Aβ) peptide derived from the Aβ precursor protein (APP). In various animal models, CR (30-40% reduction in caloric intake) has been shown to retard pathogenesis of AD, and epidemiological studies have indicated that dietary calories consumed are inversely related to risk of the disease. CR mimetics are being designed to provide the anti-aging effects of CR without requiring actual reduction in calories. A few candidate CR mimetics have been successfully evaluated in transgenic mouse models of AD by other investigators, including resveratrol as an activator of SIRT1, rapamycin, as an inhibitor of mTOR, and 2-deoxyglucose (2DG) as a glycolytic inhibitor. Using a double transgenic mouse model of AD ($APP_{swe}/PS1_{\Delta\varepsilon 9}$), we are evaluating an inhibitor of glucose metabolism, the 7-carbon sugar mannoheptulose (MH), and a citric acid cycle intermediate, oxaloacetate (OX), targeted to increase intracellular levels of NAD+. These compounds were separately incorporated into the diet at 3 mo of age. We also employed a CR group by feeding mice every-other-day (EOD). At
8-9 months mice were evaluated for cognitive function and pathology. Notably, similar to EOD fed mice, those on MH diet (1700 mg/kg delivered as an extract of avocado) had reduced body weights relative to mice on control diet as well as reduced body fat and blood cholesterol. However, unlike mice on EOD feeding, mice fed MH diet ad libitum had food intake similar to controls. Mice on OX diet (350 mg/kg) also did not differ from controls in food intake nor in cholesterol levels, body weight or
composition. Mice on EOD, MH, and OX diets exhibited improved learning ability in a complex

maze task (Stone maze) compared to mice on control diets. Preliminary stereological analysis of Aβ pathology reveal a significant positive correlation between hippocampal Aβ plaque load and maze errors, with mice on experimental diets showing reduced pathology and less errors. Separately, secreted fraction of the hippocampal extract was prepared at 100,000xg for 1 hr, and subjected to different biochemical analyses. Western immunoblotting reveals that the non-amyloidogenic
sAPPα level was significantly increased in the EOD and OX groups relative to controls, consistent with behavioral results. Thus, these candidate CR mimetics appear to ameliorate both functional and pathological manifestations of AD modeled in these mice similar to CR, and these beneficial effects on the disease do not require reduced food intake or necessarily changes in body composition.

Disclosures: P.J. Pistell: None. **K. Manaye:** None. **T. Utsuki:** None. **J. Dowden:** None. **B. Ray:** None. **D. Lahiri:** None. **D.K. Ingram:** Ownership Interest; GeroScience, Inc.. Consultant/Advisory Board; P&G, Inc..

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

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Program#/Poster#:851.10/D52

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R21 Grant AG031387-02 (MAO)

Title: Effects of estradiol-quinol treatment on a murine model of Alzheimer’s disease.

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Abstract: The neuroprotection offered by estrogens provide a potential candidate for therapeutic interventions applicable for Alzheimer’s disease (AD). However, clinical applications must provide the benefits of estradiol without deleterious peripheral effects of estradiol. Estrogen-derived para-quinol compounds are prodrugs of estrogens that deliver estradiol centrally; no stimulatory effects of e-quinol are observed. The transgenic murine AD model used in this study over-expresses human mutant forms of amyloid precursor protein (APP) and presenilin 1 (PS1). Female APP/PS1 transgenic mice and non-transgenic litter mates (6 months) were treated with

vehicle (propylene glycol), estradiol (E2; 2ug/day), or E2-quinol (E2Q, 2ug/day). Treatments were administered over 8 weeks using Alzet osmotic pumps (0.25ul/min); behavioral tests were conducted and tissue collected. Uterine wet weight was examined as an indirect measure of circulating E2. Amyloid beta 1-40/1-42 levels were measured in whole brain homogenates using an ELISA and full length APP (6E10) was assessed by western blot. Superoxide dismutase, and catalase enzyme activity and protein carbonylation were measured to assess oxidative damage. Results showed behavioral measurements were not significantly different between groups. APP/PS1 mice treated with E2-quinol have reduced uterine wet weight compared to APP/PS1 mice treated with E2, indicating low circulating E2. Additionally the ratio of 40:42 amyloid fragments is reduced in E2Q treated mice indicating a positive effect of treatment on overall amyloid load. Finally, oxidative stress markers are altered in E2Q treated mice as compared to control animals, suggesting a beneficial role for the quinol. Our findings support the possibility that E-quinol compounds have potential as an intervention that would benefit AD patients.

Disclosures: A.E. Schlappal: None. **R. Schuh:** None. **L. Prokai:** None. **M. Ottinger:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.11/D53

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Intraperitoneal and intranasal formulations of erythropoietin (EPO) showed potent protective activity against amyloid toxicity in the oligomeric Aβ25-35 nontransgenic mouse model of Alzheimer's disease

Authors: *T. MAURICE[1], M.-H. MUSTAPHA[1], M. DE LA C GARCÍA-BARCELÓ[2], Y. RODRIGUEZ CRUZ[2], J. GARCIA RODRIGUEZ[3]
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Abstract: Human recombinant erythropoietin (rHu-EPO) has been employed in the treatment of renal insufficiency associated anemia and in cancer patients suffering chemo- and radiotherapy-induced anemia. Besides its role in erythropoiesis, rHu-EPO is a potent cell protectant and it showed a strong potential in protecting brain cells from ischemic injury or from toxicity observed in neurodegenerative pathologies, including Alzheimer's disease (AD). Different types of EPO without erythropoiesis-stimulating activity have been developed, including rHu-EPO with low sialic acid content (Neuro-EPO). This latter form allows intranasal (IN) delivery, which

avoid systemic administration related potential side effects. In this study, we compared the neuroprotective ability of intraperitoneal (IP) administration of rHu-EPO and IN injection of Neuro-EPO against amyloid toxicity in mice, that received an intracerebroventricular injection of oligomeric amyloid(25-35) peptide (oAß), a validated model of AD-like pathology. oAß was administered at day 0, rHu-EPO, 125-500 µg/Kg IP, was administered once a day between day 1 to 4 and Neuro-EPO, 62-250 µg/Kg IN, was injected three times a day between day 1 to 4. Memory deficits were analyzed between day 7 to 12, using the spontaneous alternation, passive avoidances and water maze procedures. In hippocampus or cortex extracts prepared at day 7, the levels of peroxidation of membrane lipids and the level of cholinesterase activity were analyzed as markers of the oxidative stress and cholinergic activity loss. A histochemical approach is used to adress brain morphological damages and inflammation. Data showed that rHu-EPO significantly protected from oAß toxicity, on all reponses and markers examined, at the doses of 250 µg/Kg IP and higher. Neuro-EPO showed a similar efficacy, at doses of 125 µg/Kg IN and higher, outlining the potential of the IN route.

Disclosures: **T. Maurice:** Consultant/Advisory Board; AMYLGEN, ANAVEX Life Sciences. **M. Mustapha:** None. **M. de la C García-Barceló:** None. **Y. Rodriguez Cruz:** None. **J. Garcia Rodriguez:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Program#/Poster#:851.12/D54

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cure Alzheimer’s Fund

AstraZeneca

Title: Peripherally administered antibody against apolipoprotein E: Effects on amyloid-β pathology in a mouse model of Alzheimer disease

Authors: *F. LIAO, T. E. MAHAN, H. JIANG, J. KIM, D. HOLTZMAN
Neurol., Washington Univ. In St. Louis, Saint Louis, MO

Abstract: In the search for a treatment for Alzheimer's disease (AD), one focus has been on decreasing accumulation of amyloid-β (Aβ) in the brain by reducing Aβ production, enhancing its clearance or inhibiting its aggregation. Apolipoprotein E (apoE) plays an important role in modulation of AD pathogenesis by interacting with Aβ to affect its clearance and aggregation.

Amyloid precursor protein (APP) transgenic mice on a mouse apoE null background have lower plaque load and much less to no fibrillar Aβ compared with APP mice expressing endogenous murine apoE. Haploinsufficiency of human and murine apoE reduces amyloid deposition in APP transgenic mice. Overexpression of low-density lipoprotein receptor (LDLR) which binds to and clears apoE results in a decrease of both apoE level and Aβ accumulation. The goal of this study is to test whether monoclonal antibodies directed against apoE can decrease Aβ accumulation as well as decrease existing Aβ aggregates and associated pathology. When the anti-apoE mouse monoclonal antibody HJ6.3 was administered on a weekly basis intraperitoneally beginning prior to Aβ accumulation it significantly decreased Aβ accumulation as assessed both histologically and well as biochemically as compared to phosphate-buffered saline (PBS) treated PS1δE9/APPswe transgenic mice. We also administered HJ6.3 at a dose of 10 mg/kg/weekly or PBS intraperitoneally to PS1δE9/APPswe transgenic mice beginning at 7 months of age (after Aβ accumulation has already started) for 21 weeks. Two days after the final injections, the brains were processed for histological and biochemical studies. Our preliminary results suggest that compared to PBS injected mice, that anti-apoE antibody HJ6.3 significantly decreases Aβ in both the hippocampus and cortex. These results suggest that apoE, a protein involved in Aβ metabolism, may be a novel target for an immunotherapy approach for Alzheimer's disease.

Disclosures: **F. Liao:** Research Grant; Cure Alzheimer's Fund. Other Research Support; AstraZeneca. **T.E. Mahan:** Research Grant; Cure Alzheimer's Fund. Other Research Support; AstraZeneca. **H. Jiang:** Research Grant; Cure Alzheimer's Fund. Other Research Support; AstraZeneca. **J. Kim:** Research Grant; Cure Alzheimer's Fund. Other Research Support; AstraZeneca. **D. Holtzman:** Research Grant; Cure Alzheimer's Fund. Other Research Support; AstraZeneca.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Program#/Poster#:851.13/D55

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ADDF Grant 291204

NIH Grant ES020715-01

Title: Restoration of mitochondrial dynamics attenuates neurological and memory phenotype in AD mice *in vivo*.

Authors: *E. TRUSHINA[1], I. MAEZAWA[2], L.-W. JIN[3], D. H. HUA[4]
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Abstract: Aggregation of intracellular amyloid beta (Aβ) has been shown to directly affect mitochondrial dynamics, morphology and function in multiple cellular models of Alzheimer's Disease (AD). However, it is not clear to what extent alterations in mitochondrial dynamics contribute to the progression AD. We have demonstrated that cell-permeable tricyclic pyrone compound CP2 efficiently prevents Aβ aggregation in cellular and animal models of AD reducing cell death associated with intracellular Aβ oligomers. CP2 penetrates blood brain barrier and do not cause adverse side effects when administered to animals. We have also demonstrated using three transgenic animal models of familial AD (FAD) that alterations in mitochondrial dynamics and function occur early in AD progression and preclude the onset of memory phenotype and amyloid plaque formation. To test whether blocking Aβ aggregation and oligomerization could attenuate the onset and progression of AD in vivo, we administered CP2 via drinking water to a cohort of FAD mice carrying a single APP or PS1 mutation, and double mutant APP/PS1. Mice were treated for 4 and 13 months. CP2-treated animals were allowed to breed producing progeny that were on CP2 from birth. CP2-treated animals gain weight, breed, groom, and behave similar to the untreated littermates. All CP2-treated mice were examined for the onset of neurological and memory phenotype, amyloid load, and mitochondrial dynamics and morphology. Mice that were on either short- or long-term CP2 treatment were indistinguishable from non-transgenic littermates in their performance in memory (novel object recognition test) and neurological assessments (rotarod and hanging bar test), while untreated FAD mice demonstrated substantial decline in all tests. As expected, CP2 treatment significantly reduced amyloid load in the brain of FAD animals. Moreover, CP2 treatment completely restored mitochondrial dynamics that was altered in embryonic neurons from FAD mice. Electron microscopy examination revealed significant reduction in mitochondria with altered morphology in the brain of CP2-treated FAD mice comparing to untreated animals. Taken together, these results demonstrate that restoration of mitochondrial dynamics early in disease progression rescues AD phenotype in vivo in multiple animal models of FAD validating mitochondrial motility as an early therapeutic target in AD.

Disclosures: E. Trushina: None. **I. Maezawa:** None. **L. Jin:** None. **D.H. Hua:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Program#/Poster#:851.14/D56

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG031846

Alzheimer's Association IIRG-09-131791

American Health Assistance Foundation A2010328

Academic Health Center of the University of Minnesota

Title: Two protein prenylation pathways exhibit differential effects in a mouse model of Alzheimer's disease

Authors: *S. CHENG[1], D. CAO[1], F. WANG[1], D. HOTTMAN[1], E. LEATHLEY[1], W. ZHANG[1], M. PARENT[1], L. HOVDE[1], M. BERGO[2], L. YUAN[1], L. LI[1]
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Abstract: Many intracellular proteins are posttranslationally modified with a 15-carbon farnesyl or 20-carbon geranylgeranyl lipid at a carboxyl-terminal *CAAX* motif. The reactions, collectively called protein prenylation, are carried out by protein farnesyltransferase (FT) and protein geranylgeranyltransferase type I (GGT) and render the protein hydrophobic, promoting membrane anchoring and protein-protein interactions. Prenylated proteins regulate a wide range of cellular processes, such as cell growth, differentiation, cytoskeletal organization, and vesicle trafficking. Isoprenoid lipids and prenylated proteins have been implicated in the pathophysiology of Alzheimer's disease (AD), including amyloid-beta (Abeta) precursor protein (APP) metabolism, tau phosphorylation, synaptic plasticity, and neuroinflammation. However, the precise role of protein prenylation in AD pathogenesis remains to be determined. In this study, we bred FT and GGT heterozygous knockout (+/-) mice with AD mice (B6C3-Tg(APPswe,PSEN1dE9)85Dbo/J; APP/PS1) to assess the impact of decreasing protein prenylation levels on the development of AD-like learning/memory impairment and neuropathology. In the Morris water maze test, we found that decreasing FT activity rescued spatial learning and memory function in APP/PS1 mice. In contrast, decreasing GGT activity had no impact on spatial learning and memory impairment. Our preliminary data also suggest that FT and GGT deficiencies exert differential effects on synaptic plasticity in the hippocampus, with an increased magnitude of long-term potentiation (LTP) in FT+/- mice but a decreased LTP magnitude in GGT+/- mice. These results are consistent with our recent findings that a farnesyltransferase inhibitor, but not a geranylgeranyltransferase inhibitor, enhances hippocampal LTP in mice. Biochemical analyses are underway to determine the effects of FT and GGT haploinsufficiency on Abeta deposition and neuroinflammation. Our findings suggest that farnesylated and geranylgeranylated proteins exhibit differential effects on the pathogenesis of AD and that inhibiting FT activity may be a viable strategy to treat AD.

Disclosures: S. Cheng: None. **D. Cao:** None. **F. Wang:** None. **D. Hottman:** None. **E. Leathley:** None. **W. Zhang:** None. **M. Parent:** None. **L. Hovde:** None. **M. Bergo:** None. **L. Yuan:** None. **L. Li:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.15/D57

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ANRT Cifre PhD Funding

Title: Mitochondrial protection is induced by the novel tetrahydrofuran derivative ANAVEX2-73 after ICV injection of oligomeric Aß25-35 peptide in mice, a nontransgenic Alzheimer's disease model

Authors: *V. LAHMY[1,2], R. LONG[3], D. MORIN[3], V. VILLARD[2], A. VAMVAKIDES[4], T. MAURICE[1]
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Abstract: Tetrahydro-N,N-dimethyl-2,2-diphenyl-3-furanmethanamine hydrochloride (ANAVEX2-73) is a novel compound binding to muscarinic acetylcholine and sigma-1 receptors with affinities in the low micromolar range. We previously reported that the drug showed anti-amnesic and neuroprotective potential in the Alzheimer's disease (AD) model in mice induced by the icv injection of oligomeric amyloid(25-35) peptide (oAß25-35). In particular, the drug attenuated the oxidative stress, caspases induction, cellular loss, Aß1-42 seeding, Tau hyperphosphorylation and learning and memory deficits observed several days after oAß25-35 injection. Excessive mitochondrial fragmentation may also be a triggering event in AD physiopathology as mitochondrial dysfunction is consistently reported in brains of AD patients and transgenic mice. Indeed, oAß induce Ca2+-dependent reactive oxygen species generation in neurons and elicit Ca2+ entry through NMDA receptors, producing abnormal Ca2+ signals that in turn promote mitochondrial fission. In mitochondrial-endoplasmic reticulum contact sites (MAM), localized Ca2+ changes occur in microdomains, in which mitochondria operate as a barrier Ca2+ buffer. Sigma-1 receptors act precisely as molecular chaperones regulating Ca2+ effluxes at MAM. We therefore analyzed ANAVEX2-73 impact on mitochondrial markers in hippocampus extracts of oAß25-35-treated mice. oAß25-35 induced a time-dependent increase in cytochrome c release, at 1, 3, 5 days after injection, with a return to basal at day 7. This was

accompanied with increases in Bax levels, significant at day 3 and 5, no change in Bcl-2 levels, and significant increases in Bax/Bcl-2 ratio at days 5 and 7 after injection. In parallel, an increase in lipid peroxidation levels, at days 5 and 7, and in caspase-9 expression, at day 7, were measured. At day 5 after injection, mitochondrial respiration was examined by oxymetry and found to be significantly altered in oAß mice. Treatment with ANAVEX2-73, in the 0.1-1 mg/kg ip dose range, restored all parameters examined. The effect of the selective reference sigma-1 agonist PRE-084 is currently under investigation. This study delineates the mitochondrial damage induced in the oAß25-35 mouse model of AD and showed that an effective mitochondrial protection is induced by the tetrafuranmethanamine derivative.

Disclosures: V. Lahmy: Employment; AMYLGEN. Other Research Support; ANAVEX Life Sciences. **R. Long:** None. **D. Morin:** None. **V. Villard:** Employment; AMYLGEN. **A. Vamvakides:** Employment; ANAVEX Life Science. **T. Maurice:** Consultant/Advisory Board; AMYLGEN, ANAVEX Life science.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.16/D58

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 AG031859-04

Title: Rosiglitazone-dependent rescue of intrinsic excitability deficits in dentate granule cells of Tg2576 mice

Authors: *M. N. NENOV[1,2], C. HERNANDEZ[1,2,3], K. DINELEY[1,2,3], F. LAEZZA[4,2,3]
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Abstract: Emerging evidence indicates a strong link between cognitive deficits that characterize early stages of Alzheimer's disease (AD) and insulin resistance. However, the mechanistic link between these two diseases is as yet unknown. Clinical studies have shown that pharmacologic agents such as Rosiglitazone (RSG) which activate the peroxisome proliferator-activated receptor-gamma (PPARgamma) improve insulin signaling and can effectively ameliorate cognitive function in AD mouse models and in a subset of AD patients. While some current PPAR gamma agonists have demonstrated adverse effects, establishing a mechanistic link between RSG and cognitive improvement could open the way to a new generation of therapeutics against cognitive decline in AD.

Building on previous results from our group demonstrating that one month RSG treatment rescues cognitive function in the Tg2576 AD mouse model, we now present new data showing that this cognitive rescue is accompanied by restored firing of granule neurons in the dentate gyrus, a critical area of the hippocampal circuit implicated in AD etiology. Passive properties and intrinsic excitability were examined in dentate granule neurons of 9 month old Tg2576 and age matched wild type mice either untreated or treated with RSG. We found that RSG treatment normalizes resting membrane potential, firing frequency, and the action potential voltage threshold partially compensating for a loss in excitability observed in Tg2576 mice. Our data suggest that RSG-dependent cognitive rescue might take place through a global functional rescue of the DG circuit by normalizing synaptic activity (see abstract from Laezza et al.) and intrinsic excitability.

Disclosures: M.N. Nenov: None. **C. Hernandez:** None. **K. Dineley:** None. **F. Laezza:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Program#/Poster#:851.17/D59

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant 021928

NIH grant AG027544

Title: Tau immunotherapy alone or in conjunction with beta-amyloid immunotherapy reduces gallyas positive neurofibillary tangles

Authors: *K. C. WALLS, R. AGER, R. MEDEIROS, F. M. LAFERLA
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Abstract: Alzheimer’s disease is pathologically characterized by the accumulation of beta-amyloid (Aß) plaques and tau-laden neurofibrillary tangles (NFTs). To date, human clinical trials in humans indicate that clearance of Aß plaques does not improve cognition, and at best may stabilize cognitive decline. These types of results highlight the need to identify and focus on other aspects of AD pathology, with tau representing a high value target that has been understudied. Since the 3xTg-AD mice develop both plaques and tangles, we investigated the utility of concomitantly targeting both Aß and tau using immunotherapy approaches. The diverse phosphorylation states that tau can undergo complicates the selection of the appropriate antibody, but a large body of work indicates that the serine/threonine targets comprising the AT8

epitope may be the most pathologically relevant. Hence, we used an antibody that recognizes serine 199 and threonine 202 phospho-tau (the AT8 epitope). As expected, Aß immunotherapy effectively reduced Aß plaques and intracellular Aß. Here we report the novel finding that tau immunotherapy reduced somatodendritic tau and silver stained (Gallyas) positive neurons, without affecting Aß pathology. This result occurred whether tau immunotherapy was administered alone or in combination with Aß immunotherapy. We assessed the temporal reoccurrence of tau pathology after immunotherapy by performing a time course. Our results showed combining Aß and tau immunotherapies delayed somatodendritic tau and NFT accumulation versus tau immunotherapy alone. In conclusion, our results show combining Aß- and tau-directed therapies has a synergistic effect on reducing tau pathology. These studies provide compelling evidence that targeting both tau and Aß represents an effective treatment strategy for Alzheimer's disease.

Disclosures: K.C. Walls: None. **R. Ager:** None. **R. Medeiros:** None. **F.M. LaFerla:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Program#/Poster#:851.18/D60

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Byrd Alzheimer Institute Start-up Fund

Title: Efficacy of an antigen sensitized dendritic cell vaccine against Alzheimer's disease is effected by injection type.

Authors: *N. R. NABAR[1,2], X. LIN[2], S.-F. ZHOU[3], C. CAO[2]
[1]Univ. of South Florida, Tampa, FL; [2]USF Hlth. - Byrd Alzheimer's Inst., Tampa, FL; [3]Dept. of Pharmaceut. Sci., USF Col. of Pharm., Tampa, FL

Abstract: As demonstrated by the AN-1792 clinical trial, vaccine development against Alzheimer's disease (AD) has shown positive results with regards to efficacy, but has faced major challenges in avoiding the potential adverse effects. Currently, all anti-amyloid beta (Aβ) vaccines in clinical trials use only the Aβ B-cell epitope in hopes of circumventing production of a Th1 inflammatory response. In the past, our lab has aimed to develop a vaccine against AD which utilizes both the B-cell and T-cell epitopes, and our efforts have led to the development of an antigen sensitized dendritic cell (DC) vaccine. In production of our vaccine, DCs are isolated from the donor, cultured with IL-4, and sensitized to either wild-type or a mutated Aβ peptide ex vivo. The sensitized DCs are then injected into mice via I.P. injection or i.v.. Studies in BALB/c,

PDAPP, and APP/PS1 mice have shown a sustained antibody titer is elicited when a mutated Aβ peptide is used for sensitization, but no antibody titer is elicited when wild type Aβ is used. In this study, we looked to investigate the difference in antibody response when our DC vaccine is injected via I.P., I.V., and subcutaneous injection in male BALB/c mice. As expected, DCs sensitized with wild-type Aβ was not able to elicit an antibody titer when administered through I.P or I.V. injection. Interestingly, when DCs sensitized with wild-type Aβ were injected into the subcutaneous region without adjuvant, an antibody titer was elicited. We hypothesize that antigen presentation mechanistically differs in the subcutaneous region compared to the peritoneum or the vasculature, allowing Aβ to be presented as an antigen by the dendritic cell. Moreover, the inability of immune cells in the subcutaneous region to recognize wild-type Aβ as a self antigen in the subcutaneous region may have broader implications related to its physiological function and regulation.

Disclosures: N.R. Nabar: None. **X. Lin:** None. **S. Zhou:** None. **C. Cao:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.19/E1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association

Thome Foundation

Title: Increased glial glutamate transporter EAAT2 expression ameliorates Alzheimer’s-like pathologies, premature death, and memory deficits in $APP_{Sw/Ind}$ mice

Authors: *Q. KONG, K. TAKAHASHI, Y. LIN, D. SCHULTE, C.-L. G. LIN
The Ohio State University, Dept. of Neurosci., Columbus, OH

Abstract: Many reports have demonstrated that the glutamate uptake system is impaired in Alzheimer’s disease (AD) brains and that decreased glutamate uptake activity is associated with decreased glial glutamate transporter EAAT2 protein expression. Excessive glutamate can cause over-stimulation of glutamate receptors and subsequently lead to excitotoxic neuronal damage. Loss of EAAT2 protein and associated function was also observed in transgenic models of AD. However, it is still unknown whether EAAT2 dysfunction contributes to the pathogenesis of AD. In the present study, we crossed EAAT2 transgenic mice, which have a 1.5-2 fold increase in EAAT2 protein levels as compared to their non-transgenic counterparts, with the $APP_{Sw/Ind}$ mice

to investigate whether supplementation of the loss of EAAT2 would have beneficial effects on disease progression. As we expected, EAAT2 protein level and the associated glutamate uptake functions were restored in APP/EAAT2 double transgenic mice compared to their APP transgenic littermates. Several striking phenomena were observed in APP/EAAT2 mice compared with APP mice. First, premature death was significantly reduced. Second, learning and memory capacity was significantly improved. Third, several Alzheimer's-like pathological changes were significantly reduced. These results indicate that loss of EAAT2 contributes to the pathogenesis of AD and suggest that enhanced glutamate uptake by increasing EAAT2 expression may be a potential therapeutic strategy for AD.

Disclosures: Q. Kong: None. **K. Takahashi:** None. **Y. Lin:** None. **D. Schulte:** None. **C.G. Lin:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.20/E2

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: ACU-193: A drug candidate antibody selectively recognizing soluble abeta oligomers.

Authors: *G. A. KRAFFT[1], F. HEFTI[2], W. GOURE[2], J. JERECIC[2], K. IVERSON[2]
[1]Acumen Pharmaceuticals, GLENVIEW, IL; [2]Acumen Pharmaceuticals, Livermore, CA

Abstract: Soluble Abeta oligomers inhibit synaptic function, trigger early memory deficits, initiate nerve cell degeneration and are now recognized as key culprits in Alzheimer's disease. Also known as ADDLs (Abeta-Derived Diffusible Ligands), these neurotoxic assemblies represent a highly attractive point of intervention for effective treatment of Alzheimer's patients. In this presentation we report on a promising new drug candidate, ACU-193, which targets ADDLs directly and with high selectivity, preventing their attack on susceptible neurons. ACU-193 is a humanized, affinity matured IgG2 monoclonal antibody, engineered to minimize cellular effector functions and immunogenicity. ACU-193 binds ADDLs with affinities ranging from <0.8 nM to 9 nM, as measured by surface plasmon resonance (Biacore) and various ELISA methods, respectively. In competitive assays with ADDLs and Abeta monomer, ACU-193 exhibits 550-fold preferential binding for ADDLs, and it does not exhibit any cross-reactive binding to other proteins.
In vitro efficacy of ACU-193 was demonstrated by potent inhibition (IC50 = 1-5nM) of ADDL binding to cultured hippocampal neurons, whereas other Abeta-directed antibodies either fail to

inhibit this binding or exhibit substantially lower potency. In vivo target engagement and efficacy of ACU-193 was demonstrated in the Tg2576 transgenic AD mouse model. Following intravenous administration of ACU-193 (10-50mg/kg), dose-dependent complex formation with ADDLs in brain tissue was demonstrated, and initial evidence of beneficial behavioral effects emerged from experiments that measured nocturnal hyperactivity of the Tg2576 mice.
In several animal species, ACU-193 showed linear, dose-dependent pharmacokinetics, biodistribution, and brain penetration in the range of values that are typical for therapeutically used antibodies. Exploratory safety and toxicity studies revealed an overall excellent safety profile of ACU-193. Stable, high-yielding cell lines have been established for efficient production of ACU-193.
Two biomarker assays have been generated to support future clinical studies of ACU-193. First, an ELISA measuring ACU-193/ADDL complexes in CSF may provide a pharmacodynamic readout, and, second, an ultrasensitive ELISA measuring ADDL levels in CSF from human subjects should enable patient selection and stratification and serve as a companion diagnostic. Because ADDLs trigger acute and persistent synaptic malfunction, ACU-193 treatment may restore normal synaptic function and improve cognitive function in Alzheimer patients.

Disclosures: G.A. Krafft: Employment; Chairman and Chief Scientific Advisor, Acumen Pharmaceuticals. Ownership Interest; Acumen Pharmaceuticals. **F. Hefti:** Employment; CEO, Acumen Pharmaceuticals. **W. Goure:** Employment; COO, Acumen Pharmaceuticals. **J. Jerecic:** Consultant/Advisory Board; Acumen Pharmaceuticals. **K. Iverson:** Consultant/Advisory Board; Acumen Pharmaceuticals.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.21/E3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VCRS

Title: Chronic treatment with the GLP1 analogue Liraglutide increases cell proliferation and subsequent differentiation to neuronal phenotype in an AD mouse model.

Authors: *V. PARTHSARATHY, C. HOLSCHER
Univ. of Ulster, Coleraine, United Kingdom

Abstract: Background: Neurogenesis is a life long process, but the rate of cell proliferation and differentiation decreases with age. In Alzheimer’s patients, along with age, the presence of Aβ in

the brain inhibits this process by affecting the pathways of stem cell proliferation, differentiation and decreasing the survival of the cells by activating pro apoptotic pathways. Neuronal progenitors are found to have incretin hormone receptors and GLP1 mimetic, Liraglutide (Victoza^{TM}, NovaNordisk) have shown to increase cell proliferation in mouse model of AD. Here we investigated acute and chronic effects of Liraglutide on progenitor cell proliferation and differentiation of these newly generated cells to neurons in the dentate of WT and APP/PS-1 mice model at different ages. For this, APP/PS1 and their littermate female mice, aged 3, 6, 12, 15 months were injected i.p. with 50mg/kg/body wt of 5-Bromo-2'deoxyuridine (BrdU) for 7 days and were subsequently injected with 0.9% NaCl (w/v) or 25nmol/Kg Liraglutide for 7 days in case of acute treatment, or 30 days for Chronic treatment. The animals were perfused and brain harvested after 4 weeks of last BrdU injections. Immunohistochemical and double immunofluorescent studies were carried out for detection of cell proliferation (BrdU) and cell differentiation (NeuN, GFAP, Iba1) in coronal sections and the cells were counted in the dentate gyrus. Our study shows that there was no difference in the number of newly generated cells in wild type littermate control in both acute and chronic treated animals at age 3, 6 months and acute treated animals of 12, 15 months of age. Chronic treatment of 12 and 15 months WT mice showed significant increase in new cells compared to saline treatment (student t test, $p<0.05$). In AD mouse brains no difference was seen in acutely treated 3 months old mice. Increase in number of new cells were seen in chronically treated 3 month group ($p<0.01$) and in both acutely and chronically treated 6, 12, 15 months groups. In the dentate, most newly generated cells differentiated into neurons with very few converting to astrocyte and microglia. No difference in number of newly differentiated neurons was seen in WT groups at all ages ($p>0.05$). Significant increase in neuronal phenotype was observed in the dentate gyrus of acute and chronic treated 6, 12, 15 month APP/PS1 groups ($p<0.05$ and $p<0.01$). These results demonstrate that GLP1 analogue Liraglutide increases neurogenesis and has a potential to be developed as therapeutic drug for neurodegenerative disorders like Alzheimer's disease.
Keywords: Alzheimer's disease, neurogenesis, Incretin

Disclosures: V. Parthsarathy: None. **C. Holscher:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

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Program#/Poster#:851.22/E4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Society, Grant 97, with support from the Henry Smith Charity.

Title: Chronic liraglutide treatment prevents amyloid plaque deposition and memory impairment in APP/PS1 mice

Authors: *P. MCCLEAN, V. PARTHSARATHY, C. HOLSCHER
Neurosci. Res. Group, Univ. of Ulster, Coleraine, United Kingdom

Abstract: Recent research has demonstrated that type 2 diabetes is a risk factor in the development of Alzheimer's disease. The incretin hormone Glucagon-like peptide-1 (GLP-1) facilitates insulin signalling, and long-lasting analogues such as liraglutide (Victoza®) are on the market as treatment for diabetes. We reported previously that liraglutide treatment effectively improved cognitive function, reduced amyloid plaque deposition, inflammation, overall APP and amyloid oligomer levels and enhanced LTP when injected for two months in 7 months old APP*swe*/PS1 ΔE9 (APP/PS1) mice. Liraglutide also reversed disease progression when injected at 14 months of age and significantly improved recognition and spatial memory, reduced beta amyloid plaque load, total APP and aggregated beta amyloid levels and inflammation after 2 months treatment. As APP levels were reduced, the current study was designed in order to elucidate if liraglutide could exert a protective effect when administered *before* plaque deposition and associated behavioural impairments are present in the APP/PS1 model. Accordingly, 8 week old APP/PS1 mice were injected with liraglutide (25nm/kg bw) or saline (0.9%w/v) for 8 months. Wild-type mice injected with saline (0.9%w/v) served as a control. After 8 months treatment (aged 10 months), both wild-type saline ($p=0.0040$) and liraglutide treated APP/PS1 groups ($p=0.0016$) had excellent object recognition performance, while APP/PS1 saline treated mice failed to discriminate novel and familiar objects (0.0679, ns). APP/PS1 liraglutide-treated mice also performed significantly better in the water maze probe trial ($p<0.001$) and reversal probe trial ($p<0.01$) than APP/PS1 saline-treated mice. LTP in APP/PS1 liraglutide-treated mice was also enhanced compared with both wild-type saline ($p=0.0238$) and APP/PS1 saline ($p=0.0186$) groups. Overall plaque load and inflammation will be assessed, alongside total brain APP and beta-amyloid oligomer level. Further results will be disclosed at the meeting. The overall results obtained with liraglutide show an impressive array of neuroprotective effects which make it a promising candidate for the treatment of AD.

Disclosures: P. McClean: None. **V. Parthsarathy:** None. **C. Holscher:** Ownership Interest; This author is named on patents associated with the use of GLP-1 receptor agonists in neurodegenerative diaseses..

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.23/E5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01 AG031859-04

Title: Rosiglitazone-dependent rescue of synaptic deficits in dentate granule cells of Tg2576 mice

Authors: *F. LAEZZA[1,2,3], M. NENOV[4,2], C. HERNANDEZ[4,2,3], K. DINELEY[4,2,3]
[1]Dept. of Pharmacol. & Toxicology, [2]Mitchell Ctr. for Neurodegenerative Dis., [3]Ctr. for Addiction Res., [4]Dept. of Neurol., The Univ. of Texas Med. Br., Galveston, TX

Abstract: Alzheimer’s disease (AD) is the most common type of the debilitating dementias, and hippocampal dysfunction underlies the cognitive deficits which are a hallmark of AD. New memories are formed and transiently stored in the hippocampus through plasticity of synaptic circuits, and synaptic deficits in AD are observed in conjunction with cognitive impairment. New evidence indicates a link between insulin signaling, cognitive enhancement and AD. In support of that, the insulin-sensitizing drug Rosiglitazone (RSG), a potent and selective agonist of the peroxisome proliferator-activated receptor gamma (PPARgamma), ameliorates cognitive deficits in AD mouse models and has been evaluated in clinical trials on AD patients with some success. Yet, the cellular mechanisms by which RSG improves cognitive function are unknown.
To begin deciphering the cellular mechanisms underlying PPARgamma-agonism in AD, we have examined the synaptic activity of granule neurons in the dentate gyrus, a critical area for learning and memory following RSG treatment in 9 months old Tg2576, a treatment known to effectively enhance cognition in this AD model. We found that the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) recorded in dentate granule neurons from untreated 9 months old Tg2576 mice was dramatically increased compared to WT control, and restored to normal levels by RSG. In contrast, sEPSC amplitude was unchanged in both WT control and RSG-treated Tg2576 mice. Our data suggest that one of the modalities by which RSG normalized cognition in Tg2576 (and possibly AD brain) is achieved through normalization of synaptic transmission in the dentate gyrus.

Disclosures: F. Laezza: None. **M. Nenov:** None. **C. Hernandez:** None. **K. Dineley:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.24/E6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Research UK, Network Collaboration Grant

Title: Acute or long term Liraglutide treatment is protective against the effects of systemically induced inflammation stimulated by LPS administration both in peripheral tissues and in the brain

Authors: *K. HUNTER, S.-L. MCELREAVEY, R. GUNNING, C. HOLSCHER
Life and Hlth. Sci., Univ. of Ulster, Coleraine, United Kingdom

Abstract: It has become apparent in the last number of years that neuroinflammation plays a major role in the pathophysiology of Alzheimer's Disease (AD). Both post mortem brain tissue from AD patients and studies using transgenic animal models have provided evidence that activated microglia and astrocytes, as well as activated complement and release of cytokines and chemokines, are all associated with the progression of AD. Furthermore, systemic inflammation such as infection or other inflammatory episodes, cause further deterioration in the cognition and memory of AD patients. Our previous studies have shown that the anti-diabetic drug Liraglutide possesses anti-inflammatory properties, such as reduction of activated microglia, with an associated reduction in beta-amyloid and an improvement in cognition and memory in APP/PS1 mice. Therefore, in the present study we aim to elucidate more fully the role of Liraglutide on cytokine levels in the brain, serum and spleen and on markers of inflammation and oxidative stress in the brain, such as IBA1, GFAP, iNOS and 8Oxoguanine, when administered with the endotoxin LPS. When administered acutely with 1 or 2 doses of LPS Liraglutide demonstrated clear anti-inflammatory effects in the periphery, as the levels of a number of proinflammatory cytokines in the spleen were significantly reduced (*P<0.05 to **P<0.01). Immunohistological analysis also revealed anti-inflammatory effects of Liraglutide in the brain. IBA1 and GFAP expression was reduced (****P<00001 and P<0.01) as well as expression of iNOS and 8Oxoguanine (****P<0.0001 and **P<0.01 to ****P<0.0001). In a longer term study APP/PS1 mice that received 3 weeks Liraglutide treatment were protected from any further damaging inflammatory effects from an LPS challenge. These animals performed as well as controls in the object recognition task and Morris water maze. Further results of this study such as LTP analysis, immunohistological analysis and MSD multispot analysis of cytokine levels in peripheral tissues and the brain are currently underway.

Disclosures: K. Hunter: None. **S. Mcelreavey:** None. **R. Gunning:** None. **C. Holscher:** Ownership Interest; Author is a named author on the patent for use of GLP-1 in neurodegenerative diseases.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.25/E7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Venture Philanthropy

Title: Symptomatic and disease-modifying effects on an AD mouse model from a dual-receptor-binding drug

Authors: P. R. SPILMAN[1], O. DESCAMPS[1], K. POKSAY[1], O. GOROSTIZA[1], A. MATALIS[1], A. PATENT[1], R. RAO[1], V. JOHN[1], *D. E. BREDESEN[2]
[1]Buck Inst. for Res. on Aging, Novato, CA; [2]Buck Inst. for Age Res., Novato, CA

Abstract: Background: In Alzheimer's disease (AD) there is a shift in the trophic to anti-trophic balance of amyloid precursor protein (APP) processing toward the anti-trophic state, reducing sAPPα relative to Aβ species. Restoration of this balance may be essential to reverse the underlying mechanisms that lead to AD.
Methods: High-throughput screening (HTS) of a clinical compound library in 7W CHO cells expressing huAPPwt was used to identify "hits" increasing sAPPα.The hit F03 was re-tested in primary neuronal culture, and was then determined to be brain-permeable. A 28 day study (0.5 mg/kg/day) was performed using J20 PDAPP (huAPP $^{Swe/Ind}$) mice and non-transgenic (NTg) littermates (N=6) ; working memory and hyperactivity were assessed using the Novel Object Recognition (NOR) task. A 56 day study (0.5 mg/Kg/day) was performed using J20 and NTg mice (N=12) wherein spatial memory was tested by Morris Water Maze (MWM). A study with I5 huAPPwt mice (10mg/Kg/day) was also performed. Biochemical analysis included sAPPα (AlphaLISA, Perkin-Elmer) and Aβ1-40 and 1-42 (ELISA, Invitrogen) of either separate or combined hippocampal (Hip) and entorhinal cortical (ECx) tissue samples.
Results: F03 increased sAPPα by 30% in HTS and by 25% in primary culture. In the 28 day J20 study, hyperactivity was reduced to NTg levels by the end of the study. Novelty preference was increased in F03-treated J20s to NTg levels at 14 days and was even higher than non-transgenic controls at 28 days. sAPPα was significantly increased and Aβ1-40 significantly decreased in the ECx, and Aβ1-42 significantly decreased in both the Hip and ECx. In the 56 day study, F03-treated mice spent more time in the target quadrant and crossed the platform area more often in the hidden and reversal probes, significantly so in the latter. sAPPα significantly increased and Aβ1-42 significantly decreased in the combined Hip+ECx tissue samples.
Conclusions: Our iterative hierarchical screening method to identify "switching" compounds that restore the trophic balance in AD - increasing sAPPα relative to Aβ - identified F03 as a hit. It was found to be effective at the human-equivalent dose of 0.5 mg/Kg/day, has an excellent

safety profile, and is suitable for immediate transition to human clinical trials as a candidate therapeutic for mild cognitive impairment (MCI) and AD.

Disclosures: P.R. Spilman: None. **O. Descamps:** None. **K. Poksay:** None. **O. Gorostiza:** None. **A. Matalis:** None. **A. Patent:** None. **R. Rao:** None. **V. John:** None. **D.E. Bredesen:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.26/E8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIMH T32-MH076690-05

NIMH R01-MH087986

Title: Efficacy of a novel proneurogenic neuroprotective small molecule, P7C3, in the 3xTg-AD transgenic mouse model of Alzheimer’s disease.

Authors: *G. L. BECKER[1], S. R. ALLY[1], A. BURKET[1], P. J. HUNTINGTON[1], W. KNOBBE[1], D. M. MCDANIEL[1], J. NAIDOO[2], J. M. READY[2], S. L. MCKNIGHT[2], A. A. PIEPER[1]
[1]Psychiatry, [2]Biochem., UT Southwestern Med. Sch., Dallas, TX

Abstract: Alzheimer’s disease (AD) is a debilitating age-related neurodegenerative disorder characterized by progressive cognitive impairment, the accumulation of β-amyloid (Aβ) peptide and neurofibrillary tangles in the hippocampus and cortex, along with neuronal loss. There are several medications (e.g. cholinesterase inhibitors) approved by the Food and Drug Administration to treat cognitive impairments associated with AD; however, none of these delay the onset or halt the progression of AD. The hippocampus is one of the earliest and most severely afflicted regions of the brain in AD. Therapeutic strategies to protect neurons from dying in AD, or to augment hippocampal functioning, might provide new treatment options for patients. We have discovered a novel small molecule with potent proneurogenic and neuroprotective properties, termed P7C3, which readily crosses the blood-brain-barrier and can be safely administered to rodents for an extended period of time. Extended treatment with P7C3 effectively restores hippocampal neurogenesis, structure and synaptic functioning in mice deficient for neurogenesis (npas3-/-mice), and also blocks apoptotic cell death and cognitive decline in aged rats. We are now evaluating the efficacy of administration of P7C3 to 3xTg-AD

mice, a rodent model of AD that with age exhibits decreased hippocampal neurogenesis and neuronal cell loss, as well as plaque and tangle pathology associated with synaptic dysfunction. P7C3 was administered twice daily (10 mg/kg) from 3 months of age until 12 months of age and mice received Morris water maze (MWM) testing every 3 months. This extended treatment with P7C3 increased performance in the MWM for 3xTg-AD mice, relative to vehicle-treated 3xTg-AD mice. Furthermore, P7C3-treated 3xTg-AD mice did not exhibit the same weight loss as compared to vehicle-treated mice. We are currently evaluating the effects of more potent analogs of P7C3 on these and other outcome measures as well. Our hope is that this work will provide a basis for the development of new pharmacologic treatment strategies for patients with AD.

Disclosures: G.L. Becker: None. **S.R. Ally:** None. **A. Burket:** None. **P.J. Huntington:** None. **W. Knobbe:** None. **D.M. McDaniel:** None. **J. Naidoo:** None. **J.M. Ready:** None. **S.L. McKnight:** None. **A.A. Pieper:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Program#/Poster#:851.27/E9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: IIRG-10-174448

Title: Ketogenic diet improves motor performance but not cognition in mouse models of Alzheimer’s disease pathology

Authors: *M. L. BROWNLOW[1], L. BENNER[2], A. JOLY[1], D. D'AGOSTINO[1], M. GORDON[1], D. MORGAN[1,2]
[1]Mol. Physiol. and Pharmacol., Univ. of South Florida, Tampa, FL; [2]Univ. of Tampa, Tampa, FL

Abstract: Like many disorders, dietary manipulations are increasingly viewed as possible approaches to treating neurodegenerative diseases. Recent studies suggest that Alzheimer’s disease (AD) patients present an energy imbalance with brain hypometabolism and/or mitochondrial deficits. Ketogenic diets (KD), widely investigated in the treatment and prevention of seizures, have been suggested to bypass metabolic deficits present in AD brain by providing ketone bodies as an alternative fuel. We investigated the effects of a ketogenic diet on two mouse models of AD. Five months old APP/PS1 and Tg4510 mice were kept on either a KD or a control (NIH-31) diet for 3 months and then submitted to a battery of behavioral tests. Body weight and food intake were monitored throughout the study and blood was collected at 4 weeks and at the end of the study for ketone and glucose assessments. Both APP/PS1 and Tg4510 mice

weigh less than nontransgenic mice, yet, surprisingly, have elevated food intake. The ketogenic diet does not affect these differences in body weight or food consumption. We found that both mouse models of AD presented hyperactivity, measured by both open field and the y-maze tests, compared to non transgenic age-matched controls and that this effect was not prevented by KD. Mice kept on KD performed significantly better on an endurance trial in the rotarod compared to mice on the control diet independent of genotype. Only a genotype effect was observed in the radial arm water maze test with no significant differences between diets. Tissue was collected at the end of behavioral testing and additional analysis of amyloid, tau and microglial markers are ongoing. These initial data suggest that the metabolic rate of the transgenic mice is considerably greater than the nontransgenic mice and that ketogenic diet may play an important role in enhancing motor performance in mice.

Disclosures: M.L. Brownlow: None. **L. Benner:** None. **A. Joly:** None. **D. D'Agostino:** None. **M. Gordon:** None. **D. Morgan:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Program#/Poster#:851.28/E10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National S&T Major Special Project on Major New Drug Innovation of China (2009ZX09303-003)

Title: Potassium 2-(1-hydroxypentyl)-benzoate promotes synaptic transmission in animal models of Alzheimer’s disease

Authors: *X. WANG, P. LI, W. WANG, S. XU, Z. LIU, L. WANG, Y. PENG
Inst. Materia Med., Beijing, China

Abstract: Potassium 2-(1-hydroxypentyl)-benzoate (*dl*-PHPB), a novel compound that was demonstrated to have neuronal protective effects and was approved for phase I clinic trial of ischemia stroke. It has been found to improve learning and memory deficits in our preliminary studies. The aim of the present study was to further investigate the ameliorative effects and the possible synaptic mechanisms of *dl*-PHPB on cognitive capability.
The effects of *dl*-PHPB on synaptic plasticity were observed through long-term potentiation (LTP) recording *in vivo* in Wistar rats and in APP/PS1 transgenic mice. *Dl*-PHPB did not affect basal synaptic transmission. But, it significantly enhanced the HFS-induced LTP when preinjected (in CSF) for 30 min and the potentiation persisted for at least 60 min. This

potentiation was prevented by the blockade of postsynaptic NMDA receptors. Application of NMDA receptor antagonist D-APV (150μM in CSF) i.c.v 40min before HFS could significantly suppress LTP. Furthermore, pre-injection of D-APV (150μM in CSF) 10min before *dl*-PHPB application completely prevented *dl*-PHPB induced LTP improvement. In addition, oral treatment of *dl*-PHPB could recess the LTP deficits induced by muscarinic inhibition (scopolamine i.p.) or $A\beta_{1-42}$ injection (cerebral ventricle). *Dl-PHPB* could also reverse the memory impairment performed in step-down passive avoidance task and improve LTP deficits in 14 month old APP/PS1 mice.
The results demonstrated an important role of *dl*-PHPB in learning and memory improvement under pathophysiological conditions *in vivo* and explained a possible synaptic basis of its effects on cognitive amelioration. *Dl*-PHPB might be a potential drug candidate for the treatment of Alzheimer's disease.

Disclosures: X. Wang: None. **P. Li:** None. **W. Wang:** None. **S. Xu:** None. **Z. Liu:** None. **L. Wang:** None. **Y. Peng:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Program#/Poster#:851.29/E11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA

GSK

Department of funds

Title: Targeting of peripheral monocytes by a nasal proteasome-based vaccine to induce Aβ clearance in an animal model of Alzheimer's disease

Authors: R. CIALIC[1], O. BUTOVSKY[1], *D. J. SELKOE[2], H. L. WEINER[2]
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Abstract: Alzheimer's disease (AD) is characterized by extracellular amyloid β-protein (Aβ) deposition (in neuritic plaques) and intraneuronal aggregates of phosphorylated tau protein (in neurofibrillary tangles). The growing evidence for the "*amyloid hypothesis*" of AD, which focuses on a key role for Aβ accumulation in initiating the pathogenic cascade, has led to intense efforts to develop Aβ reduction strategies. We previously found that nasal treatment of APP-Tg

mice with a proteasome-based adjuvant (Protollin) reduced Aβ deposits that co-localized with activated CD11b+ cells. In that study, Protollin was detected only in the periphery, which led us to propose a non-CNS effect of Protollin on Aβ burden mediated by peripheral monocytes. Thus, we hypothesized that the effect is mediated via activation of blood-derived monocytes, either via their infiltration into CNS and direct uptake of Aβ or indirectly by their induction of Aβ uptake by resident CNS microglia. To characterize the origin of the CD11b+ microglia present in the brain of Protollin-vaccinated APP-Tg mice, we generated chimeric mice by reconstitution of the BM of 20-month-old APP-Tg-mice with wild-type BM cells expressing GFP under the CX3CR1 promoter. Two months after this BM-transplantation, AD-chimera mice (age 22 mos) were treated intranasally with Protollin (1 µg/mouse) for 2 or 6 weeks. In addition, we used our novel microglia-specific antibodies to distinguish and characterize the phenotype of resident microglia, recruited monocytes and splenic monocytes in control and Protollin-treated APP-tg mice. We used Nanostring quantitative technology for 179 inflammation-related genes to profile the microglia and monocytes. We have found that nasal treatment of the AD-chimera mice with Protollin: 1) significantly reduced Aβ plaque levels accompanied by an increase in the numbers of recruited CD11b-GFP BM-derived monocytes having intracellular immunoreactivity for endogenous Aβ. CD11b was expressed by all GFP+ cells adjacent to residual Aβ plaques, but by hardly any of the non-GFP+ resident microglia; 2) activated splenic $Ly6C^{Hi}$ monocytes having increased expression of inflammation-related genes related to the M1 phenotype. Resident microglia were not affected by Protollin treatment. Thus, nasal treatment with proteasome-based adjuvants targets peripheral monocytes, modulates their pro-inflammatory M1 phenotype and induces their recruitment to Aβ-plaques in the brain of APP-tg mice to facilitate Aβ plaque clearance.

Disclosures: R. Cialic: None. **O. Butovsky:** None. **D.J. Selkoe:** None. **H.L. Weiner:** None.

Poster

851. Alzheimer's Disease: In vivo Therapeutics III

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:851.30/E12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA UO1 AG032225

NINDS F30 NA051971

NIH NS049442

The Veteran's Administration

Jean Perkins Foundation

Richard M. Lucas Foundation

Title: A small molecule p75NTR ligand prevents cognitive deficits and neurodegeneration in a mouse model of Alzheimer's disease

Authors: *J. K. KNOWLES[1], T.-V. NGUYEN[2], D. SIMMONS[2], L. VANDER GRIEND[2], Y. XIE[3], T. YANG[2], H. ZHANG[4], J. POLLAK[5], T. CHANG[2], O. ARANCIO[4], S. M. MASSA[6], M. BUCKWALTER[2], T. WYSS-CORAY[7], F. M. LONGO[2]
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Abstract: The p75 neurotrophin receptor ($p75^{NTR}$) is one of a few receptors associated with multiple mechanisms specifically related to neuronal degeneration in Alzheimer's disease (AD). In earlier studies, crossing Thy-1 $hAPP^{Lond/Swe}$ ($APP^{L/S}$) mice with $p75^{NTR}$ mutant mice resulted in decreased neuritic degeneration and dystrophy (Knowles et al, JNS, 2009). Modulation of $p75^{NTR}$ function might therefore constitute an important therapeutic strategy. Previous *in vitro* studies demonstrated that a non-peptide small molecule, LM11A-31, binds to $p75^{NTR}$ and induces Akt signaling to promote hippocampal neuron survival (Massa et al 2006, JNS). Further, LM11A-31 prevented amyloid-β-induced degeneration and deleterious signaling in neuronal and hippocampal slice cultures (Yang, Knowles et al PLoS ONE, 2008).The present study assessed the effects of LM11A-31 on neuronal dysfunction and degeneration in $APP^{L/S}$ mice. Following oral administration, LM11A-31 was found to cross the blood brain barrier and reach brain concentrations known to provide neuroprotection *in vitro*. Once daily oral administration of LM11A-31 over a three month period prevented deficits in novel object recognition and Y-maze performance, without detectable toxic effects. While the number and size of basal forebrain cholinergic neurons were unaffected in mice expressing the $APP^{L/S}$ gene, they exhibit neuritic degeneration and dystrophy in basal forebrain cholinergic neurons and hippocampus. These pathologic changes were prevented by LM11A-31 without affecting amyloid levels. These findings indicate that $p75^{NTR}$ is an important therapeutic target for AD, and that LM11A-31 or its derivatives represent a class of compounds with potential applications as protective agents in neurodegenerative disease.

Disclosures: J.K. Knowles: None. **T. Nguyen:** None. **D. Simmons:** None. **L. Vander Griend:** None. **Y. Xie:** None. **T. Yang:** None. **H. Zhang:** None. **J. Pollak:** None. **T. Chang:** None. **O. Arancio:** None. **S.M. Massa:** None. **M. Buckwalter:** None. **T. Wyss-Coray:** None. **F.M. Longo:** Ownership Interest; Dr. Longo is a founder of PharmatrophiX, a company focused on the development of neurotrophin receptor ligands..

Poster

852. Alzheimer's Disease: Therapeutics

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:852.01/E13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NHMRC

Title: Do metallothionein and its derivatives protect against Aβ toxicity by an LRP mediated mechanism?

Authors: *E. D. EATON[1], B. BENNETT[2], V. BEREZIN[3], R. CHUNG[2], A. WEST[2]
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Abstract: Introduction
The metal binding protein metallothionein (MT) protects neurons against toxic agents including copper-bound amyloid-β (Cu-Aβ). With others, we have shown that this is primarily by a metal swap mechanism by which zinc bound by MT is exchanged for copper bound by Cu-Aβ. However, MTs are also known to bind to Lipoprotein Receptor-Related Protein (LRP) receptors including LRP1 and LRP2 thus promoting neuronal regenerative growth and survival. MT does not cross the blood brain barrier, and therefore peptide analogues derived from the MT sequence have been designed, termed emtins. We asked whether emtins also protect neurons against Cu-Aβ and if so, by which mechanism.
Method
Embryonic rat hippocampal neurons in primary culture were treated with Cu-Aβ (10μM) for 24 hours and neuronal viability tested by an Alamar Blue assay. The protective effects of MT, and the emtin variants emtinAc and emtinB were compared by co-incubation with Cu-Aβ. EmtinB action in the presence of the LRP inhibitor Receptor Associated Protein (RAP) (1μM) was also examined. The ability of emtin Ac and B to block the copper-mediated aggregation of Aβ into SDS insoluble aggregates was also examined by SDS-PAGE after 72 hours, and compared to the well characterised inhibitory action of native MT.
Results
Cu-Aβ treatment reduced neuronal viability to 53% (+/-1.0) compared to control, but co-incubation with emtinB (10μM and 25 μM) restored viability to 71% (+/- 2.0) and 82% (+/- 2.3) of control respectively. In contrast, emtinAc (10μM and 25 μM) increased cell viability from 67% (+/-9.3) to 79% (+/-7.5%) and 85% (+/-14.2%) respectively, but this was not significant. Inclusion of the LRP inhibitor RAP (1μM) significantly blocked the protective effect of emtinB (Plus RAP 76% +/-3.3, Minus RAP = 94% +/- 2.3). Neither EmtinAc nor emtinB (25μM) were

able to prevent the formation of insoluble Cu-Aβ during a 72 hour incubation, however MT (25μM) abolished the formation of insoluble Cu-Aβ, as assessed by SDS-PAGE.
Conclusion
The MT analogue emtinB protected cultured hippocampal neurons against Cu-Aβ toxicity and this action was blocked by an LRP receptor inhibitor. This is similar to the action of native MT, however we further showed that emtinB was not able to block copper-induced aggregation of Aβ, suggesting that it does not participate in a metal exchange mechanism. In contrast, the related MT analogue emtinAc was neither neuroprotective nor prevented Aβ aggregation. We are investigating the action of emtinB further in animal models of AD.

Disclosures: E.D. Eaton: None. **B. Bennett:** None. **V. Berezin:** None. **R. Chung:** None. **A. West:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Program#/Poster#:852.02/E14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant

Title: Glutamine deprivation may contribute to neurodegenerative disease: Cell cycle reentry, reduced autophagy and altered mitochondrial function

Authors: *J. CHEN, K. HERRUP
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Abstract: We have previously reported that glutamine acts as a neuroprotectant against DNA damage, beta-amyloid and H_2O_2-induced stress, and that neurons depend on both endogenous and exogenous glutamine for survival (Chen and Herrup, PLoS one, 2012). Here we report the results of our recent studies aimed at learning the pathways that may contribute to this protection. One key element is the effects of glutamine on autophagy. Normally, in untreated mature neurons
(DIV12-15), we find low amounts of LC3II, one index of the autophagic process. In mature neurons treated with H_2O_2, by contrast, we find marked increase of LC3II after an oxidative challenge. The involvement of glutamine in this process is seen through our observations that if glutamine was removed from the media before H_2O_2 treatment, then no increase of autophagy is induced. A second key pathway by which glutamine acts as a neuroprotectant is through its impact on mitochondrial function. Without glutamine, mitochondria form a long fused network

and this effect is seen in both primary hippocampal neurons and neuroblastoma cells. The strong suggestion of these findings is that adequate levels of glutamine are essential to guard the cell against deficiency of mitochondrial function. The final key element in the neuroprotection afforded by glutamine is the protection it offers against unscheduled neuronal cell cycle reentry. We find, as have others, that cells deprived of glutamine fail to complete a normal cell cycle. Again the effect is best seen when the cells are under stress. In the absence of glutamine, etoposide treatment caused a significant increase of the numbers of cyclin A -positive neuroblastoma (N2a) cells. Death follows quickly, but there was a telling absence of cyclin D1 expression. The suggestion is that a mitotic catastrophe is the proximal cause of death. Further glutamine deficiency in a second neuronal cell line, HT22 cells, leads to a significant increase in indices of cell death. Analysis of cell cycle kinetics suggests a mitotic catastrophe. In summary, glutamine is important for multiple cellular functions that are intimately related to diseases such as AD. Given that chronic inflammation is one of the central pathogenic features of AD and systemic inflammation causes dramatic loss of glutamine from brain, our data further suggest that glutamine deprivation may play a role in these diseases.

Disclosures: J. Chen: None. **K. Herrup:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant PPG14930

Burke Med Res Inst

Title: Increasing activity of one mitochondrial enzyme is neuroprotective.

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Abstract: Multiple reports indicate that the mitochondrial enzyme complex α-ketoglutarate dehydrogenase (KGDHC) is diminished in brains of patients that died with Alzheimer’s disease (AD). The decline is highly correlated to the clinical dementia rating scale of the patients before they died. The enzyme complex consists of three subunits (E1k, E2k and E3). *In vivo,* a reduction of E2k or E3 increases oxidative stress, reduces neurogenesis and makes the animals more sensitive to metabolic insults. *In vitro,* activities have also been reduced in vitro with

specific inhibitors, by shRNA or by using neurons from transgenic mice. The decline makes the cells more sensitive to oxidative insults and causes AD-like changes in calcium signaling. These experiments raise the possibility that increasing KGDHDC may be protective. Treatments known to be protective also increase KGDHC activity. For example, treatment with the vitamin E analogue trolox increases KGDHC by 120%. Increasing sirtuin activity by increasing NAD^+ with nicotinic acid riboside is protective. A six hour treatment with NR increased KGDHC activity by about 175%. Thus, we tested whether increasing just KGDHC could be neuroprotective. Neurons were transfected with AV expressing E1k. This increased E1k message four fold, protein level by 80% and increased KGDHC activity by 70%. The cells over-expressing KGDHC were resistant to hydrogen peroxide induced cell death. Thus, increasing KGDHC by either non-specific or specific means is neuroprotective.

Disclosures: G.E. Gibson: None. **H. Chen:** None. **H. Xu:** None.

Poster

852. Alzheimer's Disease: Therapeutics

Location: Hall F-J

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Program#/Poster#:852.04/E16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG018884

Baxter Healthcare grant

Title: Intravenous immunoglobulin (IVIg) protects neuronal viability and synaptic markers in cultured degenerating primary hippocampal neurons

Authors: *J. A. BAILEY, B. RAY, D. K. LAHIRI
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Abstract: Alzheimer’s disease (AD) is characterized by deposition of plaques in the brain comprised primarily of the neurotoxic amyloid-β (Aβ) peptide. High levels of Aβ lead to abnormal synaptic pruning and neuron death which is thought to produce the disabling cognitive deficits seen in AD. Therefore, a promising treatment strategy for AD is to reduce levels of the Aβ peptide in the brain. We have shown previously that several neuroprotective compounds, including memantine (J Neurosci Res, 2010) and rivastigmine (J. Neurochem, 1010; PLoS One, 2011), can reduce Aβ levels. Since Aβ clearance may be slowed in AD, reduced production may be insufficient, so strategies to remove Aβ more directly are under investigation. These include several immunotherapy approaches, including intravenous immunoglobulin (IVIg). It has been

hypothesized that the circulating immunoglobulins from healthy donors will assist in clearing Aβ from the blood, and this treatment is currently in clinical trials. To investigate potential mechanisms of IVIg action on neuronal tissues, we cultured degenerating primary hippocampal neurons as previously described (Bailey and Lahiri, J Neurochem, 2010) and treated them with IVIg in order to assess its effects on neuronal viability and synaptic protein marker levels. Viability was determined by measuring levels of intracellular ATP, which increased significantly with IVIg treatment. Additionally, levels of the glycolytic enzyme lactate dehydrogenase (LDH) were measured. Cells treated with IVIg showed higher LDH levels in the lysates and lower LDH levels in media, indicating increased viability. Using Western immunoblotting of protein extracted from treated neuronal cells, we observed that neurodegeneration in the model reduced presynaptic markers SNAP-25 and synaptophysin to undetectable levels. Notably, treatment with IVIg increased levels of both markers. Similarly, the postsynaptic marker PSD95 was reduced to nearly undetectable levels with the vehicle, while increased significantly by IVIg treatment. However, the glial activation marker GFAP was not altered by IVIg treatment. Together, these results indicate that IVIg may act to improve survival of degenerating hippocampal neurons as well as protect synaptic connections between neurons. Further investigation of the effects of IVIg on secreted APP proteolysis products and other possible targets is ongoing. We are also testing the effects of IVIG in our recently developed human fetal brain cultures. This work provides a potential mechanism for cognitive improvements seen in early clinical trials of IVIg in AD patients.
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Disclosures: J.A. Bailey: None. **B. Ray:** None. **D.K. Lahiri:** Research Grant; Baxter Healthcare.

Poster

852. Alzheimer's Disease: Therapeutics

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Program#/Poster#:852.05/E17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5R01AG018884

Alzheimer's Association IIRG

Title: Rivastigmine promotes non-amyloidogenic α-secretase pathway in human fetal brain culture by increasing the levels of ADAM 9 and 10: Implications in Alzheimer’s disease (AD) therapeutics.

Authors: *B. RAY[1], J. A. BAILEY[2], N. CHOPRA[2], N. H. GREIG[4], K. SAMBAMURTI[5], D. K. LAHIRI[3]
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Abstract: AD is characterized by deposition of amyloid β (Aβ) plaques, neuroinflammation and hyperphosphorylation of tau protein . Aβ is cleaved from the amyloid precursor protein (APP) by enzymatic actions of β-site APP cleaving enzyme (BACE-1) and γ secretase. APP is also processed by α-secretase to generate a p3 fragment and precludes Aβ production. Thus, promotion of the α-secretase pathway represents a rational therapeutic approach for drug development in AD. Rivastigmine is a cholinesterase inhibitor (ChEI) and one of the FDA-approved treatment options for mild-moderate AD. We have observed non-cholinergic, non-catalytic functions of ChEIs, including APP modulation (Greig et al, PNAS 2005; Lahiri et al JPET 2007. We have recently characterized rivastigmine's ability to increase levels of sAPPα in rat primary neuronal culture (Bailey et al 2011). To evaluate the mechanism, here we have treated human fetal brain (HFB) cultures with rivastigmine (100nM to 10 μM). Cultured cells were also treated with a non-selective ADAM inhibitor (TAPI2) alone or in combination with rivastigmine. Conditioned media (CM) samples and cell lysates (CL) were collected after the treatments and assayed for APP metabolites. Notably, rivastigmine treatment increased levels of sAPPα in HFB cultures. Concomitantly, rivastigmine decreased levels of sAPPβ, Aβ40 and Aβ42 vs controls by ELISA in CM samples. Further, rivastigmine significantly decreased levels of β-CTF vs control by ELISA in CL samples. Interestingly, these effects of rivastigmine were reversed by TAPI 2 co-treatment, suggesting rivastigmine's effects on ADAMs. Indeed, the rivastigmine-treated group showed increased levels of ADAM9 and ADAM10 vs controls by Western immunoblotting of CL samples. Levels of ADAM17 were unchanged between the treatment groups. Some of these findings were also observed in the hippocampal samples of APP-Tg mice treated with rivastigmine for three weeks. Taken together, our data suggest that rivastigmine can promote non-amyloidogenic α-secretase processing of APP by increasing levels of ADAM 9 and ADAM 10, resulting in decreased levels of Aβ. This novel property of rivastigmine warrants further studies in in vivo settings and can also be utilized in developing newer ChEIs and/or butyrylcholinesterase inhibitors in the treatment of AD.

Disclosures: B. Ray: None. **J.A. Bailey:** None. **N. Chopra:** None. **N.H. Greig:** None. **K. Sambamurti:** None. **D.K. Lahiri:** None.

Poster

852. Alzheimer's Disease: Therapeutics

Location: Hall F-J

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Program#/Poster#:852.06/E18

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Regulation of APP processing using antibody fragments reduces stress induced toxicity in cell models of Alzheimer’s Disease

Authors: *V. R. SURYADI, S. EMADI, S. BODDAPATI, M. SIERKS
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Abstract: Background: One of the most important neuropathological characteristics of Alzheimer’s disease is the aggregation and deposition of the protein beta-amyloid. Beta-amyloid is produced by proteolytic processing of the Amyloid Precursor Protein (APP). Production of beta-amyloid from APP is increased when cells are subject to stress since both APP and beta-secretase are upregulated by stress. An increased beta-amyloid level promotes aggregation of beta-amyloid into toxic species which cause an increase in reactive oxygen species (ROS), caspase-3 activity and a decrease in cell viability. Therefore reducing beta-amyloid generation is a promising method to control cell damage following stress. We have isolated a recombinant antibody fragment, iBSec1, which selectively blocks beta-secretase processing of APP by binding the APP substrate as opposed to blocking the enzyme active site. Here we show that iBSec1, when added extracellularly to mammalian cells overexpressing APP, reduces stress induced toxicity.
Methods: Different mammalian cells expressing APP treated with different concentrations of hydrogen peroxide to induce stress. Cells were also simultaneously treated with different concentrations of iBSec1 to inhibit beta-secretase processing of APP. Differences in toxicity of cells treated with or without iBSec1 were assayed by measuring changes in levels of ROS, Caspase 3 and mitochondrial health (XTT).
Results: Addition of hydrogen peroxide to cells showed a concentration dependent increase in ROS and Caspase 3 activity and a decrease in cell viability as determined by XTT. The increase in stress induced toxicity caused by addition of hydrogen peroxide was dramatically decreased by simultaneously treating the cells with iBSec1 to block the increase in beta-amyloid levels resulting from the upregulation of APP and beta-secretase.
Conclusions: Cellular stress increases ROS generation, Caspase-3 activity and decreases cell viability. Levels of beta-amyloid increase in cells following stress due to the upregulation of APP and beta-secretase activity, resulting in the formation of toxic aggregate species. Here we show that we can selectively control proteolytic processing of APP to inhibit beta-amyloid generation and reduce stress induced toxicity in cell models of AD. Since stress also upregulates APP and beta-secretase levels in healthy neurons, tailoring APP processing away from beta-amyloid generation may be an effective way to reduce stress induced toxicity in healthy neurons as well.

Disclosures: V.R. Suryadi: None. **S. Emadi:** None. **S. Boddapati:** None. **M. Sierks:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1R01NS076117-01

Alzheimer Association Grant IIRG-08- 90824

Institutional Training Grant T32 GM073546-01A1

Title: Determining the mechanism of gamma-secretase modulators

Authors: C. J. CRUMP[1], D. S. JOHNSON[3], K. R. BALES[3], *Y. LI[2]
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Abstract: One of the major hallmarks of Alzheimer's disease is senile plaques, which are composed of amyloid beta peptides. These peptides are derived from the amyloid precursor protein (APP) after sequential processing by β- and γ-secretase. The two most predominant forms of amyloid beta peptides are Aβ40 and Aβ42, which are 40 or 42 amino acids long. The more hydrophobic longer peptides are thought to be the toxic and detrimental form. Mutations in APP or γ-secretase cause the rarer autosomal dominant form of Alzheimer's disease. These mutations result in an increase in the ratio of Aβ42 to Aβ40 peptides. Therefore, γ-secretase inhibition has been considered as a potential therapeutic mechanism to slow or prevent disease progression. However, γ-secretase activity is required for the processing of many transmembrane proteins in addition to APP, including activation of the notch pathway, which is essential for cell fate determination. Therefore, emphasis has now turned towards the development of γ-secretase modulators (GSMs), which not only prefer the APP substrate over Notch, but also specifically abrogate Aβ42 production over Aβ40 production. We have developed GSM based photo-labile probes for investigating the mechanism of action of these therapeutically promising small molecules. We have identified presenilin-1, the catalytic component of γ-secretase, as a target of GSMs. Furthermore, using active site based photo-probes we are investigating GSM-induced conformational changes in the active site of γ-secretase upon binding.

Disclosures: C.J. Crump: None. **D.S. Johnson:** None. **K.R. Bales:** None. **Y. Li:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Program#/Poster#:852.08/E20

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The regulation of neuronal cell fate and neurogenesis by MSCs in Alzhimer`s Disease

Authors: *H. PARK, S. OH, H. KIM
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Abstract: Alzhimer`s Disease (AD) is a progressive neurodegenerative disease that result in neuronal death and exacerbation of cognitive functions. Neurogenesis occurs in the subventricular zone (SVZ) of lateral ventricles, and in the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus. Although the neurogenesis mechanism is impaired in AD, it remains unclear. Bone morphogenetic proteins (BMPs) and its antagonist Noggin affect differentiation in the neural progenitor cells (NPCs) of adult brain. In particular, it is known that BMP4, the member of the BMPs family, induce glial differentiation of NPCs. But Noggin inhibits these mechanism and induce neuronal differentiation. The role for noggin in expasion and differentiation of NPCs has been debated. Because noggin is not the only factor inhibiting differentiation of BMP4-exposed NPC. Also, Wnt/β-catenin signaling regulates neuronal differentiation of NPCs. These signaling is activated by moving into nucleus β-catenin. In this study, We identified that β-catenin level is decreased, while BMP4 level is increased in the NPCs of AD brain. And exogenous application of mesenchymal stem cells (MSCs) increased noggin and β-catenin level. When Noggin is released by MSCs, β-catenin and BMP4 levels completely reversed. We thought that β-catenin promotes neuronal differentiation and induces activation of BMP4. Also, glial differentiation caused by expression of BMP4 is inhibited by noggin, which means neuronal differentiation. We progressed our experiment in vitro and in vivo. In vitro, we showed expression levels of BMP4, noggin, and β-catenin with RT-PCR and immunocytochemistry (ICC) and intracerebroventricular infusions of Ab into C57/BL6 mice triggered the brain of AD patient in vivo, so we measured exprssion level of neurogenesis by perfusion. These results suggest that noggin and β-catenin secreted by MSCs regulates neurogenesis while inhibiting BMP4. Thus, we demonstrate that noggin and β-catenin signaling is a key component of neurogenesis.
Keyword : Alzhimer`s Disease, Mesenchymal stem cells, Neurogenesis, Bone morphogenetic proteins, Noggin, Wnt/β-catenin signaling

Disclosures: H. Park: None. **S. Oh:** None. **H. Kim:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Program#/Poster#:852.09/E21

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CREST

NEDO

J-ADNI

Title: Suspected limited efficacy of γ-secretase modulators

Authors: *N. KAKUDA[1], K. AKAZAWA[2], H. HATSUTA[3], S. MURAYAMA[3], Y. IHARA[1]
[1]Doshisha Univ., Kizugawa City / Kyoto, Japan; [2]Niigata Univ., Niigata, Japan; [3]Tokyo Metropolitan Inst. of Gerontology, Tokyo, Japan

Abstract: γ-Secretase modulator-like effect is found in the brains affected by mild cognitive impairment (MCI) and sporadic Alzheimer's disease (AD) brains. Due to this alteration in the activity, both levels of Aβ42 and Aβ43 in the cerebrospinal fluid (CSF) are profoundly decreased in MCI/AD. To compensate these decreases, CSF Aβ38 and Aβ40 are increased, and nevertheless Aβ42/43 seem to slowly progressively accumulate in the brain. Aβ38/42 and Aβ40/43 are distinct product/precursor pairs, and Aβ38/42 vs Aβ40/43 ratio plots clearly divided MCI/AD subjects and controls (Kakuda et al, 2012). This γ-secretase self-modulating seems to be planned to prevent further accumulation of Aβ42 and Aβ43 in the brain. We asked when this alteration would occur in the brain? Raft-associated γ-secretase activities prepared from autopsied brains in various Braak senile plaque (SP) stages were quantified. As a result, γ-secretase self-modulation was obvious in the stage of amyloid deposition, decades prior to clinically overt AD stage. Although stage A brains showed similar γ-secretase activity as controls, γ-secretase self-modulating seems to be related to the extent of amyloid-deposition in the brain.
We thus pointed out that the efficacy of Aβ42-lowering γ-modulator could be minimal in sporadic MCI/AD patients, as modulation of γ-secretase already occurs in amyloid-bearing brains. We also measured modulator-treated raft-associated γ-secretase activities in sporadic AD and control brains. GSM-1, a NSAIDs-derived Aβ42-lowering modulator, profoundly lowered Aβ42 and increased Aβ38 in the γ-secretase assay system. GSM-1 treated AD γ-secretase showed a similar trend but only to much less extent compared with control. Thus, we claim that the true efficacy of γ-secretase modulator should be evaluated in amyloid-bearing and γ-secretase-modulating brains.

Kakuda et al. Altered γ-secretase activity in mild cognitive impairment and Alzheimer's disease. EMBO Mol. Med. 4, 344-352 (2012)

Disclosures: N. Kakuda: None. **K. Akazawa:** None. **H. Hatsuta:** None. **S. Murayama:** None. **Y. Ihara:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant NS077239

NIH grant AG032611

Title: Tau antibody uptake into neurons strongly correlates with degree of tau pathology and is primarily receptor-mediated via low affinity Fcγ receptors.

Authors: *E. E. CONGDON[1], A. BOUTAJANGOUT[1,2], H. B. R. SAIT[1], E. M. SIGURDSSON[1,2]
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Abstract: Active and passive tau immunotherapy has been shown by us and more recently by others to be effective in animal models. In addition, we have previously reported that tau antibodies are able to enter into neurons and co-localize with intracellular tau aggregates within the endosomal/lysosomal pathway. However, the mechanism of uptake remained to be determined.
To this end, we have administered I^{125}-labeled tau antibodies into brain slice cultures prepared from transgenic (htau/PS1 and 3xTg) and wild type mice over 15 months of age. Slices were evenly distributed among the treatment groups, thus allowing each animal to serve as its own control. Following treatment, slices were washed with acidified buffer and subsequently several times with ice cold reaction buffer to minimize signal from extracellular binding of the antibodies.
To determine if antibody uptake is saturable, slices from htau/PS1 mice were incubated at 37°C with increasing concentrations (0.01-10 ug/ml) of I^{125}-labeled 4E6G7, a monoclonal tau antibody that we generated against the pSer396/404 epitope. Its uptake was rapid (< 1 h) which fits with our prior findings, and saturable between 5 and 10 ug/ml. Furthermore, incubating the sections

with a ten-fold excess of unlabeled mouse IgG resulted in 41% reduction in radioactivity ($p = 0.04$).
As receptor endocytosis is temperature-dependent, additional slices were incubated with I^{125}-4E6G7 for 1 hour at 4°C. Lowering the temperature produced a 63% decrease in radioactivity relative to slices at 37°C ($p = 0.002$). Inhibition of phagocytosis at 37° led to a small but non-significant decrease in uptake, suggesting that most of the antibodies were being taken up into neurons. To clarify if antibody internalization is mediated by low affinity Fc gamma receptors, slices were incubated with 0.5ug/ml I^{125}-4E6G7 and increasing concentrations of an antibody that blocks these receptors (FcBlock ; anti CD16/CD32; 0.05-50 ug/ml). It resulted in a dose-dependent decrease in radioactivity (26-56%, $p = 0.11$-0.04), indicating involvement of these receptors in the uptake of the antibody.
Interestingly, uptake strongly correlated ($r = 0.88$, $p = 0.003$) with tau pathology as determined by immunoblotting with PHF-1 in all three mouse lines used. These results suggest that tau aggregates strongly influence receptor-mediated endocytosis of tau antibodies and/or their retention within affected neurons.
In summary, our findings show that antibody uptake into neurons strongly correlates with degree of tau pathology, and is primarily receptor-mediated via low affinity Fc gamma receptors.

Disclosures: E.E. Congdon: Research Grant; NIH grants NS077239 and AG032611. **A. Boutajangout:** Research Grant; AA MNIRGD-09–133917. **H.B.R. Sait:** Research Grant; NIH NS077239, AG032611. **E.M. Sigurdsson:** Research Grant; NIH NS077239, AG032611. Consultant/Advisory Board; H. Lundbeck A/S. Other; NYU technology on tau immunotherapy is licensed to and is being co-developed with H. Lundbeck A/S.

Poster

852. Alzheimer's Disease: Therapeutics

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:852.11/E23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Field Neurosciences Institute (to GLD)

John G. Kulhavi Professorship (to GLD).

Title: Mesenchymal stem cells as treatment for cognitive and neuropathological deficits in the 5xFAD mouse model of Alzheimer's disease.

Authors: *J. J. MATCHYNSKI[1,4], J. ROSSIGNOL[2,3], C. PAPPAS[2], K. FINK[2], A. CRANE[2], S. A. LOWRANCE[1], J. J. MATYAS[1], G. L. DUNBAR[1,5]

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Abstract: Alzheimer's disease is characterized by a progressive loss of memory as well as increases in amyloid-β (Aβ) plaques, neurofibrillary tangles, inflammation, and neurodegeneration. Mesenchymal stem cells (MSCs) would likely be effective in alleviating the symptoms of Alzheimer's disease; however an optimal location of transplantation has yet to be determined. The present study compared the efficacy of various routes of administration of MSCs in alleviating symptoms in the 5xFAD transgenic mouse model of Alzheimer's disease. 5xFAD positive mice (AD) received transplantations of a total of 800,000 MSCs via bilateral injections into the lateral ventricles (LV), the hippocampus (Hipp), or divided between said locations (LV-Hipp), and were compared to AD mice and wild-type littermates (WT) injected with vehicle. After a post-operative period, the mice underwent a series of behavioral tasks, and their brains were analyzed using either immunohistochemistry (IHC) to assess levels of Aβ42 (the most aggregative form of Aβ), inflammation, and neuron loss, or enzyme-linked immunosorbant assays (ELISA) to assess levels of Aβ42 and synaptophysin proteins. In addition to improving learning, results of IHC analysis indicated that the MSCs appeared to have decreased the levels of Aβ42-labeling specifically in the fornix of all groups, as well as in the hippocampus of the Hipp and LV-Hipp groups. There also appeared to be a decrease in inflammation in the fornix of the LV-Hipp group. Therefore, MSCs should be considered as a viable treatment for AD, especially when injected into the LV and Hipp, which appeared to lead to a decrease in Aβ42 and inflammation in surrounding regions.

Disclosures: J.J. Matchynski: None. **J. Rossignol:** None. **C. Pappas:** None. **K. Fink:** None. **A. Crane:** None. **S.A. Lowrance:** None. **J.J. Matyas:** None. **G.L. Dunbar:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS077239

NIH Grant AG032611

Title: Efficacy and mechanisms of antibody-mediated clearance of tau aggregates in a brain slice model

Authors: *J. GU[1], E. M. SIGURDSSON[1,2]
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Abstract: Pathological tau proteins are hallmarks of Alzheimer's disease (AD) and other tauopathies. These aggregates are hyperphosphorylated and form neurofibrillary tangles, with at least some of these assemblies leading to neuronal dysfunction and death. In AD, tau lesions correlate better with cognitive decline than Aβ burden. Therefore, clearing tau aggregates may be more effective to ameliorate cognition. Our previous studies and more recently those of others have shown that both active and passive immunization targeting phospho-tau epitopes reduces tau pathology and prevents cognitive decline in transgenic (Tg) mice. We have since developed several monoclonal antibodies (mAbs) targeting key pathological epitopes of tau. To assess their efficacy to clear tau aggregates, we established an ex vivo brain slice model of tau pathology. Slices were obtained from pups of tau P301L Tg mice and cultured for up to 6 weeks. In control experiments, 60% increase of hyperphosphorylated tau (PHF1/total tau ratio) was detected on western blots of slices from 2 to 6 weeks in culture. In treatment studies, the mAbs (10 µg/ml) were added twice per week from 2 to 6 weeks. The two mAbs tested, 4E6G7 (targeting phospho-S396/404 epitope) and 6B2G12 (targeting non-phospho S396/404 epitope), significantly reduced hyperphosphorylated tau by 25% and 38%, respectively, compared to control ($p<0.05$). Additionally, 4E6G7 reduced the amount of sarkosyl-insoluble tau (PHF1) in the slices by 28% ($p<0.01$). Analysis of the effect of 6B2G12 on this fraction is underway. We also examined the cellular mechanisms of mAb-mediated clearance of tau pathology in slice cultures from 1 year old P301L mice, which have extensive tau pathology. Fluorescent labeled mAbs entered neurons and bound to tau aggregates after 2 hour incubation. Lesser uptake of mAbs was evident in microglia but not in astrocytes or oligodendrocytes. Within neurons, the mAbs were largely co-localized with markers of the endosomal/lysosomal pathway. Our prior studies in neuroblastoma cells overexpressing human tau did not reveal any antibody uptake, presumably because these cells did not have tau aggregates. Cell line models remain, however, ideal to study spread of extracellular tau and related interventions. Slice models are closer to in vivo conditions and allow studies of the cellular mechanisms of mAb-mediated clearance of tau aggregates, which likely include both neurons and microglia. In summary, the ex vivo brain slice model provides a useful tool for screening mAbs before in vivo studies, and provides a valuable insight into the cellular mechanisms involved in clearing these aggregates.

Disclosures: J. Gu: Other; NYU technology on tau immunotherapy is licensed to and is being co-developed with H. Lundbeck A/S. **E.M. Sigurdsson:** Consultant/Advisory Board; H. Lundbeck A/S. Other; NYU technology on tau immunotherapy is licensed to and is being co-developed with H. Lundbeck A/S.

Poster

852. Alzheimer's Disease: Therapeutics

Location: Hall F-J

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSFC-RGC Joint Grant N-CUHK433/08

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Title: The therapeutic effect of Huperzine A on Alzheimer's disease involves a novel mechanism on brain iron regulation

Authors: *Y. KE[1], X. HE[1], M. S. C. TAM[1], Z. M. QIAN[2]
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Abstract: The accumulation and deposition of amyloid-β (Aβ) peptides in the brain is a pathological hallmark in Alzheimer's disease (AD). Recent studies suggest a role of brain iron accumulation in the pathogenesis of AD. Huperzine A (HupA), a new alkaloid isolated from the Chinese herb *Huperzia serrata*, is a potent and reversible inhibitor of acetylcholinesterase. HupA is neuroprotective and improves cognitive functions. In this study, we found that the beneficial effect of HupA is related to a novel action on brain iron regulation. APPsw/PS1dE9 transgenic AD mice were treated with HupA (0.1mg/kg, po, once per day) or saline. We found that HupA treatment reduced insoluble but not soluble Aβ levels and markedly ameliorated amyloid plaques formation in these animals at ninth month of age. These effects of HupA were reduced by feeding the animals with a high iron diet starting from the second month. In parallel, we found that HupA decreases iron content in the brain. This effect was probably related to the action of HupA in decreasing the expression of transferrin-receptor 1 (TfR1) leading to reduced transferrin-bound iron uptake into cultured neurons. Taken together, these results suggest that, in addition to facilitated cholinergic transmission and neuroprotection, regulation of iron metabolism is a novel target of HupA in its therapeutic action on dementia.

Disclosures: Y. Ke: None. **X. He:** None. **M.S.C. Tam:** None. **Z.M. Qian:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Marian S. Ware Alzheimer Program

Fundación Caja Madrid

Instituto de Salud Carlos III

Title: Conditional knock out of GSK-3αdecreases Aβ amyloid deposition and prevents memory deficits in PDAPP mice

Authors: *L. MOLINA PORCEL, D. E. HURTADO, J. C. CARROLL, C. MACDONALD, A. K. ABOAGYE, J. Q. TROJANOWSKI, V. M.-Y. LEE
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Abstract: Glycogen synthase kinase-3 (GSK-3) is linked to the pathogenesis of Alzheimer's disease, but the specific contributions of each of the GSK-3 α and β isoforms in plaque formation in vivo have not been clarified. In this study, we sought to elucidate the role of each GSK-3 isoform using a conditional knock out approach. We generated triple transgenic mice using the CaMKIIα-Cre (α-calcium/calmodulin-dependent protein kinase II-Cre) system to knock down *GSK-3*α in PDAPP$^{+/-}$ mice (GSK-3α cko;PDAPP), however we were not able to generate viable GSK-3β offspring that survived beyond 6 months of age. GSK-3α cko;PDAPP mice were aged to 17 months and compared to their GSK-3α wt;PDAPP control litter-mates. Behavioral, biochemical and histological techniques were employed to ascertain both neuropathological and cognitive changes. We observed a dramatic reduction of GSK-3α immunostaining and protein levels measured by western blot, with no effect on GSK-3β levels in GSK-3α cko brains. Moreover, GSK-3α knockdown showed a significant effect on reducing senile plaques and ameliorating memory deficits in 17 month old PDAPP mice. These findings highlight the potential importance of GSK-3α as a possible therapeutic target for ameliorating behavioral impairments linked to Alzheimer´s disease senile plaques.

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Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Colciencias 110151928994

We thank Dr. Jon Collins (GSK, Stevenage, UK) for providing the GW683965A.

Title: Activation of liver X receptors restores cognitive decline in the triple transgenic mouse model of Alzheimer´s disease

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Abstract: Introduction:The activation of Liver X Receptors(LXR) has become in an important target for treatment of Alzheimer disease (AD). LXR activation up-regulates apolipoprotein E (APOE) that promotes the proteolytic degradation of Aβ, reduces amyloid plaque pathology, neuroinflammation and improve memory inamyloid models of AD (APP and PS1 mutantions). However the capacity of LXR agonist to improve the pathological characteristics in the triple AD models (APP, PS-1 and tau mutations: 3xtg) that form neurofibrillary tangleremains unclear.Aims:to analyze the potential of activation of LXR to improve the cognitive behavior in 3xtg model of AD. Methodology: We used de LXR activator GW3965 in the 3xtg model of AD which replicates more closely pathologic characteristics of AD including plaques, tangles, and synaptic dysfunction. We used two groups of animals: Group 1, short-term (ST) treatment for 6 days (50mg/kg/day) in 24-months old mice; Group 2, long-term (LT) treatment for 12 weeks (33mg/kg/day) in 12-months old mice. Cognitive performance was evaluated by using hippocampal dependent spatial memory: Morris water maze. Initially, learning tests were performed for 5 days, two trials for day; retention test were performed 48 hour after learning test; and transference test were performed for 2 days, two trials for day, 24 hour after retention test. Results: LT Litermates animals perform significantly better than 3xtg mice on 5 days of trials

($p<0.001$: learning, retentionand transference), as untreated 3xtg mice did not show any significant decrease in escape of latency over 5 days of trials. When 3xtg mice were treated with LT GW3965 there was no significant difference with litermates in these tasks. ST GW3965 treated 3xtg mice perform significant better than untreated 3xtg mice on learning and 4x retention tests in 5 days of trials ($p<0.01$). Conclusion: Both ST and LT GW3965 treatment restored memory functions in the 3xtg of AD. Further studies are underway to analyze the molecular mechanism associated to these cognitive changes.

Disclosures: A.G. Sandoval Hernandez: None. **J.I. Muñoz:** None. **G. Arboleda:** None. **P. Cardona Gómez:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Field Neurosciences Institute (to GLD)

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Undergraduate Research and Creative Endeavors grant from the Office of Research and Sponsored Programs at CMU (to TR)

Title: Antioxidants from tart cherries and essential fatty acids as treatment for cognitive deficits in the 5xFAD mouse model of Alzheimer’s.

Authors: *T. J. REINKE[1], J. J. MATCHYNSKI[1], S. ABDELAZIM[1], S. A. LOWRANCE[1], J. ROSSIGNOL[2], G. L. DUNBAR[1,3]
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Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by progressive memory loss, anxiety and personality changes, deterioration of cognitive abilities, and eventual motor and language dysfunction. Pathologically, AD is characterized by high levels of inflammation and oxidative stress, as well as a buildup of diffuse neuritic amyloid-β (Aβ) plaques and neurofibrillary tangles (NFTs). Studies have shown that humans with diets high in antioxidants are associated with lower levels of AD. In addition, because the brain contains a high amount of lipids, it is subject to peroxidation. As such, essential fatty acids, such as omega-

3, 6, and 9, are likely to be beneficial as a prophylactic treatment. A new compound, that conveniently offers a rich supply of both antioxidants and essential fatty acids, is Cerise Total Body Rhythm© (TBR; Cerise Pharmaceuticals, Traverse City, MI). TBR is a combination 17 antioxidants from tart cherry extract combined with omega-3, 6, and 9 from fresh water fish oil and ultra emu/kalaya oil. In the present study, TBR or vehicle (methylcellulose) was administered to the 5xFAD male mouse model of AD and wild-type littermates for 3 weeks. The 5xFAD mouse model was derived from transgenic mice that co-express five familial AD mutations. This model has proven to be well-suited for this study, as the mice show accelerated plaque development and very high levels of cerebral Aβ. A battery of sensitive behavioral tasks was used to test the effects of TBR as well as to provide a comprehensive profile of potential cognitive and behavioral deficits that emerge in male 5xFADs. Testing included the water t-maze (to assess learning deficits), rotorod (to assess balance), elevated plus-maze (to measure anxiety), pre-pulse inhibition (to measure sensory functioning), clasping (the withdrawal of the limbs to the torso), and open field (to assess spontaneous motor activity) tasks. Immunohistochemistry (IHC) was utilized to assess the levels of Aβ42-labeling, inflammation as measured by CD11b-labeling, and mitochondrial dysfunction as measured by cytochrome oxidase in the brain. The major findings of the current study indicate that: (1) TBR, at 60 mg/kg, was tolerated well in both normal WT and 5xFAD mice when taken daily for a 3 week period; (2) treatment with TBR reduced learning deficits in 5xFAD mice; and (3) IHC results suggest TBR may reduce levels of oxidative stress and plaque formation within the brain. Overall, the present study demonstrates the potential of TBR as an adjunct treatment for AD, as well as justifies future research on its long-term effects.

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Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA (U01 AG032225), the Jean Perkins Foundation, and the Horngren Family Alzheimer’s Research Fund to FML

Title: The effect of a novel small molecule p75NTR ligand on locus coeruleus degeneration and related psychiatric behaviors in a transgenic mouse model of Alzheimer’s disease

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Abstract: Psychiatric symptoms such as anxiety, depression, aggressive/submissive behavior, social withdrawal, and psychoses frequently occur in Alzheimer's disease (AD). Most pharmacological treatments for managing these symptoms include the use of drugs that result in further disruption of cognition. Noradrenergic neurons of the locus coeruleus (LC) contribute to the regulation of mood and behavioral states and in AD undergo significant degeneration. The p75 neurotrophin receptor ($p75^{NTR}$) regulates a number of degenerative signaling mechanisms relevant to AD and has been reported to be expressed by LC neurons. Previously, our group demonstrated that non-peptide, small molecule $p75^{NTR}$ ligands prevent basal forebrain and hippocampal degeneration and improve performance on a battery of cognitive tests (Y-maze, novel object recognition, contextual fear conditioning, and water maze) in an amyloid precursor protein mouse model of AD (Thy1-$hAPP^{Lond/Swe}$) (SFN 2011). In this study, we first confirmed the specificity of a $p75^{NTR}$ antibody by western blot analysis of mouse brain extracts. This antibody was then used to confirm significant expression of $p75^{NTR}$ by LC neurons in wild-type (wt) and transgenic (Tg) mice. We then compared the effects of a $p75^{NTR}$ ligand (L1) and vehicle on noradrenergic neuron degeneration and psychiatric-related behaviors using wt and Tg mice. Tyrosine hydroxylase immunohistochemistry combined with blinded, unbiased Stereoinvestigator and Neurolucida imaging analyses showed no change in LC noradrengeric neuron number or size in Tg mice but did reveal a significant degeneration of neurites. L1 administered by oral gavage once daily for 3 months beginning at age 3-4 months prevented LC neuritic degeneration. Using a panel of behavioral tests designed to model psychiatric endpoints, we established that Tg mice exhibited significant anxiety-like behavior in the light-dark transition test, the elevated plus maze, and the elevated zero maze; submissive behavior in the test tube dominance assay; social withdrawal in an automated social phenotyper; and a sensorimotor gating deficit in prepulse inhibition. We did not detect depression-like behavior in Tg mice using the forced swim and tail suspension tests. Administration of L1 significantly improved sociability and on-going studies indicate a strong trend towards reducing submissive behavior and a sensorimotor gating deficit. The ligand did not reduce anxiety-like behavior when tested on the elevated plus maze. These findings point to a role for small molecule $p75^{NTR}$ ligands in slowing or preventing LC degeneration with potential amelioration of certain behavioral disturbances in AD.

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Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer’s Drug Discovery Foundation

Cullen Family Trust for Health Care

Michael J. Fox Foundation

Mitchell Center for Neurodegenerative Diseases

Title: Modulation of Aβ oligomers in the Tg2576 Alzheimer’s disease mouse model by a novel generic anti-oligomer specific antibody

Authors: *M. J. GUERRERO[1], D. L. CASTILLO-CARRANZA[2], C. A. LASAGNA-REEVES[2], S. KRISHNAMURTHY[2], K. DINELEY[2], G. R. JACKSON[2], R. KAYED[2]
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Abstract: Amyloid oligomers have been implicated as the primary toxic species in the pathogenesis of a broad range of degenerative diseases, including Alzheimer’s disease (AD). We were able to mimic these intermediates in the form of conformationally constrained peptides. Here we used these oligomer mimics and generated the first generic mouse monoclonal anti-oligomer conformation specific antibody that we called F11. This antibody does not recognize monomer or fibrillar structures. Therefore, it is a valuable reagent to investigate the benefits of specific oligomer clearance in mouse models of AD by passive immunization. We used this novel antibody and evaluated the benefits of Aβ oligomer modulation in fourteen month old tg2576 transgenic mice that express full length amyloid precursor protein with Swedish point mutations. Three days prior to the injection, tg2576 mice were tested using a novel object recognition (NOR) test, then vaccinated intravenous (two weeks apart) with 30 μg of F11 antibody; control mice received injections with control antibody. Behavioral analysis showed significantly improved performance on the NOR test after immunization. Immunohistochemical and biochemical analyses on the brains extracted from these animals one week after the second injection showed a reduction of amyloid oligomers in the animals immunized with F11.
The benefits of oligomer clearance by passive immunization validate oligomers as a potential target for the development of disease-modifying immunotherapy to treat AD and other conformational diseases.

Disclosures: M.J. Guerrero: None. **D.L. Castillo-Carranza:** None. **C.A. Lasagna-Reeves:** None. **S. Krishnamurthy:** None. **K. Dineley:** None. **G.R. Jackson:** None. **R. Kayed:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NINDS 1R01NS064879

Wooten Foundation Grant

Title: Geniposide improves spatial learning, inhibits tau phosphorylation, and decreases apoptosis in streptozotocin-induced Alzheimer disease model

Authors: C. GAO[1], Y. Z. LIU[2], *J. M. DING[3], L. LI[1]
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Abstract: Alzheimer's disease (AD) is a debilitating neurodegenerative disorder without effective treatment. Despite intensive studies, the exact pathogenesis of AD remains unknown. However, recent studies suggest that intra-cerebral-ventricular (icv) injection of streptozotocin (STZ) can induce an insulin resistant brain state that may underlie the neural pathogenesis of sporadic AD. Systemic administration of STZ induces pancreatic β cell dysfunction; whereas STZ-icv impairs glucose metabolism and redox function in the brain, resulting in learning and memory deficit accompanied with elevated amyloid protein and tau protein phosphorylation that are characteristic of AD pathology. This led us to attempt to treat AD with therapeutic agents that are known to ameliorate diabetes. Our recent work demonstrated that prior treatment with Val^8-glucagon-like peptide-1 (GLP-1) could prevent STZ-induced learning impairment and tau phosphorylation in the rat brain (li *et al* 2012).
The Chinese herbal medicine genipin is known to relieve symptoms of type-2 diabetes. Since genipin is thought to act as a GLP-1 receptor agonist, we investigated the potential therapeutic effect of geniposide on STZ-induced AD rats. Our result, using sprague dawley rats (200 gram), showed that a single injection of geniposide (50 μM, 10 μ l) to the lateral ventricle prevented STZ-induced spatial learning deficit by about 40% and reduced tau phosphorylation by about 30% with Morris water maze test and immunocytochemical analysis, respectively. Hyperphosphorylation of tau protein plays an important role in the neurofibrillary tangle development, one of the distinctive AD pathological hallmarks in the postmortem brain. Tau

protein is phosphorylated by a host of kinases, including glycogen synthase kinase-3 (GSK3), a serine or threonine kinase. STZ is known to increase the activity of GSK3β. Our result with western blot analysis showed that geniposide treatment prevented STZ-induced GSK3β hyperactivity. In addition, ultrastructure analysis showed that geniposide decreased the presence of a unique electron density that is thought to be an indicator for early apoptosis.
There are 5 FDA-approved medicines currently used for the treatment of AD, tacrine, rivastigmine, galantamine, donepezil, and memantine, but none provide any indication for halting or delaying the progression of the disease. Our study with the water soluble and orally active monomer of Chinese herbal medicine genipin may lead to the development of a potentially novel therapeutic agent for the treatment of both sporadic and familial AD.

Disclosures: C. Gao: None. **Y.Z. Liu:** None. **J.M. Ding:** Employment; Wooten Laboratory. Research Grant; 5R01NS064879-03. Other Research Support; Wooten Foundation Grant. **L. Li:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RO1 AG020670

RO1 NS076117

The Belfer Neurodegeneration Research Consortium

Title: Modulating cellular clearance as a potential treatment for Alzheimer’s disease

Authors: *V. A. POLITO, L. HONGMEI, B. WANG, A. BALLABIO, H. ZHENG
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Abstract: Cellular clearance is a fundamental process required by all cells in all species. Important physiological processes, such as aging, and pathological mechanisms, such as neurodegeneration, are strictly dependent on cellular clearance. In eukaryotes, most of the cellular clearing processes occur in a specialized organelle, the lysosome. This project is based on a recent discovery, of TFEB (Transcription Factor EB) gene, that is a master gene for lysosomal biogenesis that coordinates expression of lysosomal genes and genes involved in autophagy; this gene participates in macromolecules clearance, moreover lysosomal

enhancement as a cellular response to pathogenic accumulation has been observed in neurodegenerative disease. The specific goal of the project is the evaluation of the potential therapeutic effect of TFEB on Alzheimer's disease pathology; to address this question an AAV-mediated gene therapy approach has been used.
A mouse model for Aβ accumulation and plaque formation (5XFAD) and a mouse model for NFT formation (Tg4510) have been used. Groups of mice from both mouse models were treated at p0 and adult age with AAV2/9CMVTcFEB-HA viral vector and analyzed after 4 months. Our preliminary data show that TFEB has no therapeutics effect of Aβ pathology. TFEB has a potential therapeutic effect on NFT pathology and a specific effect on pTAU reflecting a decrease in the accumulation of NFT; moreover there was an improvement in the learning process as we observed through the Morris-water maze test.

Disclosures: V.A. Polito: None. **L. Hongmei:** None. **B. Wang:** None. **A. Ballabio:** None. **H. Zheng:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Bundy Foundation

Title: Stimulating endogenous neurogenesis as a mechanism for functional repair in degenerating brain.

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Abstract: Decreased levels of neurogenesis occur with age and perhaps in neurodegenerative conditions. Drugs that halt the degenerating process still leave the brain with dramatically

reduced neuron numbers and damaged neural circuits. Transplanting stem cells may not be successful because the specialized microenvironment of the neurogenic niche may be toxic in diseased brain so that neural precursor cells (NPCs) may not survive to become mature neurons. Therefore it is necessary to both increase nerve cell numbers and create an environment that is neuroprotective and promotes nerve cell differentiation. This situation presents a unique opportunity for CNS drug discovery in which the therapeutic stimulation of neurogenesis might contribute to functional 'repair' of the degenerating brain by increasing levels of proliferative and differentiation signals, while at the same time protecting newborn cells from a toxic environment. Reduced brain plasticity, loss of memory and reduced neurogenesis could be preserved by manipulating the self-renewal, proliferation, migration, survival and differentiation of NPCs. We have used a novel drug discovery paradigm to select drug candidates for the treatment of neurodegenerative diseases that is based on drug efficacy in cell culture models of multiple, age-associated pathologies, including stimulation of neurogenesis and neuroprotection. This scheme has identified a potent, orally active compound, J147 that facilitates memory in normal rodents, prevents behavioral deficits and synaptic protein loss in Alzheimer’s Disease (AD) transgenic mice, and reverses cognitive loss in aged transgenic AD mice. J147 is also neurogenic in both very old and young mice and reduces the significant loss in dendritic spines that occurs with age. J147 induces progenitor cell division and differentiation of NPCs derived from human ES cells into more nerve-like cells. J147 also directly stimulates endogenous neurogenesis as demonstrated by a neurogenesis ablation experiment. The very potent neurotrophic and memory-enhancing activities of J147 are associated with the induction of BDNF, a growth factor that is reduced with age and in AD brain, that is required for cognitive function, and is implicated in neurogenesis. Results suggest that J147, which has the medicinal chemical properties of a good CNS drug, is a promising compound for promoting neurogenesis in the aged brain and potentially for restoration of neuronal populations in brains recovering from neurodegenerative disease.

Disclosures: M. Prior: None. **P. Maher:** None. **D. Schubert:** None.

Poster

852. Alzheimer's Disease: Therapeutics

Location: Hall F-J

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Program#/Poster#:852.22/E34

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SNSF Grant PBELP2 140643

Title: Locally induced Alzheimer’s disease in 3D microengineered neuronal cell cultures

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Abstract: Aggregations of hyper-phosphorylated tau proteins, called neurofibrillary tangles, are major features in Alzheimer diseased (AD) brains. Tau proteins are responsible for maintaining microtubules structures in axons and dendrites (neurites). Now, when tau gets hyper-phosphorylated, microtubules become unstable, leading into degeneration of the neurite network. The progression of neurofibrillary tangles follows specific patterns in AD brains; although these patterns have been reported, their prediction is unknown. To study mechanism based on hyper-phosphorylated tau in vitro, neuronal cells are commonly exposed to okadaic acid (OA), which inhibits protein phosphatase (P-1 and PP-2A). Today's neuronal cell culture methods, however, lack controlled local cell position, cell micro-environmental architecture and local protein modification. We present, therefore, a novel cell culture method that combines the generation of locally hyper-phosphorylated tau proteins with the formation of controllable 3D micropatterned cell hydrogel layers. Primary neurons were prepared from cerebral cortexes of Wistar rat brains at embryonic day 19 (E19) and gently mixed with a liquid hydrogel solution of agarose / alginate (0.5 % / 0.3 % w/v) at 37 °C. Cell-loaded and cell-free hydrogel solutions were injected into microfluidic devices, which form four laminar 3D hydrogel layers in a main channel. After gelation of hydrogel layers, primary neurons were cultured up to 8 days (8 DIV). Neurons were loaded into the 1st and 4th hydrogel layers, facilitating neurite outgrowth into cell-free hydrogel layers across a distance of 320 μm. Our microfluidic devices allow the formation of molecular gradients through the hydrogel layers. After neurite outgrowth, cell layers were locally exposed to an okadaic acid (OA) gradient of 0.3375 nM/μm for 1h at 37°C. Neural cell activity was determined using Fluo-4 loading and calcium imaging. We quantified aggregates of hyper-phosphorylated tau proteins by immunofluoresecent labelling of pS262 sites and z-stack confocal imaging. Our results showed 7.5 times higher portions of hyper-phosphorylated tau aggregates in the 1st cell layers (high OA concentrations), compared to neurons in the 4th layer (low OA concentrations). Neural cells in the 1st layer, furthermore, showed reduced calcium activity. Further studies using this microfluidic based cell culture method may reveal the underlying impact of the microenvironmental architecture on the propagation of AD in diseased brains.

Disclosures: A. Kunze: Research Grant; Swiss National Science Foundation, Grant # PBELP2 140643. **P. Renaud:** None.

Poster

852. Alzheimer's Disease: Therapeutics

Location: Hall F-J

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Program#/Poster#:852.23/E35

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG37337-1

Title: Characterization of abeta oligomers from alzheimer’s disease brain with braak stage v/vi individuals and their effects on membrane trafficking in hippocampal neurons with cogrx compounds

Authors: *C. REHAK, R. YURKO, N. IZZO, K. MOZZONI, C. SILKY, G. RISHTON, H. SAFFERSTEIN, H. SAFFERSTEIN, M. MILLER, C. JOHANSON, E. STOPA, S. CATALANO
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Abstract: Abeta 1-42 oligomers accumulate in the brains of patients with Mild Cognitive Impairment (MCI) and begin to disrupt the balance of associative and dissociative synaptic plasticity processes that underlie memory formation (Knobloch and Mansury ’08, Selkoe ‘08). Published reports describe biological activity and cognitive deficits induced by several species of Abeta 1-42 oligomers ranging from dimers to high molecular weight assemblies isolated or prepared from a variety of sources (Reed et al.,’11). We isolated Abeta species from hippocampus (N=3) or neocortex (N=5) from Alzheimer’s patients (Braak stage V/VI, <24 hours post-mortem, median age 79) or age-matched histologically normal individuals (Braak Stage I, < 24 hours post-mortem, median age 74) using detergent free conditions and measured relative sizes of Abeta species via non-denaturing Westerns. Tissue was homogenized in 1:7 (w/v) in detergent-free Tris-HCl buffer, ultra centrifuged then immunoprecipitated with high salt elution (Pierce Gentle Elution Buffer), then separated by Amicon 100kDa and 10kDa MWCO spin filters. The human hippocampal (N=3 AD, 3 normal) and cortical (N=5 AD, 5 normal) derived oligomers were visualized by non-denaturing Western blot and resulted in a wide range of oligomer sizes that varied substantially between individuals and tissue location. Neocortical Abeta species consisted of a continuous band from 100-250k, and well defined bands corresponding to monomer and dimer. The higher molecular weight band was seen in both AD and normal individuals but was 30.5% more intense in AD patients. Hippocampal Abeta species were more heterogeneous both between all individuals and between AD and normals as a group, and consisted of well-defined bands at 75-85 kDa and a higher molecular weight band centering on 250kDa. There were lower concentrations of monomer (AD, -34%, normal -45%) and dimer in hippocampal (AD, -20%, normal -34%) samples than in neocortical samples. All human oligomer species were potently bio-active on DIV 21 hippocampal neurons inhibiting membrane trafficking with an EC50 of 3.5 pM following only one hour of exposure. This supports previous findings that several Abeta species exhibit biological activity in memory-relevant contexts.

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Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG20206

Title: Therapeutic potential of endogenous cholesterol metabolites in alzheimer's disease.

Authors: J. JUNG[1], T. B. LADD[1], A. R. PRICE[1], B. D. MOORE[1], Y. TANG[2], G. SHAPIRO[3], G. HOCHHAUS[2], T. E. GOLDE[1], *K. M. FELSENSTEIN[1]
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Abstract: $A\beta_{42}$ accumulation is a key etiological trigger for Alzheimer's disease (AD) and its reduction remains a promising therapeutic target. The rate limiting enzymatic step in the formation of Aβ is catalyzed by gamma(γ)-secretase and its modulation is a target for therapeutic intervention. Small molecules, that selectively lower $A\beta_{42}$ have been identified and are referred to collectively as γ-secretase modulators (GSMs). Although GSMs appear to be mechanistically safe 'anti-amyloid' compounds, many potent GSMs suffer from non-mechanistic liabilities that limit their potential usefulness in clinical trials. Discovery of new GSM chemotypes without these liabilities would be enabling. Recent studies have shown that cholesterol, its oxidized metabolites, and other sterols, have complex effects on the processing of amyloid precursor protein (APP) to Aβ. However, little is known about numerous other endogenous cholesterol metabolites and their effects on Aβ production; therefore, we explored whether these metabolites could modulate Aβ production. We tested a collection (~200) of acidic oxysterols and related non-acidic oxysterols for their ability to alter $A\beta_{42}$ levels. We identified several endogenous oxysterol metabolites as potent GSMs with EC50s of ~200nM- 1μM for lowering $A\beta_{42}$. These compounds had the classic signature of GSMs; they not only lower $A\beta_{42}$ but increase shorter Aβ peptides, and are active in broken cell assays. Several of these oxysterols are produced in the brain; therefore, studies are currently in progress to establish whether these are endogenous modulators of $A\beta_{42}$ formation, by examining Aβ production in CYP knockout mice that regulate metabolism of these oxysterols. In addition we are currently testing whether exogenous administration of these sterols and/or novel analogs can selectively lower brain $A\beta_{42}$ levels. The data suggests that acidic oxysterols may function as endogenous GSMs and these sterols or synthetic analogs could be potential therapeutic candidates for the treatment or prevention of AD.

Disclosures: J. Jung: None. **T.B. Ladd:** None. **A.R. Price:** None. **B.D. Moore:** None. **Y. Tang:** None. **G. Shapiro:** None. **G. Hochhaus:** None. **T.E. Golde:** None. **K.M. Felsenstein:** None.

Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH Grant R01 NS36126

NIH Grant P01 NS31492

NIH Grant 2R01 NS034239

NIH Grant P20 RR15635

Title: Multimodal Neuroprotective Strategies for Alzheimer’s disease

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Abstract: The aggregation of amyloid-β (Aβ) affects glial activation and neurotoxic responses during Alzheimer’s disease (AD) is linked, in measure, to production of pro-inflammatory factors and reactive oxygen species (ROS) influencing neurodestructive activities. We posit that improved therapeutic outcomes can be achieved through halting multiple stages of disease pathobiology (combination targeting) and at the level of protein aggregation, inflammation and neuronal functions. To this end we analyzed combinations of antioxidants (catalase) to effect Aβ aggregation and oxidative stress; A2a, a novel mixed-lineage kinase 3 (MLK-3) inhibitor with potent anti-inflammatory antioxidant activities; and noncompetitive N-methyl-D-aspartate

(NMDA) receptor antagonist that effect GluN2A, 2B and 2D and neuronal excitotoxicity. The overarching goal is to inhibit excitotoxic neuronal injury and Aβ aggregation to improve therapeutic neuroprotection. First, catalase alone or captured in a nanoformulation inhibited high molecular weight aggregates or Aβ oligomers protected against neuronal cell death. Second, catalase decreased nitric oxide synthase 2 (NOS2) in expression in BV-2 microglial cells after interferon-γ and Aβ oligomer stimulation. Third, parallel results were observed with our MLK-3 inhibitor or A2a was observed in regards to inhibition of neuronal demise. Combined treatment with catalase and the MLK-3 inhibitor in BV-2 microglial cells showed significant inhibition of NOS2 expression beyond the effects of each of the drugs or proteins alone. These results suggest a more than additive effect of both catalase and MLK-3 inhibitor as a therapeutic strategy for AD. The role of NMDA receptor antagonists in these drug mixtures remains underway.

Disclosures: G. Zhang: None. **E.V. Batrakova:** None. **H.A. Gelbard:** None. **C.L. Loweth:** None. **V.S. Goodfellow:** None. **T. Kiyota:** None. **H.E. Gendelman:** None.

Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Research Foundation 2009-0081673

Korean National Institute of Health R&D Program Project (A092058)

MRC at SNU (2011-0030738)

Title: HDAC6 inhibitor recovers amyloid beta-induced impairment of mitochondrial transport in hippocampal neurons

Authors: *H. CHOI[1], C. KIM[1], C. JANG[1], S. OH[2], N. JEON[2], I. MOOK-JUNG[1]
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Abstract: The axonal transport of vesicles or organelles, which occurs along microtubules, is important for neuronal functions and survival. α-and β-tubulin, which make up the microtubules, are acetylated. The acetylated level of alpha tubulin is related to axonal transport. The disruption of axonal transport, especially mitochondrial transport, has been shown to be a factor in neurodegenerative diseases like Alzheimer’s disease (AD) and Huntington’s disease. Histone

deacetylase 6 (HDAC6), which can deacetylase α-tubulin, is also known to be increased in AD patients. Therefore, we examined how the acetylation of α-tubulin in the presence of Aβ affects axonal transport, including mitochondrial transport. We found Tubastatin A (TBA), an HDAC6 inhibitor, significantly restored Aβ-induced reduction of axonal transport, including the velocity and motility of mitochondria. Also, TBA enhanced the elongation of Aβ-induced fissioned mitochondria, implying the inhibition of HDAC6 may play a role in recovering impaired mitochondria from Aβ toxicity. In addition, the brains of 5XFAD mice, AD animal model, showed a reduction of acetylated α-tubulin. This study revealed that HDAC6 is important in the axonal transport of mitochondria. HDAC6 inhibition was shown to elevate acetylated α-tubulin levels, resulting in improved mitochondrial transport, as well as the elongation of the mitochondria that had been damaged by Aβ. As reduced axonal transport and increased mitochondrial fission are closely related to AD pathogenesis, occurring before the appearance of senile plaque, the inhibition of HDAC6 could be a candidate for therapeutic target for AD, especially in the early stage.

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Poster

852. Alzheimer's Disease: Therapeutics

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Program#/Poster#:852.27/E39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DGAPA IA201511

CONACyT 151261

Alzheimer’s Association NIRG-11-205443

Title: Amyloid beta inhibits entorhinal cortex activity through the activation of GSK-3

Authors: B. ORDAZ, A. SOLIS-CISNEROS, H. BALLEZA-TAPIA, J. LOPEZ-GUERRERO, *F. PENA
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Abstract: The cognitive decline observed in Alzheimer Disease has been related to amyloid beta-induced disturbances in several oscillatory patterns generated in the forebrain, specially in

the hippocampus. However, little is known about the alterations in the entorhinal cortex produced by the amyloid beta peptide. Thus, we tested the effects of the acute application of amyloid beta on the network activity of the entorhinal cortex slices in vitro. Moreover, we also explored the biochemical mechanisms involved in this effect. Our results show that bath application of amyloid beta (10 nM) activates the glycogen synthase kinase 3 (GSK-3) and reduces the network activity in the entorhinal cortex. The reduction network activity by amyloid beta is blocked by inhibiting GSK-3 either with lithium or with SB 216763. Our results suggest that amyloid beta-induced inhibition of entorhinal cortex network activity involves GSK-3 activation, which may provide a molecular mechanism for amyloid beta-induced neural network disruption and support the use of GSK-3 inhibitors to treat Alzheimer Disease.

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Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CREST, JST

Seeds Innovation, JST

Title: Substrate-specific dual inhibition of β- and γ-secretases by peptides binding to C99

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Abstract: γ-Secretase is a promiscuous aspartic protease that cleaves transmembrane domain of type I membrane proteins, suggesting that inhibition of γ-secretase activity to suppress Aβ production causes harmful side effects. Thus substrate-specific inhibition of γ-secretase still remains as an unsolved important issue to overcome Alzheimer’s disease. We previously found that a subset of anti-Aβ antibodies raised against N-terminus of human Aβ significantly inhibited Aβ production without interfering Notch S3 cleavage in CHAPSO solubilized γ-secretase assay and that those antibodies also suppressed β-cleavage of human APP-derived fragment in vitro β-secretase assay. Our results indicate that N-terminus of carboxyl terminal fragment of β-amyloid

precursor protein (C99) is a potential target for substrate-specific dual inhibition of β- and γ-cleavages. In this study, we selected a subset of Cys-rich peptides binding to N-terminus of C99 out of our peptide library generated by RAPID display system. Those peptides exhibited inhibition of Aβ production from the C99 substrate and negligible effect on Notch S3 cleavage in the γ-secretase assay system. In addition, those peptides inhibited β-cleavage of APP-derived fragment (633-685, numbering from APP751) without interfering cleavage of sialyltransferase 1 (st6gal1), one of β-secretase substrates, in the β-secretase assay system. To test whether those peptides suppress Aβ production even in cell-based assay, we treated the CHO cells overexpressing human APP, Notch and st6gal1 with each peptide. Interestingly, all of those peptides exhibited significant reduction of sAPPβ, C99 and Aβ productions, but no effect on st6gal1 and Notch S3 cleavages. Importantly, peptides with reverse sequence showed no influence on sAPPβ and Aβ productions and cleavages of st6gal and Notch. Our results strongly suggest that N-terminus of C99 substrate is a novel potential target for substrate-specific dual inhibition of β- and γ-cleavages and that generating small molecules that specifically bind to N-terminus of C99 would be a novel strategy for substrate-specific dual inhibition of β- and γ-secretases without affecting proteolysis of other substrates for anti-AD approaches.

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Poster

852. Alzheimer's Disease: Therapeutics

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: COBRE grant 2P20RR17699 from NCRR, a component of NIH

Title: Caffeine blocks neuronal uptake of LDL-cholesterol

Authors: *X. CHEN, S. LI, L. HUI, N. H. GEIGER, J. D. GEIGER
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Abstract: Elevated circulating LDL-cholesterol, as an extrinsic factor, has been robustly linked to the pathogenesis of sporadic Alzheimer’s disease (AD), the major form (95%) of AD for which no effective treatment is available. Our recent in vivo and in vitro studies demonstrated that circulating LDL-cholesterol enters brain parenchyma via a leaky blood-brain barrier and is internalized by neurons. Such up-taken LDL-cholesterol appear to contribute directly to the pathogenesis of AD by promoting amyloid precursor protein (APP) internalization and

disrupting neuronal endolysosome function, both of which contribute to overproduction of amyloid beta (Aβ), one of the major pathological factor in AD. Thus, blocking neuronal uptake of LDL-cholesterol could provide a novel therapeutic strategy against AD. Recent epidemiological and experimental data suggest that caffeine is protective against AD, but by poorly understood mechanisms. Here, we tested the hypothesis that caffeine blocks neuronal uptake of LDL-cholesterol via its actions on adenosine receptors. Using receptor-mediated endocytosis assays, immunoblotting, immunostaining, ELISA, and RNA interference methods we found in primary culture neurons that caffeine pretreatment concentration-dependently blocked LDL-cholesterol internalization. Using a pharmacological approach, we demonstrated that only adenosine A3 receptor antagonists mimicked caffeine's effects on neuronal uptake of LDL-cholesterol. Furthermore, knocking down adenosine A3 receptors with siRNA blocked LDL-cholesterol internalization. In addition, caffeine pretreatment increased surface protein levels of both LDL receptors and APP. Moreover, caffeine attenuated LDL-cholesterol induced overproduction of Aβ. Our findings suggest that caffeine exerts its protective effects against AD by blocking neuronal uptake of LDL-cholesterol, thus attenuating APP internalization and decreasing Aβ generation.

Disclosures: X. Chen: None. **S. Li:** None. **L. Hui:** None. **N.H. Geiger:** None. **J.D. Geiger:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Neuroplasticity of Aging training grant

LLHF

DSRTF

Title: Gamma-secretase inhibitors, but not gamma-secretase modulators, alter brain-derived neurotrophic factor trafficking and signaling

Authors: *A. M. WEISSMILLER[1], M. PEARN[1], P. NGUYEN[1], R. E. TANZI[2], S. L. WAGNER[1], W. C. MOBLEY[1], C. WU[1]
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Abstract: The amyloid theory of Alzheimer's disease (AD) proposes that Aβ deposit leads to neuronal dysfunction and overall toxicity. An attractive strategy for AD treatment is to suppress Aβ production by targeting the secretases that cleave the amyloid precursor protein (APP) to give rise to Aβ. As such, gamma-secretase inhibitors (GSIs) have been developed to treat AD. However, clinical trials with GSIs had to be discontinued due to the worsening conditions of patients and significant undesired side effects, which may be due to excessive accumulation of the C-terminal fragments of APP, Notch and many other gamma-secretase substrates. In contrast to GSIs, gamma-secretase modulators (GSMs) modulate the processitivity of gamma secretase to decrease Aβ40/42 production, while increasing the smaller Aβ38 form. Furthermore, GSMs do not appear to cause accumulation of APP-CTFs and CTFs of other gamma secretase substrates. While many studies have documented the ability of both GSIs and GSMs to function in lowering Aβ levels, nearly any evidence exists on the ability of these compounds to improve neuronal functioning at the *in vitro* level. To investigate the effect of GSIs versus GSMs on neuronal functions at molecular and cellular level, we used two structurally different GSMs (NGX 97555 and sGSM41) and one GSI (BMS-299897) to treat both mixed primary cortical and hippocampal neurons. Specifically, we assessed if and how signaling and axonal trafficking of BDNF was impacted in GSI- versus GSM-treated neurons. We found that GSI treatment of rat E18 hippocampal neurons cultured in microfluidic compartmentalized chambers resulted in a 60% decrease in retrograde transport velocity of quantum-dot conjugated BDNF and a 25% increase in anterograde movement, indicating trafficking of neurotrophic signals is significantly impaired. We further show that in GSI-, but not GSM-treated mixed cortical cultures activation of ERK1/2 by BDNF is selectively inhibited. Finally we demonstrate that upon GSI treatment, Rab7-marked late endosomes were translocated from soma to processes, an event that may lead to premature inactivation of the BDNF signal. Using the two structurally different GSMs we show that they differ slightly in their effect on normal neuronal function in that sGSM41-treated neurons function similar to vehicle, while NGX 97555 shows slower retrograde transport velocity, normal overall signaling, and little or no impairment of the endocytic pathway. Our results thus provide evidence that GSMs may make a more optimal choice in anti-amyloid based drug therapies and offer a possible mechanism for the deleterious effects of GSIs on both human and mouse cognition.

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Poster

853. Alzheimer's Disease: Abeta and Tau

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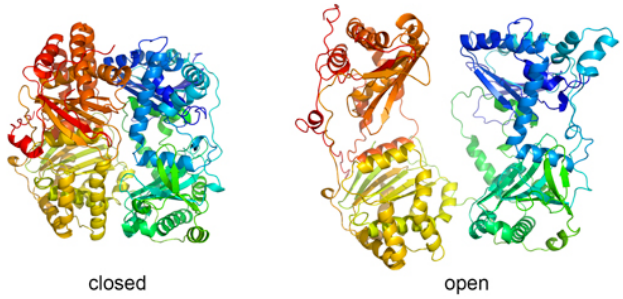
Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Mayo Foundation Grant ML01

Title: Molecular dynamics modeling of IDE reveals striking conformational changes involved in substrate binding and catalysis

Authors: T. R. CAULFIELD, *S. G. YOUNKIN, M. A. LEISSRING
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Abstract: Insulin-degrading enzyme (IDE) has long been known to be the principal protease responsible for inactivating insulin and also avidly degrades the amyloid ß-protein (Aß) that accumulates abnormally in Alzheimer disease (AD) . IDE has a bipartite active site formed from portions of the N- and C-terminal halves of the protease (IDE-N and IDE-C). The two halves are bowl-shaped and are connected by a flexible linker, allowing them to adopt “open” and “closed” conformations. When in the closed state, IDE encapsulates a large, 13,000-cubic Angstrom internal chamber. Numerous crystal structures of human IDE have been solved, but all of these have captured the protease in the closed conformation. The crystal structure of a bactierial homolog of IDE, pitrilysin, captured the protease in only a partially open conformation. Here, we present the full transition from closed to open state in a catalytically active form of human IDE containing insulin within its internal chamber. Utilizing molecular dynamics (MD), we have investigated how IDE transitions from the closed to the open state over a time interval of 250 ns. We have also shown that the binding a novel inhibitor we developed, Ii1, acts by maintaining the protease in the closed conformation. The combined MD studies are >1 microsecond and confirm the effect of having an apo IDE enzyme versus substrate-bound enzyme versus substrate-bound enzyme plus inhibitor. Our open conformation for IDE reveals that the open state exhibits a unique tetra-domain architecture, resembling the opening of a flower. IDE-N and IDE-C first separate, then each half separates further into a total of four subdomains. The end result reveals a possible mode for binding substrates quickly and efficiently. In on-going studies, we are now probing the conformational dynamics of various mutants, including the heterodimeric form of human IDE.



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Poster

853. Alzheimer's Disease: Abeta and Tau

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Support: National Institutes of Health/NIA (K01 AG029524 and P01 NS074969 DMH/JRC)

McDonnell Center for Cell and Molecular Biology (JRC)

Charles F. and Joanne Knight ADRC at Washington University (JRC)

Title: Measuring brain interstitial fluid Apolipoprotein E using *in vivo* microdialysis "MegaProbes"

Authors: *J. L. RESTIVO, D. K. VERGES, W. D. GOEBEL, J. M. BURCHETT, D. M. HOLTZMAN, J. R. CIRRITO
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Abstract: Apolipoprotein E (apoE) is a significant risk factor for late-onset Alzheimer's disease, altering clearance of the amyloid-β peptide that forms plaques in the disease, as well as altering the plaque's tertiary structure. ApoE's normal role is to shuttle cholesterol between cells in the periphery and in the brain. Interestingly, apoE found within the CNS is typically generated within that compartment as opposed to coming from the periphery. In vivo the apoE protein is rarely found in its free 35 kiloDalton form; instead it is typically associated to lipids which make the lipoprotein, HDL-like particles have a hydrated diameter in the 6-20 nanometer range. ApoE particles are found within the cerebrospinal fluid, though they appear to be produced within the brain parenchyma and diffuse through the brain interstitial fluid (ISF) to reach that compartment. Understanding apoE dynamics and metabolism over time within the brain may be important for understanding cholesterol transport within the brain, as well as apoE's role in neurodegenerative diseases such as Alzheimer's disease. Small molecules, such as neurotransmitters and small peptides, within the brain ISF can be measured using in vivo microdialysis. Large proteins, however, are typically not amenable to this technology due to steric hindrance which prevents large molecules from readily diffusing across a semi-permeable membrane to enter the microdialysis probe. We have optimized conditions to use microdialysis probes with a 1,000 kiloDalton (1 megaDalton) semi-permeable membrane to recover ISF apoE within the brains of wildtype and APP/PS1 transgenic mice (AtmosLM probes, Eicom, USA). These probes utilize a unique pressure-equalizing system to reduce pressure that typically forms when using large-membrane microdialysis probes. The inlet into the probe is essentially open to atmosphere while the dialysis is run using a peristaltic pump that "pulls" artificial CSF through the

probe/membrane. ISF murine apoE is detectable every hour over several days via ELISA to measure levels as well as using native non-denaturing gel electrophoresis to determine lipid particle size.

Disclosures: J.L. Restivo: None. **D.K. Verges:** None. **W.D. Goebel:** None. **J.M. Burchett:** None. **D.M. Holtzman:** None. **J.R. Cirrito:** Employment; Washington University. Research Grant; National Institutes of Health/NIA.

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:853.04/E45

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA AG031764

Title: Beta-amyloid immunization with behavioral enrichment in a canine model of aging: Antibody titers and CSF Beta-amyloid

Authors: P. R. DAVIS[1], *A. L. S. DOWLING[1], G. GIANNINI[2], X. WANG[1], T. BECKETT[1], T. PLATT[1], K. RUDOLPH[2], C. ROYER[2], D. PAGANI[3], F. BRESCH[4], M. P. MURPHY[1], E. G. BARRETT[2], E. HEAD[1]
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Abstract: Alzheimer’s disease (AD) is characterized by the cognitive decline of an individual and the presence of hallmark pathology, including senile plaques (SP) and neurofibrillary tangles. SPs contain β-amyloid protein (Aβ), resulting from cleavage of the amyloid precursor protein (APP). A number of therapeutic strategies being developed today are focused on reducing the production or deposition of Aβ in the brains of patients with AD. The canine model is useful for testing potential therapeutic agents. Canines produce APP that has 98% homology with human APP. In addition, canines naturally develop Aβ neuropathology and show cognitive decline with age, similar to AD patients. Recent studies have shown that active immunization for 2 years with fibrillar Aβ1-42 (IMM) in aged canines significantly decreased brain Aβ and maintained executive function, while other measures of cognition remained unchanged (Head et al., 2008). However, behavioral enrichment (BEH) improves cognition (Milgram et al 2005) without reducing brain Aβ (Pop et al., 2010). We hypothesized that IMM combined with BEH would provide larger cognitive benefits and reduce neuropathology to a greater extent, as

compared to either controls or individual IMM and BEH treatments alone.Aged beagles (10.5-13.6 y) were placed into one of four groups: controls with Alum adjuvant only, fibrillar Aβ1-42 + Alum vaccine, BEH with Alum, and combination treatment (IMM+BEH). Dogs receiving BEH were housed with a kennel mate, had novel play toys each week, and were taken for a 20 minute walk outdoors three times a week. At baseline, animals were given a series of learning tasks and a spatial memory task and matched into equivalent groups on the basis of cognitive test scores. Animals were treated for over 15 months. Fibrillar Aβ1-42 antibody titers in serum and Aβ levels cerebral spinal fluid were measured by ELISA. When compared to non-IMM groups over the first 6 months of the study, anti-Aβ1-42 IgG responses in IMM groups increased significantly ($F(2,64)=3.24$ $p=0.046$) and were maintained. No systematic effects on CSF Aβ1-42, Aβ1-40, or total Aβ have been observed in response to 12 months of treatment. On a test of spatial attention (landmark task), BEH dogs performed significantly better than IMM dogs ($t(16)=2.7$ $p=0.016$). Additional measures of cognition are being collected. Upon completion of the study, changes in brain Aβ, extent of neurogenesis, and growth factor levels will be measured using immunohistochemistry and western blot analysis.

Disclosures: P.R. Davis: None. **A.L.S. Dowling:** None. **G. Giannini:** None. **X. Wang:** None. **T. Beckett:** None. **T. Platt:** None. **K. Rudolph:** None. **C. Royer:** None. **D. Pagani:** None. **F. Bresch:** None. **M.P. Murphy:** None. **E.G. Barrett:** None. **E. Head:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:853.05/E46

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant from the National Center for Research Resources (5P20RR017699, Centers of Biomedical Research Excellence).

Title: Role of adenosine A1 receptor in cholesterol-induced Alzheimer’s disease like pathology

Authors: *J. P. RANTHAM PRABHAKARA[1], G. FEIST[1], M. SCHRAG[2], J. GEIGER[1], O. GHRIBI[1]
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Abstract: Objective: Hypercholesterolemia is a risk factor for Alzheimer’s disease (AD) by increasing the production of β-amyloid (Aβ), accumulation of which is deleterious to neurons. However, the mechanisms by which hypercholesterolemia cause Aβ accumulation and

neurodegeneration are not well known. Data from our laboratory has demonstrated that feeding rabbit with a diet rich in cholesterol leads to Aβ accumulation and down regulation of the adenosine A1 receptor (A1R). However, the extent to which down-regulation of A1R contributes to AD pathology is not well known. In the present study we have incubated organotypic slices from hippocampus of adult rabbit brains with agonist and antagonist of A1R in the presence of Aβ25-35 and 27-hydroxycholesterol (27-OHC), a cholesterol metabolite that increases Aβ production.
Methods: Organotypic slices were treated with 1μM of the A1R agonist 2-Chloro-N6-cyclopentyladenosine (CCPA) or 1μM of the A1R antagonist 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) in the presence or absence of 10μM Aβ25-35 or 25μM 27-OHC. After 72 hours of treatment, samples were harvested for Western blot and confocal microscopy. The media was collected and supplemented with protease and phosphatase inhibitor and used for quantification of Aβ by ELISA.
Results: We have found that Aβ25-35 and 27-OHC increase Aβ levels. Aβ25-35 and 27-OHC also increase levels of amyloid precursor protein (APP) and β-secretase (BACE-1) and decreased the levels of soluble amyloid precursor protein (sAPPα). Aβ25-35 and 27-OHC also increased phosphorylated tau, endoplasmic reticulum (ER) stress, inflammation, reactive oxygen species and the pro-apoptotic Bax levels. Treatment with the A1R receptor agonist reversed the Aβ25-35 and 27-OHC-induced AD pathology. Treatment with the A1R antagonist did not affect Aβ25-35 and 27-OHC -induced AD pathology.
Conclusion: Our results suggest that agonist of A1R may protect against AD like pathology.

Disclosures: J.P. Rantham Prabhakara: None. **G. Feist:** None. **M. Schrag:** None. **J. Geiger:** None. **O. Ghribi:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:853.06/E47

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NMRC/1184/2008

Title: Effects of Apolipoprotein E in insulin signaling pathway

Authors: M. LIM[1], *F. TSANG[3], B. WONG[2]
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Abstract: Although Apolipoprotein E (apoE) is highly expressed in the brain and liver its functions remain unclear. It is widely known that apoE deficiency causes atherosclerosis and cardiovascular disease (CVD). Patients with type II diabetes mellitus (T2DM) are found to have other complications such as atherosclerosis, CVD and Alzheimer's disease (AD). In recent studies, apoE level is also found to affect the removal of b-amyloid (Ab) in AD mouse models. Therefore, it is interesting to find out how apoE plays a role in the cellular and molecular level. In this study, we choose to examine the effects of apoE on the regulation of insulin signaling by comparing the ApoE knockout (KO) mice to the wild-type (WT) mice brain during aging. We found that in the absence of apoE, it could cause down-regulation of the insulin signaling pathway which leads to several physiological and pathological problems. By probing the main targets of the pathway, we found that KO mice have a lower amount of insulin receptor substrate 1 (IRS1) and the phosphorylation of the serine residues in comparison to WT counterparts. PI3K which is the downstream effector of the IRS is not affected by the upstream activity. Furthermore, the KO mice at 32weeks expressed a higher amount of phosphorylated AKT. GSK3B has a higher activity as there is less phosphorylation at Ser9 in KO mice. These findings provide an insight on how apoE affects insulin signaling pathway. There will be more studies and experiments to be carried out in near future to better understand the pathway especially with respect to conditions like T2DM and AD.

Disclosures: M. Lim: None. **F. Tsang:** None. **B. Wong:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:853.07/E48

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The role of GSK in regulating DNA methylation of genomic DNA in Alzheimer's Disease

Authors: *K.-L. CHEN[1], W.-H. CHEN[2]
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Abstract: Background: Glycogen synthase kinase-3 (Gsk-3) isoforms, Gsk-3alpha and Gsk-3beta, are constitutively active, largely inhibitory kinases involved in signal transduction. Underscoring their biological significance, altered Gsk-3 activity has been implicated in diabetes, Alzheimer disease, schizophrenia, and bipolar disorder.
In previous data, we had proved that that Abeta reduces global DNA methylation whilst increasing neprilysin (NEP) DNA methylation and further suppressing the NEP expression in mRNA and protein levels. Our previous results also support that Abeta induces epigenetic effects, implying that DNA methylation may be part of a vicious cycle involving the reduction in NEP expression along with a resultant increase in Abeta accumulation, and that Abeta may induce global DNA hypo-methylation. Epigenetic changes marked by DNA methylation have been proposed to play a role in age-related disease. This year, Popkie et al. had report that Phosphatidylinositol 3-Kinase (PI3K) Signaling via Glycogen Synthase Kinase-3 (Gsk-3) Regulates DNA Methylation.
Methods: We used the SH-SY5Y cells to study Aβ-GSK and GSK-DNA methylation relationship. On the other hand, the inhibitors of GSK, lithium ion, or GSK interfering RNA were used to reverse GSK and verify the relationship between methylation and GSK pathway.
Result: We had known Abeta-induced DNA methylation can cause brain cells aging and apoptosis. The cells' methylation occurred on Alzheimer's disease have a certain impact. The gene is methylated, the performance decline, indirectly caused by reduced degradation of Aβ, and so a vicious cycle that will result in the accumulation of Aβ and promote the generation of AD. According to the published papers, we proved that Aβ affect DNA methylation is through GSK pathway.
Discussion: Understanding the impact of Abeta physical mechanism of DNA methylation and we used demethylation agents or GSK inhibitors to prevent the accumulation of Abeta in cell line first. In the future, it will further test in mice in order to find new ways to treat AD.

Disclosures: K. Chen: None. **W. Chen:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:853.08/E49

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant AG029481to RD.

Title: Copper induces LRP-1 degradation by interacting with cellular prion.

Authors: *R. DEANE, I. SINGH, T. KASPER, J. CISZEWSKI
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Abstract: Alzheimer's disease (AD) is associated with the deposition of senile plaques containing aggregates of amyloid-β peptide (Aβ) bound to metal ions, such as copper (Cu), zinc, and iron, and other molecules such as cholesterol and apolipoprotein E. Of these metal ions, Cu avidly binds Aβ and facilitates its aggregation and toxicity. The low-density lipoprotein receptor-related protein-1(LRP1) is a receptor for Aβ clearance. LRP1 levels in brain endothelial cells (BEC) are down-regulated with ageing and in AD. However, the mechanism for this down-regulation is unclear. Since brain Cu levels are increased with aging we hypothesized that increased Cu accumulation in BEC caused the reduction in LRP1 levels. Mice (C57BL6) were treated with tracer levels of Cu via their drinking water for 3 months to enhance the accumulation of Cu in brain. In contrast to capillary-depleted brain, Cu concentrations in plasma and brain capillaries were significantly ($p<0.05$) greater in the Cu-dosed mice compared to vehicle treated mice. LRP1 levels in brain microvessels were lower ($p<0.05$) and brain Aβ40 and Aβ42 concentrations were higher in the copper-dosed mice compared to vehicle treatment. Using immunoprecipitation, we show that Cu caused prion-LRP1 interaction, and reduction of LRP1 levels in brain endothelial cells. Therefore, Cu binding to prion may contribute to the specific down-regulation of LRP1 levels in brain endothelial cells. Cu-induced down regulation of LRP1 levels may reduce Aβ clearance leading to the accumulation of potential toxic Aβ species in brain. Supported by AG029481to RD.

Disclosures: R. Deane: None. **I. Singh:** None. **T. Kasper:** None. **J. Ciszewski:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:853.09/E50

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: A model of Alzheimer's disease using human induced pluripotent stem cell derived cortical neuron cultures, astroctyes and microglia: Development, characterization and therapeutic approaches

Authors: *S. WRIGHT, S. HUSSAIN, J. BRIGHT, B. COOPER, E. ROSE, L. NGUYEN, V. DANG, C. RAMOS, U. SHOUKAT-MUMTAZ, J. GARNES, M. GRSKOVIC, J. DIMOS, E. BEATTIE, N. STAGLIANO, I. GRISWOLD-PRENNER, S. IRION
iPierian, S SAN FRAN, CA

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disease and the 6th leading cause of death in the U.S. The histopathological hallmarks of the disease are extracellular amyloid-beta protein (Aβ) plaques and intracellular aggregates of tau protein, with the two acting in concert to destroy neurons, leading to a decline in memory function. Multi-transgenic mouse models are commonly used to mimic human disease and while these models have helped our understanding of AD, they have been less successful in identifying new treatments. The reprogramming of adult fibroblasts to create induced pluripotent stem cells (iPSCs), and the subsequent redifferentiation to cell types of interest, offers a unique opportunity to study human disease using actual patient material. We collected skin fibroblasts from over 50 AD patients and controls, including familial presenilin mutants (PSEN1 and PSEN2) and generated iPSC lines from 18 individuals by retroviral transduction. The predominant cell types affected in, or believed to affect disease, in AD patients are pyramidal cortical neurons, microglia, and astrocytes. Methods have been developed and rigorously substantiated that differentiate iPSC into each of these cell types. Each cell type is identified by staining of specific markers and gene expression, and then assayed for functional endpoints (e.g., electrophysiological activity of neurons, stimulation of cytokine production for astrocytes and microglia). We then compared cortical neurons derived from AD to healthy controls and can recapitulate several features of AD. Familial PSEN1 and PSEN2 AD cortical neurons produce an increased ratio of secreted Aβ42/Aβ40 compared to both healthy and sporadic AD neurons, just as is observed in familial PSEN AD patients. Both familial and sporadic AD neurons show elevated levels of a subset of the AD associated phospho-tau epitopes which are elevated in AD cortex. Interestingly, elevated levels of total tau are also found in both sporadic and familial AD cortical neurons compared to healthy controls. Elevated phospho-tau is reduced through inhibition of Aβ production using BACE or gamma secretase inhibitors. We are using the changes in phospho-tau as a read out of disease state and have developed assays to test compounds for effects on phospho-tau as a therapeutic approach. Importantly, in addition to studying neurons in isolation, iPSC technology allows us to apply a "systems approach" to AD, combining patient derived neurons with patient derived astrocytes and microglia. We have successfully established neuron-glia cocultures and are using these culture systems to study cell non-autonomous pathologies of AD.

Disclosures: S. Wright: Employment; iPierian employee. **S. Hussain:** Employment; iPierian employee. **J. Bright:** Employment; iPierian employee. **B. Cooper:** Employment; iPierian employee. **E. Rose:** Employment; iPierian employee. **L. Nguyen:** Employment; iPierian employee. **V. Dang:** Employment; iPierian employee. **C. Ramos:** Employment; iPierian employee. **U. Shoukat-Mumtaz:** Employment; iPierian employee. **J. Garnes:** Employment; iPierian employee. **M. Grskovic:** Employment; iPierian employee. **J. Dimos:** Employment; iPierian employee. **E. Beattie:** Employment; iPierian employee. **N. Stagliano:** Employment; iPierian employee. **I. Griswold-Prenner:** Employment; iPierian employee. **S. Irion:** Employment; iPierian employee.

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:853.10/E51

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Howard Hughes Medical Institute

Title: Identification of MT-038, a tissue selective LXR modulator that inhibits apoE secretion in human astrocytoma cells

Authors: C. MORDAUNT[1], J. SCHOTT[1], A. VARGAS[1], S. MAITRA[2], *N. V. PATEL[1]
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Abstract: Apolipoprotein-E (apoE) synthesis and secretion by astrocytes has implications for Alzheimer's Disease (AD) by facilitating amyloid-β (Aβ) clearance from the brain. However, the hypofunctional apoE ε4 isoform can accelerate AD pathology in vivo by differentially modulating Aβ clearance. Liver X receptor (LXR) and its obligate heterodimer retinoid X receptor (RXR) are among the best know regulators of apoE in the brain. Thus, these nuclear receptors are considered desirable targets for AD therapeutics. The objective of this study was to identify a novel class of small molecule inhibitors of apoE production in astrocytes. CCF-STTG1 cells (human astrocytoma cell line) were treated with a library of synthetic compounds for 24 hours and medium was analyzed for apoE content by ELISA. Our initial screen uncovered triarylmethylamines as inhibitors of apoE production. One compound of interest, MT-038, reduced extracellular apoE by more than 50% with an IC50 of 2.7 µM. We further characterized the biological effects of MT-038 to identify its mechanism of action. Q-PCR experiments demonstrate that MT-038 effectively inhibits apoE mRNA expression in CCF-STTG1 cells stimulated with LXR ligands. Consistent with an LXR-dependent mechanism, MT-038 also reduced steady-state ATP-Binding Cassette Transporter 1 (ABCA1) mRNA levels by more than 80%. Similar effects were seen in H4 cells (human neuroglioma cell line), but not HepG2 (human liver carcinoma cell line), suggesting that these effects are cell-type specific. Luciferase transcriptional assays showed that MT-038 repressed ABCA1 promoter activity (IC50 = 138 nM) and antagonized promoter activation by synthetic LXR ligands (GW3965 and T1317) and endogenous LXR ligands (22R-hydroxycholesterol and 24S-hydroxycholesterol). Antagonism of LXR ligands was also observed in HEK cells co-transfected with a GAL4-responsive reporter and fusion constructs of the GAL4 DNA-binding domain linked to the ligand binding domain (LBD) of LXRα/β. MT-038 did not show any activity against a panel of other GAL4-nuclear receptor fusion constructs, suggesting its activity is specific to LXR among related nuclear receptors. Furthermore, MT-038 exhibited inverse agonist effects in a TR-FRET

cell-free biochemical assay, as it inhibited the constitutive interaction of LXR LBDs with co-activator peptides. Taken together, our results strongly suggest that MT-038 inhibits apoE production by modulating the activity of LXR, and thus may be an important chemical probe used to study AD based therapeutics.

Disclosures: C. Mordaunt: None. **J. Schott:** None. **A. Vargas:** None. **S. Maitra:** None. **N.V. Patel:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

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Program#/Poster#:853.11/E52

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA #AG037337

ADDF #20100501

Title: Receptor antagonists inhibit abeta oligomer-induced memory deficits.

Authors: *S. M. CATALANO, N. IZZO, C. REHAK, R. YURKO, K. MOZZONI, C. SILKY, G. LOOK, G. RISHTON, H. SAFFERSTEIN
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Abstract: Small molecules that rapidly block the negative effects of Abeta oligomers on the molecular mechanisms of synaptic plasticity underlying memory have the potential to be disease-modifying Alzheimer’s therapeutics. We have discovered first-in-class receptor antagonists that block the toxic effects of Abeta oligomers by binding with high affinity to CNS receptors. These molecules 1) pharmacologically compete with oligomers for access to receptors regulating membrane trafficking rate, 2) eliminate Abeta oligomer-induced abnormal membrane trafficking and synapse loss/regression without affecting normal trafficking or synapse number, 3) prevent Abeta oligomer-induced associative memory failure in an acute AD model (Abeta 1-42 oligomer injection in wild-type mouse brain) as measured by fear conditioning, 4) reverse memory failure in both genders of a transgenic animal model at nanomolar brain concentrations following shorter duration of treatment than previously published therapeutics, and 5) exhibit a high brain/plasma ratio following long term administration and no neurotoxicity or glial toxicity in vitro, and no behavioral toxicity in vivo. The receptor antagonist mechanism of action will allow the design of disease-modifying therapeutic clinical trials based on receptor occupancy at critical receptor binding sites together with cognitive outcome.

Disclosures: **S.M. Catalano:** Employment; Cognition Therapeutics Inc. **N. Izzo:** Employment; Cognition Therapeutics Inc. **C. Rehak:** Employment; Cognition Therapeutics Inc. **R. Yurko:** Employment; Cognition Therapeutics Inc. **K. Mozzoni:** Employment; Cognition Therapeutics Inc. **C. Silky:** Employment; Cognition Therapeutics Inc. **G. Look:** Employment; Cognition Therapeutics Inc. **G. Rishton:** Employment; Cognition Therapeutics Inc. **H. Safferstein:** Employment; Cognition Therapeutics Inc..

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:853.12/E53

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: George M. Leaders endowment

Title: H63D-HFE induces brain atrophy and cognitive impairment by reducing brain cholesterol and synaptic function: implications for Alzheimer Disease

Authors: *F. ALI-RAHMANI[1], S. LEE[2], P. S. GRIGSON[3], E. NEELY[2], C.-L. SCHENGRUND[4], J. R. CONNOR[2]
[2]Neurosurg., [3]Neural and behavioral sciences, [4]Biochem. and Mol. Biol., [1]Pennsylvania State Univ., HERSHEY, PA

Abstract: The H63D variant of the hemochromatosis (HFE) gene, associated with deregulation of iron homeostasis, has been implicated as a risk factor for Alzheimer disease (AD). Apolipoprotein epsilon 4 (APOE4), a known risk factor for AD, is associated with disruption of cholesterol metabolism. Recent studies showed that individuals with both the APOE4 allele and the H63D variant had a much greater risk and earlier onset of AD than individuals carrying either APOE4 allele or H63D variant of HFE alone. Our global hypothesis is that the altered cholesterol metabolism associated with expression of H63D-HFE leads to disruption of cellular cholesterol homeostasis which will ultimately impact fidelity of lipid raft-mediated signaling pathways. We investigated the effect(s) of H63D-HFE on expression of cholesterol, major lipid raft component, using SHSY5Y human neuroblastoma cells transfected to stably express either wild type WT- or H63D-HFE. Total cholesterol content was ~ 50% reduced in cells expressing H63D-HFE with concomitant increase in expression of cholesterol 24S hydroxylase (CYP46A1), an enzyme involved in cholesterol efflux. Depletion of cholesterol by exposing cells to simvastatin significantly decreased survival of H63D-HFE expressing cells compared to WT-HFE cells, suggesting a genotype-dependent response to statins. Consistent with in vitro studies,

H67D (homologous to human H63D) knock-in mice, showed a genotype and age dependent decline in brain cholesterol and changes in the expression of proteins regulating cholesterol metabolism. A significant reduction in cholesterol, decrease in brain synaptophysin and increase in caspase-3 were observed in the brains of 18 and 24 month old homozygous H67D mice. Performance on learning and memory tasks (novel object recognition and Barnes maze) revealed memory deficits in H67D-HFE mice relative to WT-HFE mice. Magnetic resonance imaging of mice brains at the age of 9 and 24 months showed reduction of brain volume in H67D-HFE mice. Combined these data indicate that H63D variant induces alterations in the expression of proteins involved in cholesterol efflux resulting in excessive loss of brain cholesterol and proteins regulating synaptic transmission, eventually causing neuronal loss, brain atrophy, and memory impairment. Further lowering of cholesterol via treatment with cholesterol lowering drugs can have detrimental effects for H63D carriers. Therefore, these data support the hypothesis that expression of H63D-HFE establishes a permissive cellular milieu for pathogenic processes, contributes to the disease process in AD by altering iron and cholesterol metabolism and impacts treatment response.

Disclosures: F. Ali-Rahmani: None. **S. Lee:** None. **P.S. Grigson:** None. **E. Neely:** None. **C. Schengrund:** None. **J.R. Connor:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

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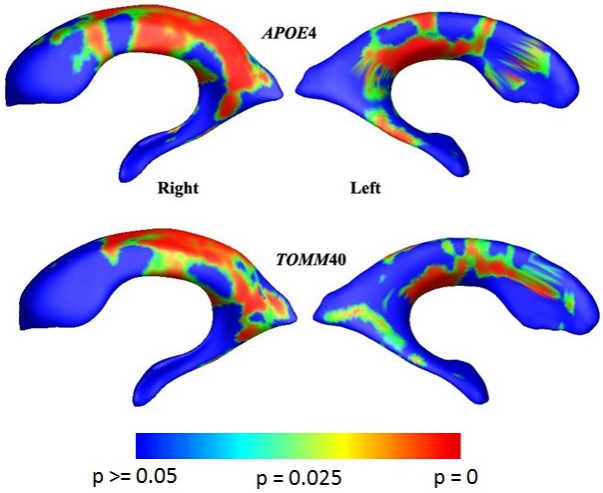
Dana Foundation

Title: Alzheimer’s risk genotypes in the APOE and TOMM40 genes are associated with faster ventricular expansion rates: An N=644 ADNI study

Authors: *B. GUTMAN[1], P. RAJAGOPALAN[2], A. TOGA[2], C. JACK[4], M. WEINER[5], P. THOMPSON[3]
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Abstract: Introduction
The epsilon 4 allele of the apolipoprotein E gene (APOE4) is a well-established risk factor for Alzheimer's disease (AD). The 'G' allele of the SNP rs2075650, located in intron 2 of the *TOMM40* gene, is also a more recently discovered risk factor for AD. *TOMM40* lies close to the APOE gene on chromosome 19 and the two alleles are in linkage disequilibrium (they are correlated). Both alleles are associated with greater brain atrophy in carriers, specifically temporal lobe atrophy and ventricular expansion. However, so far, it is unknown how *APOE4* and *TOMM40* SNPs influence *rates* of ventricular expansion. This was tested in our study.
Methods
We used 644 of the 738 Caucasian subjects (187 controls, 317 with mild cognitive impairment, 187 with AD; mean age: 75·4±6·8 years) who received a brain MRI at 1.5 Tesla at both baseline and 1-year follow-up and were genotyped as part of the ADNI cohort. All 644 subjects had *APOE*4 genotype data; of these, 616 had rs2075650 genotype data. The following multiple regression model was fitted at each point on the ventriclular surface:
$y = \beta_0 + \beta_{Predictors} Predictors + \beta_{Confounders} Confounders + \varepsilon$
APOE4 and *TOMM40* were tested individually, each coded as 0,1,2 after adjusting for age, sex and diagnosis. After registering all ventricular models, we created 3D maps reflecting profiles of neurodegeneration (expansion) associated with the genotypes. Permutations tests were used to correct for multiple comparisons.
Results
Both *APOE*4 (Corrected *P*-value: Right-0.0004; Left <0.0004) and *TOMM*40 (Corrected *P*-value: Right-0.0008 Left-0.0036) risk alleles are significantly associated with accelerated rate of ventricular expansion. 3D statistical maps show the associations predominantly in the body and occipital horn, stronger on the right than the left.
Discussion
This is the first study to reveal a dynamic 3D pattern of ventricular expansion associated with carrying Alzheimer risk alleles in *APOE4* and *TOMM40* genes. These anatomical profiles may

help to empower drug trials using various biomarkers to evaluate efficacy of interventions for Alzheimer's Disease.



Disclosures: B. Gutman: None. **P. Rajagopalan:** None. **A. Toga:** None. **C. Jack:** None. **M. Weiner:** Ownership Interest; Synarch, Elan. **P. Thompson:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:853.14/E55

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA/NIH, AG039625

Department of Veterans Affairs

Title: Spatial acquisition learning is remarkably restored after intranasal administration of WGA-conjugated NU4 antibody in 5XFAD mice during early plaque stage

Authors: C. XIAO[1], F. J. DAVIS[1], K. VIOLA[2], P. LACOR[2], V. P. DRAVID[2], *N. B. CHAUHAN[1], W. L. KLEIN[2]
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Abstract: Alzheimer's disease (AD) is a devastating incurable disease currently afflicting >5 million Americans that awaits effective disease-modifying treatment(s). Recognition of synaptotoxic Aß oligomers (also known as ADDLs) as a seeding event and as a primary

therapeutic target in AD pathogenesis, is emerging. Our ongoing collaborative work has demonstrated that intracerebral immunization with oligomer-specific anti-ADDL antibody (NU4) more efficiently reversed synaptic and behavioral deficits in a transgenic model of AD, than the conventional anti-Aß antibody (SFN/ICAD 2008). Currently, we used intranasal (IN) route of delivery to bypass brain barrier (BBB) and systemic adversities for transfer of immunotherapeutics directly to the brain. Further advancement included the use Wheat Germ Agglutinin (WGA) as an efficient vehicle to enhance delivery of immunotherapeutics by facilitating endocytic active intraneuronal uptake while simultaneously promoting passive extraneuronal uptake of unconjugated candidate drug due to nasal mucosa-like properties of WGA. This investigation was undertaken to examine if intranasal passive immunization usinga 1:1 mixture of WGA-conjugated plus unconjugated NU4 (*w*NU4) will improve spatial acquisition learning in 5XFAD transgenic mice modeling AD, and if there exists any gender difference. 8 weeks old male and female 5XFAD transgenic mice (Tgs) were intranasally injected with *w*NU4 [(10µg/5ul/naris) X 2 = (20µg/20µl/mouse/day), twice/week, for 8 weeks]. Age-matched male and female Tg/Lt controls received equal amount of saline (10ul/naris) for 8 weeks. Spatial acquisition learning was evaluated by Morris water maze (MWM). Data were analyzed using RMNOVA, Tukey posthoc test. Results showed that there was no significant difference in the learning pattern of males or females, both among Lts or Tgs, with or without treatment. Baseline data prior to beginning immunization indicated that the spatial learning in 2-month-old Tgs was significantly impaired compared to 2-month-old Lts (↓42%, RMANOVA, Tukey posthoc test, $p<0.0001$), which remained impaired to almost the same level in the 4-month-old age-matched saline injected Tgs compared to the 4-month-old age-matched saline injected Lts (↓41%, RMANOVA, Tukey posthoc test, $p<0.0001$). Intranasal immunization with *w*NU4 significantly restored spatial acquisition learning compared to saline injected age-matched Tgs (↑35%, RMANOVA, Tukey posthoc test, $p<0.0001$). Conclusion: Intranasal immunization with *w*NU4 efficiently reversed spatial learning deficits in the 5XFAD mice at an early plaque stage without exhibiting any gender difference.

Disclosures: C. Xiao: None. **F.J. Davis:** None. **K. Viola:** None. **P. Lacor:** None. **V.P. Dravid:** None. **N.B. Chauhan:** None. **W.L. Klein:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

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NIH Grant AG005119

Alzheimer's Association Grant IIRG-10-172905

CART Foundation

Title: Bin1 expression is altered in Alzheimer’s disease

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Abstract: Recent genome wide association studies (GWAS) have implicated bridging integrator 1 (amphiphysin 2, Bin1) as a late-onset Alzheimer’s disease susceptibility gene. Bin1 was originally discovered as MYC-interacting protein that functions as a tumor suppressor; however, it also plays a critical role in cellular lipid metabolism. There are at least 15 different isoforms of Bin1 known, with many being expressed in the brain including the longest isoform (isoform 1), which is brain-specific and localizes to axon initial segments and nodes of Ranvier. In the central nervous system, Bin1 interacts with proteins such as clatherin, dynamin, amphiphysin 1, synaptojanin, and endophilin, and may function as an adapter protein that regulates synaptic vesicle endocytosis. It is currently unknown what role Bin1 may play in Alzheimer’s disease. Our lab set out to determine the expression pattern of Bin1 in control and late-stage AD brain tissue. By western blot, we discovered that the largest form of Bin1 (brain-specific isoform 1) was significantly decreased in AD in four separate brain regions: hippocampus, inferior parietal cortex, inferior temporal cortex, and frontal cortex (BA9). In contrast, several smaller isoforms of Bin1 were significantly increased in AD tissue, resulting in no significant difference in total Bin1 between control and AD cases. In human neuroglioma (H4wt) cells, overexpression of brain-specific full-length Bin1 or ubiquitously expressed Bin1 isoform 9 lead to an accumulation of full-length APP and a corresponding decrease in CTFs and total Aβ. Knock-down of Bin1 (total) using shRNA constructs is ongoing and we hypothesize that a decrease in Bin1 will increase Aβ levels possibly through a disruption in endocytic trafficking, which is important for Aβ generation. In addition, using quantitative real-time PCR, we are analyzing the levels of the various Bin1 isoforms from AD and control brain tissue to determine if Bin1 splicing is altered in AD. Finally, we examined the localization of Bin1 in human control and AD cases using immunohistochemistry to determine if the location of Bin1 in axons and neuronal synapses is changed in AD. These experiments will be useful in determining if Bin1 plays a critical role in the development of Alzheimer’s disease and may implicate Bin1 as an important biomarker or therapeutic target for the disease.

Disclosures: C.J. Holler: None. **E. Head:** None. **M.P. Murphy:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH grant P01AG030128

NIH grant P50AG16574

Title: Amyloid-β endocytic trafficking and recycling: Implications for its degradation, aggregation and toxicity

Authors: *J. LI, T. KANEKIYO, G. BU
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Abstract: Alzheimer’s disease (AD) is the most common neurodegenerative disease and the leading cause of dementia in elderly. Accumulation of amyloid-β (Aβ) peptides in the brain leads to its aggregation and toxicity, and is a major pathogenic event for AD. Aβ is cleaved from amyloid precursor protein and secreted into extracellular space. A portion of secreted Aβ can be re-internalized and metabolized by neurons and glia. To assess Aβ endocytic trafficking and the underlying implications for Aβ toxicity, we examined the specific roles of several small GTPases that regulate various endocytic trafficking steps. While Rab5 and Rab7 regulate early and late endosomal trafficking, respectively, Rab11 is involved in endocytic recycling. We found that overexpression of Rab5 or Rab7 significantly increases cellular Aβ accumulation, in particular in the presence of lysosomal inhibitors, suggesting that Rab5/Rab7-mediated lysosomal trafficking and subsequent degradation are major trafficking events for neuronal Aβ. Expression of a Rab5 dominant-negative mutant, which blocks early endosomal trafficking, leads to increased cell-associated Aβ. Interestingly, a blockage of the Rab11-mediated recycling pathway with a constitutively-active mutant also significantly accelerates cellular Aβ accumulation. Apolipoprotein E (apoE) further accelerates neuronal Aβ uptake and lysosomal trafficking in a manner that depends on apoE isoforms with apoE3 more efficiently facilitates Aβ lysosomal trafficking and degradation than apoE4, a risk factor for AD. Finally, we found that impaired lysosomal degradation of Aβ leads to its intracellular accumulation and aggregation. Taken together, our results demonstrate that Rab5, Rab7 and Rab11 regulate endocytic trafficking of Aβ through the lysosomal degradation and recycling pathways. A disturbance of these endocytic

trafficking and metabolic pathways can lead to intracellular Aβ accumulation, aggregation, and toxicity.

Disclosures: J. Li: None. **T. Kanekiyo:** None. **G. Bu:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

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Program#/Poster#:853.17/E58

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: N-pyroglutamate Abeta oligomer toxicity and its prevention by inhibition of glutaminyl cyclases

Authors: *H. U. DEMUTH
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Abstract: Alzheimer's disease (AD) is characterized by neuron loss and neuroinflammation. Although N-truncated and in particular N-pyroglutamated Aβ-peptides (pEAβ) are known as prominent constituents of plaques in AD brain, their importance has been ignored for some time and pathways leading to their formation not understood. Because of their abundance, resistance to proteolysis and neurotoxicity, such N-terminally truncated and modified peptides can be potentially important for initiation of pathological cascades leading to AD. Our recent work uncovers, that the N-terminal pE-formation is catalyzed by glutaminyl cyclase (QC) in vivo. QC expression was found upregulated in the cortex of individuals with AD and correlated with the appearance of pE-modified Aβ. Oral applications of QC inhibitors resulted in reduced pE3Aβ42 burden, but surprisingly also to the attenuation of the 1.000fold higher amounts of total Aβ in transgenic models of AD. Besides showing a diminished plaque formation and gliosis, we found improved performance in context memory and spatial learning tests. These observations led to the hypothesis that pEAβ can seed Aβ oligomerization by self- and co-aggregation with other monomeric Aβ species. In recent in vitro, in situ and in vivo studies amounts of less than 10nM pE3Aβ42 generated cytotoxic oligomers which are over 20 fold more stable than oligomers of the classical full-length Aβ-peptides. Such mixed pE3Aβ42 oligomers propagate their toxic structure in a prion-like manner. Moreover, the neurotoxicity unfolds to be strictly tau-dependent in cell culture as well as in new animal models. There, specific neuronal expression of pEAβ provides in vivo evidence for profound pEAβ neurotoxicity and gliosis induction.
Based on these data, we launched a broad QC-inhibitor development program which has entered the regulatory drug testing phase 2010. A phase 1 single (SAD) and multiple ascending dose

(MAD) study of PQ912 in healthy volunteers has been conducted since spring 2011. PQ912 is a glutaminyl cyclase (QC) inhibitor for the treatment of AD and it is the first QC inhibitor to enter clinical development.
The phase 1 trials, conducted in Switzerland, demonstrated that PQ912 is safe and well tolerated after oral dosing. Dose-proportional pharmacokinetics and a strong pharmacokinetic and pharmacodynamics relationship based on QC inhibition were observed in plasma and cerebrospinal fluid. The combined SAD/MAD studies involved more than 120 volunteers in a blinded, placebo controlled randomized trial.

Disclosures: H.U. Demuth: Employment; Probiodrug AG.

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FIRB RBNE08HWLZ001

PRIN 2008F5A3AF005

Title: The role of metalloproteases in neurodegeneration: Recent findings

Authors: F. BELLIA[1], G. GRASSO[2], *E. RIZZARELLI[3]
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Abstract: Carnosinases belong to the M20 metalloprotease family and they are mainly studied as carnosine-degrading enzymes. In mammals, two isoforms have characterized: the serum-circulating form (CN1), secreted by brain cells into the cerebrospinal fluid [1] and the cytosolic isoform (‘tissue carnosinase’, CN2), a non-specific dipeptidase distributed in several human tissues and in the rodent brain [2]. The role in the homeostasis of carnosine, a multi-functional endogenous dipeptide [3], makes the impairment of these enzymes very dangerous for the health of humans and other mammals.
In recent years, CN1 has attracted the interest of many scientists because of its correlation with aging and neurological disorders. CN1 is essentially inactive in newborn and its activity gradually rise with the age [4]. Moreover, the extent of the enzyme activity depends on the cerebral areas and the enzyme activity was significantly higher in all brain regions of senescent rats compared with that in aged animals [5]. Reduced CN1 activity in serum has been reported in

patients with neurological disorders, such as dementia and Alzheimer's disease [6].
In our lab we have found CN1 is able to degrade A-beta (1-40). The CN1 activity towards the A-beta hydrolysis has been studied in vitro in the presence of potentially new activators, inhibitors or competitors, such as transition metal ions, endogenous carnosine-related compounds and synthetic derivatives of carnosine.
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Disclosures: F. Bellia: None. **G. Grasso:** None. **E. Rizzarelli:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:853.19/E60

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Trafficking of exogenous macrophages to and within the brain

Authors: *A. F. CINTRON[1], A. F. CINTRON[2], E. HEUER,[2], R. NAIR[2], J. DOOYEMA[2], L. C. WALKER[2]
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Abstract: The introduction of Aβ seeds into the peritoneal cavity induces cerebral Aβ deposition in APP-transgenic mice (Eisele et al., Science 330:980-2, 2010). To determine how the seeds are conveyed from periphery to brain, we have investigated two potential cellular mechanisms,

intraneuronal transport and transport by macrophages, based on studies suggesting that the interaction of these cell types may facilitate the neuroinvasion of prion disease (Heikenwalder et al., J Mol Neurosci 23:3-12, 2004). Using the retrograde tracer fluorogold, we confirmed that the dorsal motor nucleus of the vagus is labeled following intraperitoneal injection, with no obvious label elsewhere in the brain. Because peripherally seeded Aβ occurs in widespread regions of the forebrain of APP23 mice, particularly around blood vessels (Eisele et al., 2010), we next investigated the disposition of exogenous macrophages labeled with fluorescent nanobeads shortly after infusion into the tail vein. We found that the macrophages migrate primarily to systemic organs such as the spleen and liver, but that infrequent exogenous macrophages also are found within the brain. When exogenous, labeled macrophages are injected directly into the brain, they tend to collect around cerebral blood vessels. Presently, we are investigating the trafficking and pathogenicity of exogenous macrophages bearing aggregated Aβ seeds derived from Alzheimer brain extracts in APP/presenilin-transgenic mice. From this work, we hope to identify the cellular mechanisms by which proteopathic seeds spread from one region to another, and thereby propagate cerebral Aβ-amyloidosis. Supported by NIH R21AG040589 and P51OD011132.
Key words: Aβ, Alzheimer's disease, amyloid, cerebral amyloid angiopathy, macrophages, prion

Disclosures: A.F. Cintron: Research Grant; Emory University. **A.F. Cintron:** None. **E. Heuer,:** None. **R. Nair:** None. **J. Dooyema:** None. **L.C. Walker:** None.

Poster

853. Alzheimer's Disease: Abeta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DGAPA, PAPIIT, UNAM IN204212-3

CONACYT 239696

Title: Role of cholesterol, IFN-γ and IL-1β on APP metabolism in astrocytes

Authors: *M. E. ÁVILA-MUÑOZ[1], P. FERRERA[2], C. ARIAS[2]
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Abstract: The neuropathology of Alzheimer´s disease (AD) is characterized by the presence of extracellular senile plaques that are mainly composed of the β-amyloid peptide (Aβ). Marked by focal activation of microglia and astrocytes in the vicinity of amyloid plaques, AD-associated inflammation has been widely described by pathological examination of brain tissue from AD patients and transgenic mouse models. The resulting neuroinflammatory processes usually involve the release from activated glia of a number of potentially neurotoxic molecules including cytokines such as IFN-γ and IL-1β that are thought can increased the production of Aβ. Other important risk factor for the development of AD is the presence of high levels of cholesterol. Although multiple lines of evidence indicate a role for cholesterol in the modulation of the lipid membrane, environment in which the proteins (APP, BACE-1 and nicastrin) for the Aβ production in neurons are located, the exact mechanisms involved in the cholesterol-induced amyloidogenic APP processing remain largely unknown in the neurons and astrocytes. In this work we aimed at evaluating the role of proinflammatory cytokines (IFN-γ 20ng/ml and IL-1β 10ng/ml for 48hrs) under the modulation of the composition of the lipid membrane with the use of cholesterol in the production of Aβ by astrocytes. Primary cultures of astrocytes were used trough the study and by Western blot, we assessed: 1) the presence of APP, BACE-1 and nicastrin proteins in astrocytes, 2) the effect of cytokines in the levels of these proteins and in the Aβ production, 3) the role of cholesterol (25 and 50μM for 48hrs) in the levels of APP, BACE-1, nicastrin and Aβ, 4) the combined effect of cytokines and cholesterol in the modulation of the amyloidogenic APP pathway, 5) the activation of astrocytes, by detection of GFAP, under the stimulation of cytokines and cholesterol. Our results so far show the presence of APP, BACE-1 and nicastrin in astrocytes. Cytokines as well as cholesterol have the ability to increase levels of APP in astrocytes and at the same time increase the expression of GFAP, indicating the activation of these cells. Furthermore, treatment of cytokines together with cholesterol, changed the APP levels but in a non-additive fashion. These results suggest that astrocytes have the proteins required for the amyloidogenic pathway and that cytokines and cholesterol can regulate Aβ production modulating the levels of APP after astrocyte activation.

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Poster

853. Alzheimer's Disease: Abeta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: COLCIENCIAS Grant 110151928994

Title: Apolipoprotein E Isoform-specific effect in the PI3K/Akt and MAPKs pathway in CAD cells

Authors: *M. A. ORTIZ, D. ORDOÑEZ, L. MORALES, A. SANDOVAL, H. ARBOLEDA, G. ARBOLEDA
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Abstract: Introduction: Apolipoprotein E (APOE) carries cholesterol and other lipids present in the plasma and in the central nervous system and binds to APOE receptors in order to control lipid homeostasis. There are three *ApoE* alleles in humans (*ApoE* ε2, ε3 y ε4). Although ApoEε4 has been implicated as a risk factor in Alzheimer's disease, the molecular effects associated to ApoEε4 have not been fully described. Aim: to study the differential effect of APOE isoforms in the AKT-mTOR and MAPKs pathways. Materials and methods: we used a murine CNS catecholaminergic cell line (CAD) as a study model. Protein expression was evaluated by Western blot. Mitochondrial metabolism was measured by MTT assay. Results: LDLR, an APOE receptor, is expressed after two days of differentiation of CAD cells. When differentiated CAD cells were treated with APOEε4 we found an increase in MTT assay associated with an increase in the phosphorylation level of AKT(Ser 473), ERK 1-2 (Thr 202/Tyr 204) and P38 (Thr180/Tyr 182) compared to APOEε2 and ε3 treatments. Phosphorylation of mTOR (Ser2448) was higher in cells treated with APOEε3 and ε4, and significantly lowers in those exposed to APOEε2. GSK-3β protein expression and phosphorylation did not show significant difference between treatments. In addition, APOEε4 also causes an increase in the expression level of hexokinase. Conclusion: These results suggest that APOEε4 raises glucose and mitochondrial metabolism and a subsequently cellular stress response associated to activation of the P38 pathway (maybe associated to an increase production of ROS). Activation of AKT and ERK pathways could be a compensatory survival mechanism. The increase in mTOR phosphorylation observed in cells treated with APOEε4 suggests and inhibition of autophagy maybe associated to the high intracellular glucose levels.

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Poster

853. Alzheimer's Disease: Abeta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG18379

NIH Grant AG18884

Alzheimer's Association-IIRG

Title: Regulation of the BACE1 gene promoter by the SP1 and STAT1/3 transcription factors: Identification of possible drug targets for Alzheimer's disease (AD) treatment

Authors: *D. K. LAHIRI, J. A. BAILEY, B. MALONEY
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Abstract: The β-amyloid cleaving enzyme (BACE1) is the rate-limiting enzyme responsible for cleavage of the amyloid precursor protein in order to produce neurotoxic β-amyloid (Aβ), which accumulates into plaque found in AD affected brain. Our goal is to study the recently proposed gene-environment interaction model, the LEARn pathway, for AD etiology (Lahiri et al, 2009). Our interest has been to study mechanisms by which the gene encoding BACE1 is regulated, with the hypothesis that reducing the rate-limiting enzyme will reduce Aβ levels, ameliorate symptoms and slow progression of AD. To this end, our recent work has identified possible feedback regulation mechanisms by which Aβ might act, itself, as a transcription factor (TF) on the BACE1 promoter, as well as multiple predicted binding sites for other TF, such as SP1 and STAT1/3 (Maloney & Lahiri, 2011; Bailey et al, 2011). We have proposed that SP1 and STAT1/3 influence BACE1 levels by direct modification of transcription through its gene promoter. To test such potential regulation of the BACE1 promoter, we stably transfected PC12 cells with a luciferase-based reporter gene system under control of a 3.3kb fragment of the 5' regulatory region of the BACE1 gene. These cells were neuronally differentiated with NGF and treated with selective pharmacological inhibitors of SP1, STAT1 or STAT3 activity. The SP1 inhibitors tolfenamic acid (an anti-inflammatory) and mithramycin (an anti-neoplastic) both dose-dependently reduced BACE1 promoter activity in these cells. Likewise, the STAT1 inhibitor fludarabine (chemotherapy agent), and the STAT3 inhibitor Stattic, both reduced BACE1 promoter activity, but to a lesser degree than either of the SP1 inhibitors. These results have important implications for the development of pharmacological inhibitors of BACE1 activity. We previously found that SP1 and BACE1 mRNA levels corresponded across the lifespan in primates (Dosunmu et al, 2009). Notably, as per the LEARn model (Latent Early-Life Associated Regulation) , environmental factors, including metals and dietary factors, operate by interfering the interaction of methylated CpG clusters with binding proteins, such as SP1. We also have found an active Aβ-interacting domain (AβID) on the BACE promoter and additional AβIDs overlap SP1 sites within the 3.3kb promoter fragment. Currently, agents aimed at reducing BACE1 activity in the brain have focused on inhibition of the enzyme, however, our cumulative data suggest that additional upstream targets at the level of transcriptional regulation may also be effective and have the added benefit of an already established array of compounds that could be developed for this purpose.

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Poster

853. Alzheimer's Disease: Abeta and Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Mayo Foundation Grant ML01

Title: Lysosomal localization of insulin-degrading enzyme

Authors: S. O. ABDUL-HAY, M. H. CHEN, F. CHEN, M. MCBRIDE, *L. H. YOUNKIN, M. A. LEISSRING
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Abstract: Insulin-degrading enzyme (IDE) is an atypical zinc-metallopeptidase responsible for the degradation of insulin, the amyloid β-protein (Aβ), and other intermediate-sized peptide substrates. The subcellular localization of IDE differs markedly from conventional zinc-metalloproteases, and the intracellular site(s) where IDE interacts with its substrates remains surprisingly ill-defined. IDE is well-known to be abundant in cytosol, secreted into the extracellular space, and targeted to mitochondria via alternative translation initiation, but the extent to which it is trafficked to other intracellular compartments is not as firmly established. IDE contains 3 residues at its extreme C-terminus (AKL), which had been believed to constitute a Type-I peroxisomal targeting sequence (PTS1). However, the complete PTS1 has recently been shown to involve adjacent residues, and bioinformatic analysis that included these residues failed to yield a strong prediction for the targeting of IDE to peroxisomes. To test this, we constructed a series of (non-mitochondrial) IDE expression constructs tagged with red fluorescent protein (DsRed) at the N-terminus and containing either the wild-type C-terminus (WT), a C-terminal V5 tag (V5), deletion of the 3 terminal residues (ΔAKL) or substitution of the 15 terminal residues with a well-established PTS1 (PTS1). None of these constructs colocalized with a genetically encoded peroxisomal marker, nor with markers for endosomes, endoplasmic reticulum, Golgi, nucleus or adiposomes. Unexpectedly, DsRed-IDE colocalized strongly with multiple lysosomal markers, a finding that was confirmed for untagged forms of IDE, as well. Significantly, in stable cell lines expressing DsRed-IDE, lysosomes appeared to be the principal site of localization, in contradistinction to transient cells, which showed a chiefly cytosolic localization. Fluorescently labeled recombinant IDE was readily internalized by cells and localized to lysosomes, suggesting that IDE may normally be trafficked to this compartment via

endocytosis of secreted IDE. Of note, exogenous application of recombinant IDE together with fluorescently tagged insulin and Aβ led to colocalization within lysosomes, suggesting that lysosomes may be an important intracellular site for degradation of peptide substrates. In sum, our study suggests that IDE is trafficked to lysosomes, but not peroxisomes, which may represent the locus of degradation of insulin, Aβ and other internalized substrates.

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Poster

854. Parkinson's Disease: Genetic Animal Models

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant NS038370

Title: Generation and characterization of a mouse model of Parkinson disease-related neurodegeneration by deletion of the lysosomal ATPase Atp13a2

Authors: *L. KETT, A. SUBRAMANIAN, W. T. DAUER
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Abstract: An increasing number of genes have been associated with familial forms of Parkinson’s disease (PD). Improved understanding of the cellular functions of these genes is likely to elucidate pathways common to both familial and idiopathic forms of PD that are amenable to therapeutic targeting. Mutations in the ATP13A2 gene cause Kufor-Rakeb Disease (KRD), an autosomal recessive parkinsonian syndrome that also features supranuclear palsy, pyramidal signs, and dementia. ATP13A2 is a lysosomal P-type ATPase linked to metal toxicity and autophagic and mitochondria defects in cell culture and yeast models of the disease. We generated Atp13a2 null mice to explore in vivo the normal function of this protein and to test the hypothesis that loss of Atp13a2 in mice will cause a neurodegenerative phenotype that models KRD and elements of lysosomal dysfunction relevant to PD-related neurodegeneration. Atp13a2 null mice are born at normal Mendelian frequencies and appear indistinguishable from littermate controls. These mice develop a mild, age-related hypokinetic phenotype as assessed by open field testing, but display no significant deficits when performing on the rotarod, DigiGait treadmill or balance beam. These data argue against a pronounced neurodegenerative phenotype, and preliminary neuropathological studies do not show evidence of overt neurodegeneration, accumulation of alpha-synuclein or abnormal proteinaceous inclusions. Ongoing studies of these

mice include additional neuropathological assessments, tests of epistasis with other PD-associated genes and in vitro studies to assess autolysosomal pathway function. While Atp13a2 null mice do not recapitalute many of the motor and pathological features of PD, they represent a valuable resource for understanding alterations in lysosomal biology associated with neurodegenerative disease.

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Poster

854. Parkinson's Disease: Genetic Animal Models

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Topic: C.03. Parkinson’s Disease

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University of Florida

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Mayo Clinic

Title: Age-dependent sensorimotor deficits and neuronal lipofuscinosis in Atp13a2 knockout mice

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Abstract: Loss of function mutations in ATP13A2, a lysosomal membrane protein of the P-type family of ion transport ATPases, underlies Kufor-Rakeb syndrome, a recently classified form of early-onset Parkinsonism with dementia (PARK9). To gain a better understanding of ATP13A2 function, we analyzed the behavioral and neuropathologic effects of the loss of this gene in mice. Knockout of the Atp13a2 gene in mice was accomplished by exchanging a region of the Atp13a2 gene encompassing exons 12-15 and the 5' half of exon 16 with a neomycin resistance gene cassette. The replaced exons include sequences encoding the phosphorylation site of the protein, which is required for enzyme activity. We then tested wildtype and Atp13a2 knockout mice on a battery of sensorimotor tests sensitive to varying degrees of neurological dysfunction including challenging beam traversal, spontaneous activity, gait, and nest building. Separate cohorts of mice were tested at 5-6, 12, and 24-27 months of age. At 5-12 months of age, there were no differences in sensorimotor function between wildtype and Atp13a2 knockout mice. However, at 24-27 months of age, the Atp13a2 knockout mice showed significant impairments compared to wildtype mice in motor performance and coordination on the beam, shortened stride length, and impairments in fine motor skills and orofacial movements involved in nestbuilding. We observed accelerated deposition of lipofuscin in the hippocampus, cortex, and cerebellum of Atp13a2 knockout mice in comparison to wildtype mice at 12-15 months of age. These results are consistent with lysosomal dysfunction that has been associated with Kufor-Rakeb syndrome. These findings indicate that loss of Atp13a2 function leads to age-dependent impairments in sensorimotor function as well as lysosomal pathology in the brain in mice. These mice will be a useful model to study the impact of the lysosomal degradation pathway in the development of features associated with Parkinson’s disease.

Disclosures: S.M. Fleming: None. **A.K. Clippinger:** None. **C. Gruber:** None. **O. Ekhator:** None. **Z. Malstrom:** None. **A. Couch:** None. **W. Thomas:** None. **A. Bailes:** None. **J. Lewis:** None. **D.W. Dickson:** None. **G.E. Shull:** None. **P.J. Schultheis:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:854.03/E67

Topic: C.03. Parkinson’s Disease

Title: Dopamine-dependence of murine LRRK2 associated behaviors

Authors: *M. VOLTA[1], A. MILNERWOOD[1], L. TAPIA[1], D. BECCANO-KELLY[1], C. VILARINO-GUELL[1], K. CO[1], H. MELROSE[2], A. HUANG[1], K. YU[1], M. J. FARRER[1]

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Abstract: Mutations in the gene encoding leucine-rich repeat kinase 2 (LRRK2) are the most common cause of familial Parkinson's disease (PD). The physiological role of LRRK2 requires further characterization although several mouse models highlight impairments in striatal dopaminergic innervation and aberrant tau phosphorylation. The LRRK2 function contributes to several neuronal phenotypes such as neuritic membrane arborization, microtubule/vesicle trafficking and autophagy. Behavioral correlates of LRRK2 genetic manipulations have yet to be rigorously tested using the same methodology across lines. In particular, behavioral paradigms reminiscent of non-motor symptoms (reflecting early pre-motor PD) have not been extensively investigated.

We report on motor, cognitive and olfactory testing for non-transgenic (NT), murine LRRK2 knockout (KO) and BAC LRRK2 wild-type overexpressing mice at 6-months of age, on C57Bl/6 background. We previously reported BAC LRRK2 G2019S L-DOPA-responsive motor deficits at 6 months, and now can directly compare results across lines.

Automated open field testing was performed to measure spontaneous horizontal and vertical locomotor activity, distance travelled, rearings and immobility time. We have also assayed physiologically-stimulated motor activity with "drag" and "accelerating rotarod" tests to assess coordination of the anterior limbs and overall motor performance, respectively.

Although PD is clinically characterized by motor deficits, depression and olfactory dysfunction are noted as early features that precede a motor phenotype, whereas mild cognitive impairment and dementia typically occur later in the course.

Herein we report initial findings in non-motor function among and across LRRK2 lines at 3 months of age. Olfactory discrimination was assayed by the habituation/de-habituation test (investigating novel odors preference) and the buried pellet test (assessing performance in finding hidden scented objects). The tail suspension test was also employed to study propensity to depressive-like states among and across LRRK2 mouse lines. Finally, we combined the use of novel object placement (spatial memory) and novel object recognition (learning abilities) to study baseline cognitive function in these mice. Comparative motor and non-motor testing will also be performed at 6 months and later time points.

Lastly, as the activity of the nigrostriatal dopamine (DA) system may correlate with both motor and non-motor phenotypes, we report on measurements of stimulated release of DA in the striatum using fast-scan cyclic voltammetry (FSCV) in acute brain slices from LRRK2 mouse lines at 3 and 6 months.

Disclosures: M. Volta: None. **A. Milnerwood:** None. **L. Tapia:** None. **D. Beccano-Kelly:** None. **C. Vilarino-Guell:** None. **K. Co:** None. **H. Melrose:** None. **A. Huang:** None. **K. Yu:** None. **M.J. Farrer:** Employment; Mayo Clinic. Other Research Support; GlaxoSmithKline, Isis Pharmaceuticals. Ownership Interest; Mayo Foundation. Consultant/Advisory Board; Teva, Genentech, H. Lundbeck A/S, Parkinson's Society Canada, Michael J. Fox Foundation.

Poster

854. Parkinson's Disease: Genetic Animal Models

Location: Hall F-J

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Program#/Poster#:854.04/E68

Topic: C.03. Parkinson’s Disease

Support: CIHR 201109 MOP 260124

Title: Retromer (VPS35) dysfunction in Parkinson’s disease.

Authors: *A. J. MILNERWOOD[1,2], L. TAPIA[2], M. VOLTA[2], D. A. BECCANO-KELLY[2], C. VILARINO-GUELL[2], M. J. FARRER[2]
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Abstract: Parkinson’s disease (PD) is a debilitating neurodegenerative disease that affects 1-2% of the population by 65 years, increasing to 4-5% by 85. Although previously thought to lack any hereditary basis, the genetic etiology of parkinsonism is becoming increasingly apparent with mutations in one protein (LRRK2) accounting for up to 40% of PD in certain ethnic populations. We recently uncovered a novel mutation causal to autosomal-dominant, late-onset parkinsonism within the vacuolar protein sorting 35 (VPS35). VPS35 is a central component of the eukaryotic retromer cargo-recognition complex, a system critical for membrane-protein recycling and endosome-trans-Golgi trafficking. A greater understanding of VPS35 biology and the pathophysiology of mutant VPS35 are essential to the development of therapeutic interventions aimed at preventing the onset and/or retarding progression of PD. Although relatively well characterized in non-neuronal cells, the physiology and regulation of the retromer system in the brain and especially in neurons is largely unstudied.
VPS35 has been shown to regulate the trafficking of several proteins involved in other neurodegenerative diseases including progranulin (PGRN, linked to fronto-temporal dementia) and the amyloid precursor protein (APP, linked to Alzheimer’s disease). VPS35 has also been implicated in actin cytoskeletal organization, synapse formation, Wnt signaling, mitochondrial peroxisome trafficking and nuclear export. To determine potential pathophysiological alterations in neuronal protein trafficking induced by mutant VPS35, we have explored the retromer binding efficiency of previously described VPS35-specific cargos in both wild-type (WT) and mutant VPS35 expressing cell lines. Furthermore, we report on the binding relationships, sub-cellular localization and the neurophysiological impact of WT and mutant VPS35 expression in primary neuronal cultures. Additionally, using a combination of yeast-2-hybrid and co-immunoprecipatation studies we have identified novel binding partners whose intracellular transport may be altered by mutant VPS35. An update will be provided on our results. Our goal

is to determine neuronal processes perturbed by mutant VPS35, and which of these disturbances are most relevant to the pathophysiology of PD. A greater understanding of these processes, in the broader context of the molecular network implicated in PD, will enable development of novel therapeutics with symptomatic and neuroprotective benefits.

Disclosures: A.J. Milnerwood: None. **L. Tapia:** None. **M. Volta:** None. **D.A. Beccano-Kelly:** None. **C. Vilarino-Guell:** None. **M.J. Farrer:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:854.05/F1

Topic: C.03. Parkinson’s Disease

Support: EraNet Neuron

Title: *In vivo* imaging of dopaminergic neuron turnover in the olfactory bulb of mouse models of Parkinson's disease

Authors: *J. NEUNER, J. HERMS
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Abstract: Dopaminergic Neurons in the adult olfactory bulb (OB) are continuously added to the pre-existing neuronal network throughout life. They are one subtype of adult-born neurons, which arrive to the OB and strive for integration as only half of them will survive. As Parkinson’s disease (PD) patients suffer from olfactory deficits from early stages of the disease on and as nerve cell loss in the PD brain mainly concerns dopaminergic neurons, we expected that this neuron population is also affected in the highly plastic OB. To follow this idea, we created a new Cre/lox mouse model of PD which both overexpresses human A30P α-Synuclein and specifically expresses the tdTomato fluorophor in its dopaminergic neurons. We showed that human A30P α-Synuclein was preferentially expressed in dopaminergic neurons of the OB. Moreover, we found that α-Synuclein overexpression notably affected the survival of specifically newborn dopaminergic neurons in our Parkinson mice. By long-term in vivo two-photon time-lapse imaging, we now followed the turnover of the tdTomato-labeled neurons through an open-skull cranial window over the OB. Thus, we traced over time the arrival of newborn dopaminergic neurons and also determined their fate to survive within the diseased OB network. Our in vivo results do not only explain olfactory malfunction in PD but also contribute to better understand the integration and the survival of the most susceptible neuron population in PD being crucial for the development of a targeted therapy.

Disclosures: **J. Neuner:** Research Grant; EraNet Neuron. **J. Herms:** Employment; DZNE.

Poster

854. Parkinson's Disease: Genetic Animal Models

Location: Hall F-J

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Program#/Poster#:854.06/F2

Topic: C.03. Parkinson’s Disease

Support: INBRE 5P20RR016463-12

NIGMS 8 P20 GM103423-12

Title: Sodium butyrate (an HDAC inhibitor) ameliorates rotenone-induced motor defects in a *Drosophila* model of Parkinson’s disease

Authors: *S. AHMAD, R. ST LAURENT
Colby Col., Waterville, ME

Abstract: Parkinsons disease (PD) is a neurodegenerative disorder primarily affecting dopaminergic neurons in the nigrastriatal pathway characterized by debilitating motor impairment. Both familial and sporadic cases of PD exhibit resting tremor, rigidity, slowness of movement, and postural instability. Histone deacetylase (HDAC) activity is misregulated in PD and other neurodegenerative diseases. Thus, HDAC inhibitors have been recently proposed as a treatment for neurodegenerative diseases. In a Drosophila model of PD, chronic exposure to the pesticide rotenone through food selectively deteriorates dopaminergic neurons and causes climbing deficits. This study investigated the effects of sodium butyrate (an HDAC inhibitor) treatment through food supplementation on motor deficits by measuring the climbing ability in a rotenone-induced PD model. We show that co supplementation of 10 mM sodium butyrate improved the climbing ability of flies exposed to 250 uM rotenone after one week. Additionally flies with the genetic knockdown of HDAC activity through Sin3A mutation, (a component of the HDAC machinery), mimicked the sodium butyrate-mediated rescue of rotenone-induced climbing deficits. Furthermore, Sin3A mutant flies raised on sodium butyrate supplemented food had an additive effect with sodium butyrate treatment for rescuing motor deficits. Therefore, sodium butyrate is presented as a treatment for rescuing rotenone-induced motor impairment in Drosophila.

Disclosures: S. Ahmad: None. **R. St Laurent:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:854.07/F3

Topic: C.03. Parkinson’s Disease

Title: Automated gait analysis of the MitoPark mouse, a genetic model of Parkinson's Disease

Authors: *S. GELLHAAR, M. ABRAMS, D. GALTER
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Abstract: In Parkinson´s Disease (PD), locomotor patterns, including gait disturbances and postural instability, are impaired. Some of these motor symptoms which develop also in rodent models of the disease can be monitored with several automated motor behavior and gait analysis systems. We used MitoPark mice, a genetic model of PD, to study changes in locomotor performance and gait parameters in response to treatment with different doses of L-DOPA. The CatWalk system, which has previously been used in toxin-induced models for neurological disorders, was used to complement the analysis of horizontal and vertical motor activity measured in locomotor boxes. To analyze postural instability and L-DOPA induced dyskinetic behavior in the mouse model, we assessed the behavior in the cylinder test.
MitoPark mice aged 25 weeks were treated daily with saline, 2, 8, or 16 mg/kg L-DOPA for three weeks and analyzed at two time points. The gait of saline treated MitoPark mice was impaired on day 16 showing a decreased stride length and swing speed and an increased step cycle. Treatment with 8 mg/kg L-DOPA reversed these impairments to control level. The drug induced locomotor activity tested the following day was increased, though did not reach control level.
Currently, we are analyzing the changes of gait parameters in MitoPark mice treated with the other doses of L-DOPA in an attempt to correlate them to data from the cylinder test.
Preliminary results suggest that the CatWalk system broadens the number of detectable gait changes that occur during the development of PD symptoms in MitoPark mice and is useful in monitoring the treatment success with L-DOPA, as well as with other potential drug treatments.

Disclosures: S. Gellhaar: None. **M. Abrams:** None. **D. Galter:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

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Title: Transgenic expression of PGC-1α protect against MPTP-induced neuronal degeneration in the mouse model of Parkinson's disease

Authors: *J. A. AGUIRRE, SR[1], V. DI LIBERTO[2], G. MUDO[2], R. CUETO[1], B. GUERRERO-STRACHAN[1], D. LINDHOLM[3], N. BELLUARDO[2]
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Abstract: Mitochondrial dysfunction and oxidative stress occur in Parkinson's disease (PD). Peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) is a transcriptional coactivator that is a master regulator of oxidative stress and mitochondrial metabolism. PGC-1α transgenic mice were generated using standard techniques at Uppsala University, Sweden. The levels of PGC-1α in brain tissue including substantia nigra (SN) and striatum (STR) were studied by immunoblotting using polyclonal anti-PGC-1α antibody. Mice received three i.p. injections of MPTP (14 mg/kg) within a time period of 3 h followed by a fourth injection using 7 mg/kg MPTP. Mice from the experimental groups: wild-type (WT), WT+MPTP, PGC-1α transgenic (PGC1-tg) and PGC1-tg+MPTP, were killed 7 days after the MPTP treatment using deep anesthesia and brains were collected, frozen and stored at -70°C until using. Serial 40-µm-thick coronal sections of the SN (bregma A 2.54 to A 4.04 mm) followed the stereological protocol and were processed for tyroxine-hidroxilase (TH) immunoreactivity (TH-IR) and NeuN-positive cells. Total number of TH-IR in the SN compacta (SNc) was estimated by means of unbiased stereology (CAST-Grid software package, Olympus). Coronal sections (20µm) from the STR were processed for TH-IR or anti-DA reuptake transporter (DAT) antibodies. Positive fibers and terminals were measured by densitometry. Inmunoblots for TH was used to compare the effects of MPTP on WT mice or in two different PGC-1α transgenic mouse lines: PGC11 and PGC12. Measurements of mitochondrial antioxidants SOD2 and Trx2 in the SN of PGC1-tg were also measured. MPTP caused a 60-70% depletion of DA cells bodies in the SNc in the WT wile

PGC1-tg mice overexpressing PGC-1α in dopaminergic neurons are resistant against cell degeneration induced by the neurotoxin MPTP. The increase in neuronal viability was accompanied by elevated levels of mitochondrial antioxidants SOD2 and Trx2 in the SN of transgenic mice. We show here that transgenic mice PGC-1α overexpression also protected against MPTP-induced striatal loss of dopamine. To modulate PGC-1α, we employed the small molecular compound, resveratrol (RSV) that protected dopaminergic neurons against the MPTP-induced cell degeneration almost to the same extent as after PGC-1α overexpression. The results reveal an important function of PGC-1α in dopaminergic neurons to combat oxidative stress and increase neuronal viability. RSV and other compounds acting via SIRT1/PGC-1α may prove useful as neuroprotective agents in PD and possibly in other neurological disorders.

Disclosures: J.A. Aguirre: None. **V. Di Liberto:** None. **G. Mudo:** None. **R. Cueto:** None. **B. Guerrero-Strachan:** None. **D. Lindholm:** None. **N. Belluardo:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

Location: Hall F-J

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Program#/Poster#:854.09/F5

Topic: C.03. Parkinson’s Disease

Title: PGC-1α is required for survival of dopamine neurons in the substantia nigra

Authors: *H. JIANG[1,4], S. KANG[1,4], S. S. KARUPPAGOUNDER[1,4], Y. LEE[1,4], S. BRAHMACHARI[1,4], V. L. DAWSON[1,2,3,4,5], T. M. DAWSON[1,2,4]
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Abstract: Parkinson’s disease (PD) is a chronic progressive neurodegenerative disorder. Genetic factors contribute to the pathogenesis of PD. PGC-1α (peroxisome proliferator activated receptor γ co-activator protein-1 α) regulates metabolic processes and brain energy homeostasis. The role of PGC-1α in the function and survival of substantia nigra pars compacta (SNpc) dopamine (DA) neurons is not clear. Here we report that there is no significant alteration of DA neurons in the SNpc of germline PGC-1α knockout mice at approximately 4 months of age. At a similar age there is only a slight loss of DA neurons in SNpc of conditional PGC-1α knockout mice using DAT-Cre. In contrast, we observe a dramatic loss of the DA neurons and down-regulation of PGC-1α target genes in mature DA neurons of the SNpc in which PGC-1α was deleted in adult animals by means of transduction of a Cre-encoding adeno-associated virus. Transgenic mice

overexpressing the PGC-1α driven by CamKII promoter show hyperactivity and a slight loss of DA neurons in the SN at approximately 4 months of age. Our findings suggest that PGC-1α is required for the survival of DA neurons in the adult SN. Moreover, appropriate levels of PGC-1α are critical for proper DA neuronal survival and function.

Disclosures: H. Jiang: None. **S. Kang:** None. **S.S. Karuppagounder:** None. **Y. Lee:** None. **S. Brahmachari:** None. **V.L. Dawson:** None. **T.M. Dawson:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

Location: Hall F-J

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant R01NS45748

Title: Glutathione deficiency in gclm knockout mice results in complex i inhibition and dopamine depletion following paraquat administration.

Authors: *L. LIANG[1], T. J. KAVANAGH[2], M. PATEL[1]
[1]Pharm. Sci., Univ. of Colorado Denver, Aurora, CO; [2]Dept. Envrn. and Occup. Hlth., Univ. of Washington, Seattle, WA

Abstract: Depletion of glutathione has been shown to occur in autopsied brains of patients with Parkinson’s disease (PD) as well as in animal models of PD. In fact, GSH depletion has been suggested to be one of the earliest detectable biomarker in the PD brains, prior to selective decreases in mitochondrial complex I activity or loss of striatal dopamine. The goal of this study was to determine if GSH deficiency resulted in complex I inhibition and/or dopamine depletion and whether these indices were further potentiated by administration of paraquat, a redox-cycling herbicide. Depletion of the rate-limiting GSH synthesis enzyme, glutamate-cysteine ligase modifier subunit (Gclm) leads to significantly lower GSH concentrations in all of the tissues including brain. Gclm-null ($Gclm^{-/-}$) mice provide a model of prolonged GSH depletion to explore the relationship between GSH, complex I inhibition and dopamine loss. Despite ~60% depletion of brain GSH in $Gclm^{-/-}$ mice of ages 3-5 or 15-16 months, striatal complex I activity, dopamine levels, 3-nitrotyroine/tyrosine ratios, aconitase activity and CoASH remained unchanged. Administration of paraquat (10 mg/kg, twice/week, 3 weeks) in 3-5 month old $Glcm^{+/+}$ mice resulted in a small but statistically significant depletion of GSH and CoASH levels and increase in 3NT/tyr ratios but no changes in aconitase activity, complex I activity or dopamine levels. Administration of paraquat (10 mg/kg, twice/week, 3 weeks) in 3-5 month old

Glcm$^{-/-}$ mice resulted in further potentiation of GSH and CoASH depletion, increase in 3NT/tyr and decreased aconitase activity, complex I activity and dopamine levels. Furthermore, paraquat-induced inhibition of complex I and aconitase activities in Glcm$^{-/-}$ mice was observed in the striatum but not cortex. The results suggest that chronic deficiency of GSH in Glcm$^{-/-}$ mice was not sufficient to result in complex I inhibition or dopamine depletion perhaps due to homeostatic mechanisms but required an additional oxidative stress insult i.e. paraquat exposure.

Disclosures: L. Liang: None. **T.J. Kavanagh:** None. **M. Patel:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

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Topic: C.03. Parkinson’s Disease

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NIH, NINDS, 5R01 NS072467-02

Title: Characterization of the role of Regulator of G-Protein Signaling 10 in aging murine substantia nigra pars compacta

Authors: *C. P. RAMSEY, M. G. TANSEY
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Abstract: Parkinson's disease (PD) is the most common progressive neurodegenerative movement disorder. The motor symptoms of PD are caused by nigrostriatal dopamine deficiency due to the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) in the midbrain. To date, a single cause for PD has yet to be identified; however, it is known that the risk for PD onset increases with age. Thus, it is of interest to understand the underlying mechanisms of PD pathogenesis, especially those related to the normal aging process. Previous studies from our group have shown that the GTPase activating protein (GAP), Regulator of G-protein signaling (RGS)-10, plays an in vivo role at protecting nigral dopaminergic neurons from extrinsic inflammatory stimuli. However, it is not known whether RGS10 is required to promote nigral neuronal health without the addition of external insult. Neither is it known whether the in vivo role for RGS10 changes during aging. To address these questions, in the study herein, young (3-5 month old) and aged (18-20 month old) RGS10 null and wild-type (WT) mice will be compared. Ventral midbrain (VMB) and striatum tissues from the mice will be analyzed by QPCR to detect changes in PD-related gene expression that are caused by aging and RGS10

function. The identified altered genes will be assessed by quantitative immunoblot using VMB and striatum tissue extracts. Additionally, co-immunoprecipitation studies will be conducted to determine whether RGS10 forms direct protein interactions with the gene products that are found to be altered in the quantitative immunoblot studies. Lastly, unbiased stereology experiments will be conducted in aged mouse SNpc to determine whether RGS10 is required to promote nigral neuronal health during aging.

Disclosures: C.P. Ramsey: Employment; Emory University. Research Grant; NIGMS (3K12GM000680-10S1). **M.G. Tansey:** Employment; Emory University, School of Medicine. Research Grant; NINDS (5R01 NS072467-02).

Poster

854. Parkinson's Disease: Genetic Animal Models

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Program#/Poster#:854.12/F8

Topic: C.03. Parkinson’s Disease

Support: NIH 1R21NS058375

Title: Expression and function of nurr1 in nurr1 null heterozygous genotype and aging

Authors: *E. KUMMARI, J. B. EELLS
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Abstract: Dopamine neurons from different dopaminergic populations like the nigrostriatal, mesoaccumbens and mesocortical regions are anatomically and functionally different. Determining the expression patterns of Dopamine genes and proteins involved in Dopamine neurotransmission in various dopaminergic populations and the regions they innervate in young and aged, heterozygous and wild type mice is the goal of this study. Nurr1 (NR4A2) is a transcription factor that is needed for the development and sustenance of dopamine neurons and has been implicated to play a major role in regulating the expression of dopamine neurotransmission genes like Tyrosine hydroxlase (TH), Dopamine transporter (DAT), Vesicular monoamine transporter (VMAT) and GTP cyclohydrolase (GTPCH). Previous research found that Nurr1 is reduced during aging and that dopamine neuron function in Nurr1 null heterozygous mice is more susceptible to aging. The current experiment investigates how the Nurr1-null heterozygous genotype affects protein expression in young and aged mice. Brain sections of both Nurr1 wild type and Nurr1 null heterozygous mice were immunohistochemically treated using Nurr1 antibody and the quantity of Nurr1 determined by studying the cumulative signal strength of the chromogen staining. The wild type showed higher expression of Nurr1 in

the Ventral Tegmental Area (VTA) regions compared to the heterozygous. We will further investigate the expression of Nurr1 in Substantia Nigra Pars compacta (SNpc) as well as the expression levels of GTP cyclohydrolase, Dopamine transporter and tyrosine hydroxylase in dopamine target regions in young and aged Nurr1 wild type and Nurr1 null heterozygous mice. Understanding the interaction of aging and expression of these proteins involved in dopamine neurotransmission could give us important insight into understanding Parkinson's disease.

Disclosures: E. Kummari: None. **J.B. Eells:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

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Topic: C.03. Parkinson's Disease

Support: 3PO1ES016731-04

5T32ES012870-08

Title: Reduced expression of the vesicular monoamine transporter (VMAT2) disrupts catecholamine homeostasis and causes progressive neurodegeneration in mice

Authors: *S. ALTER[1], T. N. TAYLOR[2], P. SULLIVAN[3], D. S. GOLDSTEIN[3], G. W. MILLER[1]
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Abstract: PD features the progressive loss of catecholamine neurons in the substantia nigra pars compacta (SNc), the locus coeruleus (LC), and the autonomic nervous system. We have previously reported that mice deficient in VMAT2 expression (Slc18a2) undergo progressive nigrostriatal degeneration, monoamine depletion (DA, NE, 5-HT), and exhibit both motor and nonmotor symptoms of Parkinson's disease (Caudle, J. Neuroscience, 2007; Taylor, J. Neuroscience, 2009).
Here, we report parallel disruptions in the norepinephrine and dopamine systems of VMAT2-deficient mice. Unbiased stereological analysis reveals that VMAT2-deficient mice undergo progressive LC degeneration beginning at 12 months of age, preceding SNc degeneration at 18 months. LC degeneration is accompanied by reductions in norepinephrine transporter (NET) immunoreactivity throughout the brain including the hippocampus and bed nucleus of the stria terminalis (BNST). Quantitative analysis of NET abundance in the brain and sympathetically

innervated peripheral tissue is being performed with 3H-nisoxetine binding.
In addition to their neurodegenerative phenotype, VMAT2-deficient mice also exhibit alterations in catecholamine homeostasis. HPLC analysis revealed increased ratios of DHPG:NE (foldchange in cortex, 1.91; striatum, 1.83; hippocampus, 1.71) and drastic increases in DOPAC:DA (foldchange in cortex, 15.2 ; striatum, 15.1; hippocampus, 18.7), indicative of increased cytosolic catecholamine metabolism. These aberrations are not limited to the central nervous system: VMAT2 deficient hearts show a 97% reduction in norepinephrine and a 10-fold increase in cardiac DHPG:NE.
These data describe an animal model of aberrant catecholamine metabolism and progressive catecholaminergic degeneration. The severity and staging of cell loss and changes in catecholamine metabolism are also observed in PD patients. The observed changes in the cardiac NE system are also noteworthy, as there is a strong comorbidity of cardiac denervation in human PD. These findings demonstrate that reduced catecholamine storage in an in vivo model is sufficient to produce progressive catecholaminergic neurodegeneration in brain and peripheral regions affected in PD.

Disclosures: S. Alter: None. **T.N. Taylor:** None. **P. Sullivan:** None. **D.S. Goldstein:** None. **G.W. Miller:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:854.14/F10

Topic: C.03. Parkinson’s Disease

Support: NINDS Grant NS064507

Alzheimer's Association

Backus Foundation

Title: Evaluation of non-motor behaviors in DJ-1-deficient mice.

Authors: V. P. CHOU, A. S. MOALEM, *J. ORDUNA, J. MONTENEGRO, T. V. KHROYAN, N. KO, A. B. MANNING-BOG
SRI Intl., MENLO PARK, CA

Abstract: To investigate the relationship between DJ-1 and non-motor features related to Parkinson’s disease (PD), we utilized a mouse model of DJ-1 deficiency (i.e. Exon 7 insertion).

At ages 6 and 12 months (n = 10-12 per genotype per age), behaviors were assessed using a battery of non-motor testing paradigms including a food retrieval task, the elevated plus maze and the novel object recognition assay. In the buried pellet task, time to locate and retrieve normal chow was compared between null DJ-1 mice and their wild-type (WT) littermates using a 10-day testing regimen. In the initial five days of testing, no apparent difference in time to find food pellet was detected between null DJ-1 and WT mice. Subsequently, WT mice displayed a steep learning curve with this cohort requiring 97% less time to retrieve food from day 5 to 7 ($p < 0.01$); this effect was sustained in the WT mice for the remainder of the paradigm. In comparison, the null DJ-1 cohort showed only modest improvements in retrieval time, and at days 7-10 required significantly more time to complete the task (approximately 3-fold compared to WT; $p < 0.01$). Such changes were noted in the aged cohort only. These data indicate that aged null DJ-1 mice may have impaired olfaction and/or cognition compared to WT. In the elevated plus maze paradigm, anxiety-like behavior and memory deficits were evaluated. While no significant differences in time spent and entries into closed arms due to genotype were detected in the younger cohort, a significant increase in time spent by WT vs. null DJ-1 in the open arm was revealed (approximately 1.9-fold; $p < 0.05$). In the aged groups, null mice had significantly fewer entries into the open space vs. WT (40% reduction; $p < 0.05$). These data indicate age-related alterations in anxiety and cognition in DJ-1 null mice.. It is also important to note that total distance travelled during the assay by DJ-1 null mice was also decreased as compared to WT, consistent with previous findings from our lab and others that motor activity is reduced. Lastly, using the novel object recognition (NOR) task, memory was assessed. In the younger cohort, both null DJ-1 and WT animals spent significantly more time with the novel vs. familiar object. However, in the aged group, while WT mice had greater exploration time with the novel object (2.7-fold increase; $p < 0.005$), the null DJ-1 cohort equivalently explored the familiar and novel object. These data suggest impaired memory in the transgenics. Taken together, these non-motor behavior assays indicate that DJ-1 deficiency in mice may promote impairment of non-motor behaviors reminiscent of those noted in PD.

Disclosures: V.P. Chou: None. **A.S. Moalem:** None. **J. Orduna:** None. **J. Montenegro:** None. **T.V. Khroyan:** None. **N. Ko:** None. **A.B. Manning-Bog:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

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Program#/Poster#:854.15/F11

Topic: C.03. Parkinson’s Disease

Support: NIH Training Grant 5T32GM00820322

American Parkinson Disease Association

Parkinson's Benefactors

Title: Analysis of dopaminergic and serotonergic systems in Parkin and DJ-1 knockout mice as models of loss-of-function mutations linked to Parkinson's disease.

Authors: *M. R. HENNIS, M. A. MARVIN, M. S. GOLDBERG
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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease, after Alzheimer's disease and afflicts over a million people in the United States alone. PD is an age-dependent disease that causes progressive death of dopamine-producing neurons within the brain. The loss of dopamine neurons results in locomotor symptoms such as slowness of movement, tremor, rigidity and postural instability. Recent studies indicate that other neurotransmitters, such as serotonin, may also be involved. There are currently no therapies proven to halt or slow down the progressive neuronal cell loss in PD. To achieve this goal, it is important to understand the cause of PD at the molecular and cellular level. Although most cases of PD are sporadic, many families show a Mendelian pattern of inherited parkinsonism and causative mutations have been identified in genes such as Parkin, DJ-1, PINK1, alpha-synuclein and Leucine Rich Repeat Kinase 2 (LRRK2). Additionally, the activity of glutathione peroxidase 1 (Gpx1), a major antioxidant protein, is reduced in PD brains. We crossed mice deficient for both Parkin and DJ-1 with mice deficient for Gpx1 to test the hypothesis that loss-of-function mutations in Parkin and DJ-1 cause PD by increasing susceptibility to chronic oxidative stress. Surprisingly, mice lacking Parkin, DJ-1 and Gpx1 have increased striatal dopamine levels in the absence of nigral cell loss compared to wild-type, single, and double mutant mice. Mice lacking Parkin and DJ-1 exhibit improved rotarod performance and have increased serotonin in the striatum, hippocampus and prefrontal cortex. We performed stereological analysis of immunostained serotonergic processes in the hippocampus to determine whether the increase in serotonin we observed by HPLC analysis is due to an increase in serotonergic projections. The results of our behavioral, neurochemical and immunohistochemical analyses indicate that PD-linked mutations in Parkin and DJ-1 cause dysfunction in neurotransmitter systems beyond the nigrostriatal dopaminergic circuit.

Disclosures: M.R. Hennis: None. **M.A. Marvin:** None. **M.S. Goldberg:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

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Topic: C.03. Parkinson's Disease

Support: Gardner Family Center For Parkinson's Disease and Movement Disorders

Selma Schottenstein Harris Lab for Research in Parkinson's

Title: Initial characterization of the behavioral phenotype of DJ-1 knockout rats

Authors: *T. L. KYSER[1,2], A. M. HEMMERLE[1], S. N. CASSELLA[1,2], O. EKHATOR[3], N. A. HOLMES-LAU[3], J. P. HERMAN[2,4], S. M. FLEMING[1,2,3], K. B. SEROOGY[1,2]
[1]Neurol., [2]Neurosci. Grad. Program, [3]Psychology, [4]Psychiatry & Behavioral Neurosci., Univ. of Cincinnati, Cincinnati, OH

Abstract: Loss of function mutations of the DJ-1 gene (Park7) are associated with a familial form of autosomal-recessive early-onset Parkinson's disease (PD). DJ-1 is a 189 amino-acid protein whose function appears to be multifunctional as it has the properties of an antioxidant, can regulate transcription and RNA binding, serve as a chaperone, and may play a role in apoptosis. In partnership with the Michael J. Fox Foundation for Parkinson's Research, Sigma Life Science, using zinc-finger nuclease technology, has developed the DJ-1 homozygous knockout (KO) rat. This novel rat genetic model, only recently available, has yet to be phenotypically characterized and presents an excellent opportunity to investigate the role of DJ-1-related mitochondrial dysfunction and oxidative stress in experimental parkinsonism in an animal model that is more amenable to behavioral, neuroanatomical, and functional study than the mouse. The present study represents the initial behavioral phenotyping of DJ-1 KO rats with the goal of shedding light on possible sensorimotor and non-motor deficits resulting from the loss of function mutation of the PD-linked DJ-1 gene in this model. Male wildtype and DJ-1 KO rats are being tested on a battery of behavioral tests to assess both sensorimotor and non-motor functions. Sensorimotor assessment includes tests of gait, response to somatosensory stimuli, movement initiation, and bracing. Non-motor assessment includes tests of anhedonia, emotional reactivity, and attention. We found alterations in several sensorimotor parameters in DJ-1 knockout compared to wildtype rats. At two months of age the stride variability is significantly different in DJ-1 compared to wildtype rats and at four months of age DJ-1 knockout rats show significantly different rearing and hindlimb stepping in the cylinder compared to wildtype controls. Body weights also differed at four months of age with DJ-1 knockout rats weighing significantly more that wildtype controls. Analysis of non-motor behavior is ongoing. The novel DJ-1 rat model may prove useful in understanding the underlying molecular mechanisms that eventually lead to the development of PD, which could inform novel therapeutic targets for treatment.

Disclosures: T.L. Kyser: None. **A.M. Hemmerle:** None. **S.N. Cassella:** None. **O. Ekhator:** None. **N.A. Holmes-Lau:** None. **J.P. Herman:** None. **S.M. Fleming:** None. **K.B. Seroogy:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

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Topic: C.03. Parkinson's Disease

Support: RO1AG031184

P20RR015583

P20RR017670

Title: Dna repair deficiency accentuates the parkinson related phenotype in the dj-1 knockout mice

Authors: M. Y. SANCHEZ-CONTRERAS[1], J. LYDA[2], *F. CARDOZO-PELAEZ[3]
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Abstract: Parkinson's disease (PD) is considered to be a multifactorial disease involving genetic, environmental or the interaction of both factors in its etiology. Loss-of-function mutations in DJ-1 gene have been associated with autosomal recessive early-onset PD. Although a precise role for DJ1 defects in PD remains unknown, DJ1 may function as a redox-sensitive chaperone and protecting neurons against oxidative stress and cell death. In previous studies, we have shown that mice lacking the DNA repair enzyme 8-oxoguanine glycosylase 1 (OGG1 KO), present an age-dependent mild parkinsonian phenotype, which supports that increased oxidative damage to the DNA may play a role in the nigrostriatal system dysfunction in PD. Furthermore, we showed that DJ-1 KO mice can be protected from MPTP-induced dopaminergic loss by inducing neuronal-specific expression and activity of Ogg1 in the substantia nigra suggesting that DNA damage plays a major role in protecting DA neurons during aging and in PD-related neurotoxicological. We generated DJ1/OGG1 double KO (DKO) mice and reported that they exhibit an early-onset and age-dependent bradikynetic phenotype consisting of reduced speed, increased immobility and decreased rearing activity. This diminished motor behavior phenotype is responsive to dopamine agonists. We treated DJ-1/OGG1 DKO mice with the dopaminergic precursor L-DOPA and with the dopaminergic receptor agonist apomorphine at different age

points. When treated with L-DOPA, 12-month-old DJ-1/OGG1 DKO mice increased 137% in speed. This L-DOPA-induced increment in the speed constitutes a significant improvement of DJ-1/OGG1 DKO mice reaching to a normal behavior level (1.02cm/s±0.2 basal to 1.12cm/s±0.2 L-DOPA). Additionally, we assessed the susceptibility of DJ-1/OGG1 DKO to the dopaminergic toxin MPTP at 3 months of age. We found that DJ-1/OGG1 DKO was more susceptible than WT mice (71.8% vs. 32% reduction in striatal DA, from saline treated). Even more, DJ-1/OGG1 DKO mice lost more striatal dopamine than the single DJ-1 KO (48.8%) and OGG1 KO (27.3%). Current studies are assessing whether neuronal-specific Ogg1 expression protects DJ-1/OGG1 DKO against MPTP. Our findings add further evidence to the role of DNA damage in the age-related dysfunction of the dopaminergic system observed in PD. Supported by NIA grant RO1AG031184, NIH P20 RR015583 and NIH P20RRP20RR017670.

Disclosures: M.Y. Sanchez-Contreras: None. **J. Lyda:** None. **F. Cardozo-Pelaez:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

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Program#/Poster#:854.18/F14

Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation Community Fast Track Grant

Title: Aged H-ferritin-deficient mice as a model for Parkinson’s Disease

Authors: *A. M. ILL SNYDER[1], E. B. NEELY[1], E. L. UNGER[2], J. R. CONNOR[1]
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Abstract: Age is one of the most significant risk factors in the onset of neurodegenerative disease and oxidative stress in the brain increases with age. A key component to oxidative stress is iron accumulation without coinciding increases in proteins that manage iron, such as ferritin. Iron deposition is particularly apparent in the substantia nigra and is thought to be a major factor in the progression of the dopaminergic cell death seen in Parkinson’s disease. Therefore, the component of age is an important factor in modeling PD in animals and current animal models of PD do not show age-dependent vulnerability.
In a mouse model that is deficient for the iron management protein H-ferritin, we found that neurotoxin challenge with paraquat and maneb does not affect cell viability in 4-month old mice; however, 12-month old mice that are deficient in H-ferritin exhibit a 35% loss of cells as compared to age-matched saline-treated controls and a 28% loss of cells compared with H-

ferritin-deficient animals treated with saline.
Our data demonstrate that the number of DAergic cells and the amount of TH protein in the unperturbed nigra is unaffected by H-ferritin deficiency at 4 or 12 months of age. Further examination of the nigrostriatal pathway and revealed no change in iron content or DAT. Taken together, these data suggest that the DAergic neurons in the aged H-ferritin-deficient mouse are vulnerable to the effects of paraquat and maneb in ways that are beyond the “classical” iron-mediated pathways.
This led to the hypothesis that changes in trophic factor profiles or alterations in mitochondrial proteins are important factors in identifying the cause of the age-dependent host susceptibility of neurons. MANF (mesencephalic astrocyte-derived neurotrophic factor) levels were increased in H-ferritin-deficient mice at both 4 and 12 months of age. Mitochondrial COX IV protein levels were unchanged due to age or genotype. Mitochondrial ferritin, which is structurally and functionally similar to H-ferritin, was not able to be detected histologically or by immunoblotting.
The collective data from these experiments suggest that the H-ferritin deficient mice offer a model of age-dependent host susceptibility for the study of PD. The increased host susceptibility is not simply due to iron accumulation with age or loss of DAT expression. Studies are in progress to continue to interrogate our hypothesis that there are compensatory mechanisms in place in the 4-month-old H-ferritin-deficient mice that enable neuronal survival following neurotoxins, but by 12 months of age, this compensatory profile is no longer in place or is no longer adequate to protect the neurons.

Disclosures: A.M. Ill Snyder: None. **E.B. Neely:** None. **E.L. Unger:** None. **J.R. Connor:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant R01-NS038065

NIH Grant R01-ES017384

NIH Grant R01-NS038377

Title: Enhanced sensitivity to chronic MPTP exposure in HuA53TαS overexpressing mice

Authors: *J. BARNES[1], Y. LIU[2], N. XIONG[1], T. N. TAYLOR[1], B. THOMAS[3], M. K. LEE[1]
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Abstract: Parkinson's Disease (PD) is the second most-common neurodegenerative disease worldwide after Alzheimer's Disease and affects ~1% of the population over 65 years of age. While there are known genetic causes of PD, the vast majority of cases are idiopathic. Of the identified heritable PD-causing factors, three mutations in the gene encoding the 140-amino acid protein α-synuclein (αS) have been shown to cause an early-onset familial form of the disease. In addition, environmental dopaminergic (DAergic) neurotoxins are implicated in PD. The relationship between αS and toxin vulnerability, however, has been uncertain because mutant αS does not increase the sensitivity of DAergic neurons to acute toxin exposure.
To study whether a more chronic stress would reveal pathological interactions between αS and DAergic neurotoxins, we tested whether mice overexpressing A53T mutant human αS (HuA53TαS, line G2-3) are hypersensitive to the neurotoxic effects of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP). We used a chronic MPTP model in which osmotic mini-pumps loaded with MPTP were implanted sub-cutaneously in male mice for 28 days (delivering 30 mg/kg of MPTP per day). Transgenic (HuA53TαS overexpressing) and non-transgenic (nTg) mice aged 4-6 months were utilized for all experiments. All mice were asymptomatic at the time of surgery. As observed previously with this chronic MPTP-induced model of PD, the treatment paradigm resulted in a marked loss of DAergic neurons in the substantia nigra pars compacta as well as a decrease in total locomotor activity in the open-field test in all mice. Importantly, however, HuA53TαS mice were more sensitive to the effects of MPTP, showing a greater loss of DAergic neurons and locomotor activity than control animals. Our results thus show that increased expression of mutant HuA53TαS leads to increased vulnerability of DAergic neurons to chronic toxin exposure but not acute toxicity.

Disclosures: J. Barnes: None. **Y. Liu:** None. **N. Xiong:** None. **T.N. Taylor:** None. **B. Thomas:** None. **M.K. Lee:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

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Topic: C.03. Parkinson's Disease

Support: SFRH / BD / 51061 / 2010

Title: The 6-ohda model of Parkinson's disease: Behavioral characterization and effects of dopaminergic and antidepressant therapy

Authors: *M. M. CARVALHO[1,2], F. L. CAMPOS[1,2,3], B. COIMBRA[1,2], A. J. RODRIGUES[1,2], P. M. JOSÉ[1,2], J. M. BESSA[1,2], J. J. CERQUEIRA[1,2], G. BALTAZAR[3], N. SOUSA[1,2], A. J. SALGADO[1,2]
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Abstract: Parkinson's disease (PD) is a chronic neurodegenerative disease of dopaminergic circuits, characterized by motor and non-motor symptoms. Animal models of PD have helped understanding basic neurodegenerative mechanisms of the disease, and are ideal tools to test new therapies for PD. One of the most used models is the 6-hydroxydopamine (6-OHDA) unilaterally-lesioned rat, in which motor deficits are well described. However, information regarding changes in other behavioral dimensions is scarce. Thus, this work aimed at improving the characterization of this model, hoping to reveal additional phenotypic hallmarks, useful in the future to address, beyond the motor level, the relevance of novel treatments. Furthermore, we were interested in assessing the effect of dopaminergic and antidepressant therapy in any changes encountered, particularly at the motor and mood levels.
Wistar-Han rats were unilaterally injected with 6-OHDA or saline in the MFB. Behavioral assessment addressed motor, emotional and cognitive dimensions, together with apomorphine-induced turning behavior. Post-mortem, we performed tyrosine hydroxylase immunohistochemistry to quantify nigral dopaminergic cell loss. To study the effect of dopaminergic and antidepressant therapy a second set of animals was used. Animals were treated daily with one of three compounds: levodopa (25mg/kg), paroxetine (6mg/kg) or bupropion (10mg/kg). During treatment behavioral analysis was repeated.
Apomorphine-induced turning behavior and histological analysis revealed the existence of two animal clusters in the 6-OHDA-lesioned group. Animals with severe lesions displayed significant motor impairments, decreased exploratory activity, depressive-like behavior and impairments in cognitive flexibility. Animals with incomplete lesions, relatively spared at the motor level, showed depressive-like behavior and changes in exploratory activity. Moreover, we found that both levodopa and antidepressant therapy was effective on improving lesion induced behavioral changes.
Our work shows that the unilateral model of PD presents behavioral changes beyond the motor level, which can be improved with both dopaminergic and antidepressant therapy. Thus, this can be a useful model to test the impact of PD-targeted therapies in a multidimensional fashion.

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Poster

854. Parkinson's Disease: Genetic Animal Models

Location: Hall F-J

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Program#/Poster#:854.21/F17

Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation

Title: The JAX Parkinson’s disease mouse model repository: Characterization of novel strains

Authors: *M. SASNER[1], M. A. OSBORNE[1], K. WARDWELL[1], C. M. LUTZ[1], R. WILPAN[1], R. LYNCH[1], D. HINERFELD[1], K. D. DAVE[2]
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Abstract: The Parkinson’s Disease Mouse Model Repository (PDMMR) at The Jackson Laboratory serves as a central resource to select and obtain PD mouse models for basic research and therapy development. In addition to distributing a large set of previously published strains, we are working in collaboration with The Michael J. Fox Foundation for Parkinson’s Research with the goals of creating improved models that more closely mimic human disease, and making mice available for both academic research and therapy development in the pharmaceutical and biotechnology sector. Extensive molecular and phenotypic characterization information is being developed to identify novel lines with highest expression levels and to compare these new mice to existing strains to enable users to choose the best model for a specific application.
The repository provides a collection of previously published strains such as alpha-synuclein (SNCA) transgenic mice that include A53T, A30P, and wild-type human alleles driven in distinct neuronal subsets by various transgenic or the endogenous promoter; novel SNCA lines have been developed and are being characterized. Some of the SNCA mutants display motor deficits, alpha-synuclein inclusions and neurodegeneration similar to that seen in PD patients. A mouse model expressing the human PARK2*Q311X allele driven in dopaminergic neurons by the Slc6a3 (DAT) promoter displays late-onset and progressive Parkinson's disease pathogenesis. Previously established BAC transgenic lines expressing mutant human alleles of LRRK2 are being compared to novel BAC transgenic lines carrying the D1994A, G2019S, and R1441G mutations. Available knockout strains carry deletions in the alpha-, beta-, and gamma-synuclein, Lrrk1, Lrrk2, Pink1 and Park2 (Parkin) and Park7 (DJ-1) genes. This collection affords users the opportunity to create novel combinations of alleles to better understand the molecular pathways involved in PD and potentially make better disease models; for example, a SNCA transgenic/Pink1 knockout strain displays an earlier phenotype than the strains with the single

alleles.
We are actively seeking additional Parkinson's disease mouse models. Submission of a strain to the repository, which can be initiated via a web form, fulfills requirements for sharing of mice in accordance with NIH's policy for sharing of research reagents. If necessary, donating investigators can place licensing restrictions on the distribution and use of their models. For more information about the PDMMR including a list of strains, phenotype descriptions, allele and genotyping information and associated references, see
http://research.jax.org/grs/parkinsons.html

Disclosures: M. Sasner: None. **M.A. Osborne:** None. **K. Wardwell:** None. **C.M. Lutz:** None. **R. Wilpan:** None. **R. Lynch:** None. **D. Hinerfeld:** None. **K.D. Dave:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

Location: Hall F-J

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Topic: C.03. Parkinson's Disease

Title: MJFF animal models 2: Generation, characterization, and distribution of LRRK2 animal models for Parkinson's research

Authors: *M. A. BAPTISTA[1], K. D. DAVE[1], S. DE SILVA[1], M. SASNER[2], A. GORODINSKY[3], M. J. BECK[4], C. QUANG[4], K. GAMBER[5], A. REITH[6], J. LYON[6], A. ROULOIS[6], Y. AZIZ[1], S. S. DAS[1], J. L. EBERLING[1], A. DUFOUR[1], M. F. FACHERIS[1], B. K. FISKE[1], N. SHETH[1], T. B. SHERER[1], A. URKOWITZ[1], M. FRASIER[1]
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Abstract: Determining the efficacy of LRRK2 inhibitor therapeutics has been a challenge due to the lack of availability and systematic characterization of LRRK2 animal models. The existing LRRK2 animal models have not closely recapitulated PD-related CNS pathology and symptomology, resulting in limited translation of preclinical findings. MJFF is generating, characterizing, and distributing LRRK2 animal models in an effort to make them available at cost without any restrictions on use for the entire research community. MJFF is generating 15 LRRK2 transgenic/knockout rodent lines, out of which 4 lines (LRRK2 WT mouse, LRRK1 KO mouse, LRRK2 KO mouse, LRRK2 KO rat) are currently available. To date, MJFF funding has facilitated distribution of over 1500 LRRK2 transgenic rodents to both non-profit organizations

and industry. To generate new animal models, founder lines are selected at Taconic based on genotyping, mRNA/protein expression, and copy number data, and transferred to Jackson Labs for re-derivation. Using this process, MJFF has recently generated a BAC human LRRK2 wild type mouse that has approximately 5 fold expression over endogenous LRRK2, with the R1441G, G2019S, D1994A and A2016T mutants to be generated soon in the hope that a robust phenotype will emerge. As part of the strategy to standardize and replicate published phenotypic data, MJFF has initiated the breeding and aging of both MJFF-generated and pre-existing LRRK2 models. For example, BAC human R1441G LRRK2 transgenic mice (Li et al., 2009) were aged to 4, 8, and 12 months and behavioral (rotarod, catalepsy, balance beam, and open field, etc.), neurochemical (striatal neurotransmitter concentrations), and histological (staining for alpha-synuclein, tyrosine hydroxylase, etc.) outcome measures were determined. MJFF preliminary results indicate that while there is no behavioral phenotype evident in 4 and 8 month BAC R1441G mice (Li et al., 2009), striatal dopamine and 5-HT levels are statistically lower than non-transgenic controls at 8 months of age. MJFF is also characterizing phenotypes associated with the toxic effects of knocking out LRRK2 in order to mitigate the risk of therapeutically targeting LRRK2. MJFF generated LRRK2 KO rats have no behavioral or neurochemical deficits up to 12 months of age. Since LRRK2 KO mice exhibit kidney pathology (Herzig et al., 2011; Tong et al., 2012), MJFF is currently investigating if there are similar morphological differences in kidneys of LRRK2 KO rats. All phenotypic data will be shared by creating a public database.

Disclosures: M.A. Baptista: None. **K.D. Dave:** None. **S. De Silva:** None. **M. Sasner:** None. **A. Gorodinsky:** None. **M.J. Beck:** None. **C. Quang:** None. **K. Gamber:** None. **A. Reith:** None. **J. Lyon:** None. **A. Roulois:** None. **Y. Aziz:** None. **S.S. Das:** None. **J.L. Eberling:** None. **A. Dufour:** None. **M.F. Facheris:** None. **B.K. Fiske:** None. **N. Sheth:** None. **T.B. Sherer:** None. **A. Urkowitz:** None. **M. Frasier:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

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Program#/Poster#:854.23/F19

Topic: C.03. Parkinson’s Disease

Title: MJFF Animal Models 2: Phenotypic characterization of the autosomal recessive (Parkin, Pink-1 and DJ-1) gene knockout rat models of Parkinson’s disease

Authors: *K. D. DAVE[1], S. DE SILVA[1], S. RAMBOZ[2], M. J. BECK[3], C. QUANG[3], S. A. BENKOVIC[4], S. AHMAD[5], S. SUNKIN[6], D. WALKER[7], K. M. GAMBER[8], Y. AZIZ[1], M. A.

BAPTISTA[1], S. S. DAS[1], A. DUFOUR[1], J. L. EBERLING[1], M. F. FACHERIS[1], B. K. FISKE[1], N. SHETH[1], T. B. SHERER[1], A. URKOWITZ[1], M. FRASIER[1]
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Abstract: Recessively inherited loss-of-function mutations in the Parkin, DJ-1 or PINK1 gene are linked to familial cases of early-onset Parkinson's disease (PD). Single knockout (KO) mice (lacking either Parkin, DJ-1 or PINK1) and triple-knockout (lacking all three genes) exhibit disruptions of dopamine homeostasis but no dopaminergic degeneration in the substantia nigra (SN). As part of the strategy to provide more tools for the research community, The Michael J. Fox Foundation for Parkinson's Research (MJFF) has funded the generation of novel rat models lacking either Parkin, DJ-1 or PINK1 genes. Additionally, MJFF sponsored a standardized characterization including behavioral, neurochemical and pathological measures to determine if these rats exhibit progressive PD-like phenotype. The PINK1 KO rats demonstrated motor dysfunction and gait impairment compared to wild-type rats (WT), and some of these deficits were progressive starting as early as 8-weeks of age and continuing up to 8-months of age. These deficits included increased hind-limb foot slips in the tapered beam test, decreased rearing in fatigue challenge test, loss of hind-limb grip strength, impaired mobility in the open-field, gait disturbances in the Neurocube, lack of hind-limb extensor strength, decreased air-righting reflex and reduced muscle tone. The DJ-1 KO rats expressed similar motor and gait abnormalities, however these deficits were less pronounced and appeared mostly between 6 and 8 months of age. At 8-months of age, both PINK1 KO and DJ-1 KO rats showed a significant two-three fold increase in both striatal dopamine and serotonin content compared to WT, however, there were no robust differences in DOPAC, HVA and 5-HIAA levels. Moreover, stereological estimation in both PINK1 KO and DJ-1 KO rats showed a progressive loss of TH-positive neurons of the pars compacta of SN with 50% loss observed at 8-months of age compared to WT. There were no significant differences observed in the Parkin KO rats compared to WT in behavior, striatal neurotransmitter content or numbers of SN dopaminergic neurons up to 12-months of age. Characterization of alpha-synuclein immunoreactivity in these rat models is currently ongoing. These results demonstrate that loss of either the PINK1 or the DJ-1 gene in the rat produces a progressive loss of dopaminergic neurons, and that this neurodegeneration may be correlated to progressive motor and gait deficits. Thus, these MJFF-generated PINK1 and DJ-1 KO rats will help elucidate the mechanisms by which these recessive genes produce PD pathology and aid in therapeutic development.

Disclosures: K.D. Dave: None. **S. De Silva:** None. **S. Ramboz:** None. **M.J. Beck:** None. **C. Quang:** None. **S.A. Benkovic:** None. **S. Ahmad:** None. **S. Sunkin:** None. **D. Walker:** None. **K.M. Gamber:** None. **Y. Aziz:** None. **M.A. Baptista:** None. **S.S. Das:** None. **A. Dufour:**

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Poster

854. Parkinson's Disease: Genetic Animal Models

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:854.24/F20

Topic: C.03. Parkinson’s Disease

Support: SYNERGY Scholars Award from Dartmouth SYNERGY: The Center for Clinical and Translational Science

The Hitchcock Foundation

Title: Id2 inactivation results in biphasic ambulatory abnormalities and age-dependent histologic changes in the substantia nigra pars compacta

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Abstract: Characterizing dopaminergic neuronal development and function may uncover strategies for researchers to develop disease-modifying treatments for neurodegenerative diseases, such as Parkinson’s disease (PD). Id2 encodes a helix-loop-helix transcriptional repressor highly expressed in the developing central nervous system (CNS). Id2-/- mice have fewer dopaminergic neurons in the olfactory bulb associated with reduced olfactory discrimination, a pre-clinical marker of PD. Behavioral, histologic and in vitro molecular biological analyses indicated that midbrain dopaminergic neurons were also affected by Id2 loss. Id2-/- mice were hyperactive at 1 and 3 months, but by 6 months, showed reduced activity. Histologic examination of Id2-/- mice showed age-dependent loss of dopaminergic neurons in the substantia nigra pars compacta (SNpC) correlated with changes in ambulatory activity. Indicators of neurodegeneration including activated caspase-3 and glial infiltration were noted in the SNpC of older Id2-/- mice. Reduced DAT expression was observed in Id2-/- mice in vivo as well as in an in vitro dopaminergic differentiation model. These findings indicate that Id2 has a critical role in the maintenance of midbrain dopamine neurons. The Id2-/- mouse is thus a suitable animal model to study the progression of neurodegenerative disorders involving the dopamine system.

Disclosures: M.C. Havrda: None. **B. Paolella:** None. **N. Ward:** None. **K. Holroyd:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:854.25/F21

Topic: C.03. Parkinson’s Disease

Title: Knockout rat models for Parkinson’s disease

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Abstract: The rat is a superior choice to the mouse for studying human neurological diseases due to a more sophisticated brain. Until recently, targeted rat genome manipulation was not possible due to the lack of experimental tools used to alter the rat genome. In 2009, Geurts et al. created the first Zinc finger nuclease (ZFN)-mediated knockout rats by microinjection of ZFNs into single-cell embryos (1). ZFNs are fusion proteins of a zinc finger protein and the DNA endonuclease domain of a type II restriction enzyme, *Fok*I. ZFNs are engineered to bind and cleave at specific chromosomal locus to generate double strand breaks, repair of which by the non homologous end joining pathway results in insertions/deletions that potentially disrupt the gene function.

Most Parkinson’s disease (PD) cases are sporadic in origin, and the less common familial forms are linked to a number of genes, including the following most studied five: Parkin (Park2), PINK1, DJ-1 (Park7), α-synuclein and Lrrk2. Mouse knockout models have been made against these genes and show alterations within the nigrostriatal dopamine system but lack Parkinsonism phenotypes such as loss of dopaminergic neurons in the substantia nigra and the development of protein inclusions called Lewy bodies (2). We created four knockouts against the above five genes using ZFN technology. Western blots analysis showed the total lack of respective proteins in these models. We will report expression profile changes via PCR array analysis as well as available brain imaging and behavior data. These models will potentially help elucidate the molecular mechanisms that underlie PD and facilitate drug development.

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Geurts, A. *et al.* Knockout rats via embryo microinjection of zinc-finger nucleases. *Science* 325, 433 (2009)

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Fleming, S. *et al.* Genetic Mouse Models of Parkinsonism: Strengths and Limitations. *The Journal of the American Society for Experimental NeuroTherapeutics*. 2, 495-503 (2005)

Disclosures: **E.V. Kouranova:** Employment; SAGE Labs, Sigma Aldrich. **D. Fisher:** None. **Y. Wu:** None. **E. Weinstein:** None. **X. Cui:** None.

Poster

854. Parkinson's Disease: Genetic Animal Models

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Topic: C.03. Parkinson's Disease

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Portuguese Foundation for Science and Technology – FCT (SFRH/BPD/69643/2010)

Title: A comparative behavioural study of Parkinson's disease rat models

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Abstract: Parkinson's disease (PD) is classically characterized by motor symptoms; however, non-motor manifestations are increasingly recognized as relevant in this condition. Given the influence of these non-motor symptoms in the well-being of PD patients it is of major interest to assess these features in studies using PD animal models. Therefore, the present study aims to compare the motor, emotional and cognitive behaviour of three rat models of PD.
The models analyzed were the 6-OHDA lesion model, paraquat (PQ) model, and a genetic model based on α-synuclein overexpression. 6-OHDA and AAV-α-synuclein vector were injected bilaterally in the substantia nigra of adult male Wistar rats. For PQ delivery, micro-osmotic pumps were implanted in the interscapular region. After surgeries the animals were allowed to recover for five weeks before starting the behavioural assessment. The motor, emotional and cognitive performances were assessed through different paradigms: spatial reference and working memory tests, forced swimming test, sucrose preference test, open field, elevated plus maze and rotarod. All the models exhibited motor deficits. In addition to motor impairments the animals of α-synuclein model presented also a reduction of exploratory activity as well as an increase in anxiety-like behavior. Animals exposed to 6-OHDA and PQ also displayed a

significant increase in anxiety-like behavior. Interestingly, only in the 6-OHDA model there was an increase in depressive-like behavior. Learning and memory tasks were not affected by treatments, suggesting that these cognitive domains are not affected in these PD models. Overall, these PD models are suitable for mimicking the motor symptoms, and some of them encompass also other relevant components of the disorder.

Disclosures: F.L. Campos: None. **M.M. Carvalho:** None. **A.C. Cristovão:** None. **Y. Kim:** None. **G. Baltazar:** None. **A.J. Salgado:** None. **N. Sousa:** None.

Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

Location: Hall F-J

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Topic: C.03. Parkinson’s Disease

Support: Swedish Research council

EU FP7 Replaces programme

UK MRC

Title: Impact of cortical alpha-synuclein overexpression on the performance in operant tasks of cortico-striatal function

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Abstract: Although Parkinson’s disease (PD) has traditionally been considered a pure motor disorder, impairments in cognition are increasingly recognized among the non-motor symptoms with significant clinical impact. More specifically, cognitive impairments typically fall within the ‘executive’ domain, affecting functions such as cognitive flexibility and rule learning. Cortical and subcortical pathological accumulation of intracellular alpha-synuclein aggregates are considered to be a key factor to this cognitive decline.
Rats were pre-trained on an operant version on the classical delayed alternation task before receiving an injection of adeno-associated viral vectors (AAV) encoding for alpha-synuclein into the medial prefrontal cortex. Another subset of animals received excitotoxic lesions of the same prefrontal region and all rats were re-tested on the delayed alternation task twelve weeks post-injection. Overexpression of alpha-synuclein in medial prefrontal cortex had no effect on

performance in this operant task with rats displaying choice accuracy in the same range as the sham-lesioned controls. In contrast, rats with an excitotoxic lesion of the medial prefrontal cortex were significantly impaired, showing a reduced choice accuracy at all delays, suggesting that the deficit is one of frontal-type executive function rather than of short-term memory. Considering the involvement of prefrontal cortex in cognitive flexibility, the rats are now being trained on an operant reversal-learning task and their performance will be assessed and compared among the different lesion-groups. After completion of the operant testing, tracer injections, histological and biochemical assessments of alpha-synuclein expression and neuronal pathology will be performed and correlated to the performance in these operant tasks.
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Disclosures: H.S. Lindgren: None. **A. Klein:** None. **A. Heuer:** None. **S.B. Dunnett:** None.

Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

Location: Hall F-J

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Program#/Poster#:855.02/F24

Topic: C.03. Parkinson’s Disease

Title: Overexpression of α-synuclein impairs learning and memory and cholinergic mini-synaptic currents in *Drosophila*.

Authors: *Z. PEI[1], X. ZHAO[2], L. CHEN[2], X. SU[1]
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Abstract: Parkinson’s disease (PD) is the most common degenerative disease of the nervous system besides Alzheimer’s disease. Both genetic and environmental factors contribute to the etiology of PD. Pathologically, the symptoms of PD are mainly caused by nigrostriatal dopaminergic nerve cell loss or the decline of remnants dopaminergic neurons in dopamine biosynthesis ability. In addition, the impairment of cholinergic system or imbalance between dopaminergic system and cholinergic system also
plays a role in the pathophysiology of PD, especially, Parkinson's disease
dementia (PD dementia, PDD). A large number of animal experiments and
clinically studies showed that change in the cholinergic system is closely
related to learning and memory. Cholinergic neuronal degeneration may be associated with attention disorders and executive function decline in PD. α-synuclein is the first PD gene and the mutation of α-synuclein has been linked to a variety of neurological disorders including PD and

PDD. Recently, α-synuclein aggregates were found to locate at the presynapses and cause synaptic dysfunction, suggesting presynaptic neurotransmitter deficiencies in Parkinson's disease, PDD and DLB. Drosophila has been widely used as a system to study neurobiology, neuropharmacology, and neuropathologic mechanisms. The projection neurons (PNs) play a critical role in learning and memory in drosophila. PNs are cholinergic neurons and are projected to mushroom body to receive cholinergic synaptic transmission from the olfactory bulbs and transduct to higher brain center. In the present study, compared with non-transfected controls, the ability of learning and memory of α-synuclein wild type and A53Tflies significantly decreased. The miniature excitatory postsynaptic currents (mEPSCs) frequency but not mean mEPSCs amplitude was also significantly lower in the α-synuclein WT and A53T flies than in the control flies. Additionally, there was a significant decrease in the calcium currents of PNs in both α-synuclein wild type and A53T flies compared with control flies.
All together, our data suggest that overexpression ofα-synuclein impair learning and memory and cholinergic mini-synaptic currents and block the calcium current of PNs in Drosophila.

Disclosures: Z. Pei: None. **X. Zhao:** None. **L. Chen:** None. **X. Su:** None.

Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

Location: Hall F-J

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Program#/Poster#:855.03/F25

Topic: C.03. Parkinson’s Disease

Title: A53T α-synuclein Parkinson’s disease model mice display early motor and anxiety symptoms, and aberrant sleep and monoamine neurotransmitter levels

Authors: *S. M. ROTHMAN[1], K. J. GRIFFIOEN[2], N. VRANIS[2], W.-N. CONG[2], B. MARTIN[2], J. L. CADET[3], B. LADENHEIM[3], M. P. MATTSON[2]
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Abstract: Parkinson’s disease (PD) is characterized by degenerative motor symptoms including resting tremor and bradykinesia. Mutations in the human α-synuclein gene lead to early onset PD making these mutations attractive targets for the design of mouse models of PD; however, phenotypes of α-synuclein mutant mice vary depending upon which promoter is used to drive transgene expression. The goal of this work was to characterize the onset and progression of behavioral symptoms and neurochemical alterations in transgenic mice expressing mutant (A53T) human α-synuclein controlled by a Thy-1 promoter. We used the line of A53T (SNCA)

mice originally described by (Chandra et al., 2005; Cell 123:383-396); the SNCA mice and wild type (WT) mice of the same genetic background were purchased from Jackson Labs and used in separate cohorts to evaluate behavioral function (n=16 WT; n=12 SNCA) and for biochemical analysis (n=30 WT; n=30 SNCA). Beginning at 8 weeks of age, mice were evaluated for motor function (rotarod and stride length) and anxiety (elevated plus maze and open field) every 2 weeks until 32 weeks of age. At 24 weeks mice were evaluated in a Continuous Lab Animal Monitoring System (CLAMS). For biochemical analysis, a separate cohort of mice was euthanized at 12, 24 and 36 weeks for: immunoblot analysis of α-synuclein, dopamine transporter (DAT), tyrosine hydroxylase (TH); ELISA of brain-derived neurotrophic factor (BDNF) (n=4 WT; n=4 SNCA); and HPLC analysis of monoamine neurotransmitters (n=6 WT; n=6 SNCA). Behaviorally, SNCA mice display consistent motor and anxiety symptoms that do not worsen over time. Rotarod and stride length analyses revealed significant motor deficits at 8-11 weeks of age and anxiety tests showed a decrease in anxiety in SNCA mice compared to littermates, consistent with other reports of mouse models of PD. CLAMS analysis revealed an increase in activity during the dark phase for SNCA mice compared to WT and a reduction in overall sleep time for SNCA mice at 6 months of age consistent with clinical reports of sleep abnormalities in PD. Changes in the expression of DAT and neurotransmitters appeared early (12 weeks) in the striatum and late (36 weeks) in the midbrain. An early rise in dopamine was associated with a rise in BNDF implying a compensatory mechanism. Increases in serotonin levels potentially explain reductions in anxiety behaviors. Results indicate that this model of PD displays consistent and relevant motor, anxiety and sleep phenotypes. Neurotransmitter data are among the first of its kind to profile longitudinal alterations in neurotransmitters in PD mice and indicate that alterations begin in the striatum and progress to the midbrain.

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Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:855.04/F26

Topic: C.03. Parkinson’s Disease

Title: Reduced hippocampal theta frequency in mice expressing the human A53T mutation of alpha-synuclein

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[1]Pfizer, Inc, Groton, CT; [2]Comparative Med., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Abnormal hyperphosphorylation and aggregation of pathogenic proteins are common features of various neurodegenerative disorders. Aggregated fibrillar α-synuclein, a presynaptic protein is a major component of Lewy bodies is thought to play a critical role in pathology of Parkinson's disease. It is known that A53T form of α-synuclein, which is associated with autosomal dominantly inherited form of Parkinson's disease have a high rate of self-aggregation, supporting this notion. The A53T transgenic mice express the human A53T alpha-synuclein variant (full-length, 140 amino acid isoform) directed by the mouse prion promoter (Giasson et al., 2002), leading to the formation of filamentous neuronal inclusions and neurodegeneration, providing an experimental tool for studying pathophysiological mechanisms underlying Parkinson's disease.

In the present experiments the impact of over-expression of alpha-synuclein on hippocampal physiology was studied in 8 month old, homozygous, male A53T transgenic and age matched wild-type control mice. Local field potentials were recorded in the hippocampus CA1 region and elicited theta oscillation recorded in response to high-frequency electrical stimulation of the brainstem nucleus pontis oralis (nPO) under urethane anesthesia. Hippocampal recordings showed a current-dependent increase in power and frequency of theta oscillation in response to stimulation of the nPO in wild-type mice. A53T transgenic mice showed a significant decreased peak frequency across the entire theta frequency range (4-9 Hz) in response to increasing stimulating current comparing to wild-type control mice. In contrast, there was no significant difference in absolute and relative theta power between A53T transgenic and control mice. Reduced frequency in elicited theta oscillation has been well characterized following administration of various anxiolytic drugs (McNaughton et al., 2007); therefore the currently described hippocampal activity of A53T transgenic mice could indicate their well characterized diminished anxiety. Furthermore, the neuroaxis involved in generation of stimulation-induced hippocampal theta oscillation also contributes to rapid eye movement (REM) sleep regulation. Alterations in elicited hippocampal oscillation predict changes in REM sleep physiology, which is known to be impacted in Parkinson's disease patients at the early stage of the disease.

Disclosures: E. Morozova: Employment; Pfizer, Inc. **J. Dunlop:** Employment; Pfizer, Inc. **M. Hajos:** Employment; Pfizer, Inc.

Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

Location: Hall F-J

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Topic: C.03. Parkinson's Disease

Title: Comparison of D-amphetamine -induced locomotor activity and brain alpha-synuclein load in two mouse strains carrying A30P alpha-synuclein

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Abstract: Alpha-synuclein (aSyn) is coded by the SNCA -gene, and a missense mutation switching Ala30 residue to Pro (A30P) has been shown to be strongly associated with an autosomal dominant familial form of Parkinson's disease (PD). The mutation is located in the amphipathic N-terminal domain of the protein, which couples the protein with phospholipid membranes. The A30P -mutated protein has enhanced propensity to form oligomers and aggregates, and decreased connection with lipids. Transgenic mouse lines have been created in attempt to model PD, but no consistent results have been obtained. Some strains exhibit generally age-dependent motor defects, but pathology of the dopaminergic system is rare. Also, while one A30P-transgenic strain is more sensitive to the effects of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, another strain does not show any sensitization. Despite contradictory data, expression of human A30P aSyn in mice can lead to neuropathological and behavioral changes. We have studied D-amphetamine -induced locomotor activity and brain aSyn load in two A30P -strains: one with inserted human A30P gene (mPrPh(A30P)asyn), and another one with an A30P-point mutated mouse aSyn gene (SNCA(A30P)). We found a significantly decreased response to 1 mg/kg D-amphetamine in the mPrPh(A30P)asyn-mutated mice when compared to wild-type (WT) littermates, which may be due to a lower capacity or inadequate recruitment of dopamine storage pool as earlier reported. The decrease was not seen in SNCA(A30P)-strain, whose mutant mice rather exhibited slight hyperactivity. With 2.5 mg/kg of D-amphetamine, no differences were observed between genotypes in either of the strains. Immunohistochemical staining of aSyn revealed a significant accumulation of aSyn in the substantia nigra, striatum and M1 motor cortex in the mPrPh(A30P)asyn mutant mice when compared to WT mice, but in SNCA(A30P) -mice the accumulation was less apparent. This is most likely due to the different total amounts of aSyn expressed by the strains, which may also partially explain the more robust behavioural changes in mPrPh(A30P)asyn -mice.

Disclosures: M.H. Piltonen: None. **M. Savolainen:** None. **T.T. Myöhänen:** None. **P.T. Männistö:** None.

Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

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Jane and Aatos Erkko Foundation to Timo T. Myöhänen

Title: Prolyl oligopeptidase inhibitor KYP-2047 reduces α-synuclein aggregation in rotenone treated α-synuclein overexpressing cell lines

Authors: *M. J. HANNULA[1], L. DOKLEJA[1], M. GERARD[2], V. BAEKELANDT[3], P. T. MÄNNISTÖ[1], T. T. MYÖHÄNEN[1]
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Abstract: Prolyl oligopeptidase (PREP) is an enzyme hydrolyzing peptides less than 30 amino acids. It is present in many different species and, in mammals, it is widely distributed in the tissues. Its physiological function is still unclear but present hypothesis suggests, that the role of PREP is to cleave neuropeptides including substance P, vasopressin and neurotensin. Recently, it was shown that PREP accelerates the aggregation of wild-type α-synuclein (α-syn) under cell-free conditions, and this action can be blocked with PREP inhibitors. Abnormal misfolding of α-syn is considered to be the most critical pathogenic event leading to neurodegeneration in Parkinson's disease (PD). Mutations in α-syn gene, such as, A30P, A53T and E46K point mutations, lead to increased accumulation of α-syn in cells and to early onset PD.
We treated SH-SY5Y cell lines overexpressing A30P or A53T mutant human α-syn with rotenone to study the effect of PREP inhibitor KYP-2047 on α-syn aggregation in cell culture. Wild type SH-SY5Y cells served as control. Roteone is an organic pesticide that causes mitochondrial dysfunction by inhibiting complex I of the electron transport chain. This leads to energy deficiency, aggregation of α-syn and to selective death of dopaminergic cells.
Cells were exposed to rotenone (50 nm) for 3 days to induce α-syn aggregation and were then treated with the PREP inhibitor (10 μm) during or after the stress. The aggregation and immunoreactive protein of α-syn were determined by immunohistochemistry and western blot. The effect of KYP-2047 on cell viability was investigated and double-label immunostaining with confocal microscopy was used to study the colocalization between PREP and α-syn.
Rotenone induced robust α-syn aggregation in both α-syn overexpressing cell lines, and low concentrations of KYP-2047 significantly reduced the number of cells with α-syn inclusions and abolished the colocalization of α-syn and PREP. KYP-2047 significantly reduced the amount of aggregated α-syn, and it had beneficial effects on cell viability.
These results show rotenone to be capable of modeling one of the main features of PD, aggregation of α-syn, in α-syn overexpressing cell lines. Results also suggest that PREP may

play role in brain accumulation and aggregation of α-syn, while KYP-2047 seems to effectively prevent these processes.

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Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

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Topic: C.03. Parkinson’s Disease

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Jane and Aatos Erkko Foundation to Timo Myöhänen

Title: Prolyl oligopeptidase inhibitor KYP-2047 reduces the high molecular weight α-synuclein forms in the brain of α-synuclein A30P mouse strain

Authors: *M. H. SAVOLAINEN[1], V. BAEKELANDT[2], P. T. MÄNNISTÖ[1], T. T. MYÖHÄNEN[1]
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Abstract: α-Synuclein is natively unfolded, abundant brain protein that consists of 140 amino acids. α-Synuclein is believed to have a physiological role in dopamine transport, storage and release but its aggregation is toxic. The hallmark findings of the brain of patients with Parkinson’s disease are α-synuclein rich inclusions called Lewy bodies. Inherited early-onset forms of Parkinson’s disease are strongly associated with autosomal mutations A30P, A53T and E46K in α-synuclein coding gene or its duplications or triplications. Prolyl oligopeptidase (PREP) is a serine protease cleaving proline-containing neuropeptides shorter than 30 amino acids. PREP has been found to accelerate α-synuclein aggregation in vitro although it cannot cleave it. Recent studies have shown that a PREP inhibitor treatment reduces aggregation of α-synulein in vitro and in vivo.
The effect of a PREP inhibitor, KYP-2047, on α-synuclein aggregation and accumulation in vivo was further studied using a transgenic knock-in mouse model. Mice had an inserted A30P point mutation on their α-synuclein gene. It has been reported earlier that mice develop age related decline in motor function. KYP-2047 was administered subchronically for five days or chronically for 28 days period intraperitoneally using Alzet® osmotic pumps. α-Synuclein

immunohistochemistry and Western blot analysis were done to study α-synuclein amounts in the brain after the treatments. The fractionation method was used to separate different molecular weight forms of α-synuclein.
After the 5- and 28- days KYP-2047 treatments of the A30P +/+ transgenic mice we observed a decrease in α-synuclein immunostaining in different brain areas (substantia nigra, cortex and striatum) compared to the vehicle treated animals. No effect was seen in wild type littermates. In Western blot analysis the protein levels of high molecular weight α-synuclein aggregates in the A30P +/+ mice were decreased in KYP-2047 treated compared to the vehicle treatment.
The formation of aggregates of α-synuclein is a key step in Parkinson's disease pathophysiology. PREP inhibitor, KYP-2047, decreases the amount of aggregated form of A30P- α-synuclein in the mouse brain. This may offer a new principle for therapeutic applications in Parkinson's disease.

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Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

Location: Hall F-J

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Topic: C.03. Parkinson's Disease

Support: NIEHS ES015777

NINDS NS007480

Title: RING finger protein 11 (RNF11) mediates inflammatory responses and alpha-synuclein-induced neurodegeneration in *Drosophila*

Authors: *E. L. PRANSKI[1,2], J. J. LAH[1,2], A. I. LEVEY[1,2], S. SANYAL[2,3], R. S. BETARBET[1,2]
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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder that is characterized by loss of dopaminergic neurons in the substantia nigra and the presence of Lewy bodies in surviving neurons. RING finger protein 11 (RNF11), a candidate gene at the PARK 10 locus, has been recently reported to be a negative regulator of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling. NF-κB is a critical regulator

of inflammation and apoptosis and thus is a key player in chronic inflammation, pathogenesis and progression of various neurodegenerative diseases including PD. The function of NF-κB pathway is well conserved and plays a major role in Drosophila immune response, including the Toll and the immune deficiency (Imd) pathways. In this study, we report the first characterization of CG32850, the Drosophila homologue of RNF11 (dRNF11), as a key regulator of Drosophila immune response signaling cascade, as well as dRNF11's role in mediating neurodegeneration in a Drosophila model of PD. To characterize dRNF11, classical infection paradigms with gram-positive and gram-negative bacteria were performed to induce activation of Toll and Imd pathways respectively. Quantitative real-time PCR was used to measure mRNA levels of antimicrobial peptides at various time points post-infection as a direct measure of immune pathway activation. Through genetic manipulation of RNF11 expression, we demonstrate that dRNF11 is indeed a negative regulator of the Drosophila immune response and knockdown of dRNF11 leads to heightened immune responses with decreased survival. Next, we examined the role of dRNF11 in a genetic model of PD where wild-type or mutant alpha-synuclein is over-expressed. This model is characterized by adult-onset loss of dopaminergic neurons, alpha-synuclein inclusions, and locomotor deficits. Expression of dRNF11 and alpha-synuclein was targeted to the eye in order to easily screen for potential interactions leading to eye degeneration phenotypes. We observe neurodegeneration in the eyes of adult animals expressing alpha-synuclein, with enhanced degeneration observed in conjunction with knockdown of dRNF11. Further experiments are being pursued to identify the contribution of immune activation in this phenotype. These findings support the hypothesis that in Drosophila, dRNF11 negatively regulates immune activation and fly survival and that reduced or functionally compromised RNF11 can exacerbate alpha-synuclein-induced neurodegeneration, a phenomenon with known implications in PD-like neurodegenerative disease pathogenesis and progression.

Disclosures: E.L. Pranski: None. **J.J. Lah:** None. **A.I. Levey:** None. **S. Sanyal:** None. **R.S. Betarbet:** None.

Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

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NIEHS ES012870

NIH AG025688

NINDS NS007480

Title: Neuronal RING finger protein 11 (RNF11) modulates susceptibility to 6-OHDA-induced nigral degeneration and behavioral deficits

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by selective loss of dopaminergic (DA) neurons in substantia nigra (SN) and presence of Lewy bodies in cytoplasm of surviving neurons. We have previously demonstrated that RING finger protein 11 (RNF11), a candidate gene at the PARK 10 locus, is localized to cortical and nigral Lewy bodies in PD brain tissue. Additionally, we have reported neuronal RNF11 as a negative regulator of the NF-κB signaling pathway. A number of studies have highlighted chronic inflammation, through activation of NF-kB pathway, as a key player in progressive pathogenesis of PD. In this report, we determined if RNF11-mediated modulation of neuronal NF-kB signaling had an impact on 6-hydroxydopamine (6-OHDA)-induced behavioral deficits and DA neuron loss associated with this rodent model of PD. In vivo levels of neuronal RNF11 were manipulated by targeted knockdown (KD) or over-expression (OE) of RNF11 in the SN through unilateral stereotaxic injections of tagged adeno-associated virus (AAV2) constructs. After 2 weeks, rats received striatal stereotaxic injections of 6-OHDA on the same side. Amphetamine-induced rotation was monitored at 3 weeks post-6-OHDA lesion. Brains were harvested 4 weeks post-6-OHDA lesion to determine the role of RNF11 on neurodegeneration using immunohistochemical and quantitative real-time PCR analysis. Nigral DA neuron counts were estimated using stereological analyses of tyrosine hydroxylase (TH)-positive neurons in SN. Additionally, mRNA expression levels of RNF11, TH, and inflammatory markers were measured from microdissected ventral midbrain (VM) regions. For quantitative analysis, the uninjected side served as an internal control for each animal. AAV2 constructs specifically infected neurons at the site of injection. Targeted OE of neuronal RNF11 in SN decreased NF-kB and TNF-alpha mRNA levels in VM samples when compared to scramble-injected samples, and resulted in significant increase in amphetamine rotations & more extensive 6-OHDA-induced DA loss. In converse, KD of neuronal RNF11 correlated with increase in NF-kB and TNF-alpha mRNA levels in VM samples and protected against 6-OHDA-induced DA loss & reduced amphetamine rotations. These findings demonstrate that neuronal RNF11 negatively regulates canonical NF-κB signaling in vivo and that chronic activation of neuronal NF-kB is neuroprotective. In contrast, others have reported glial activation of NF-kB to enhance neurodegeneration. This diverse role of NF-kB within the brain has important implications for targeted therapeutics aimed at controlling neuroinflammation and neurodegeneration.

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Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

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Michael J. Fox Foundation

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Title: Phenothiazine analogues as potential α-synuclein imaging ligands for Parkinson's Disease: Synthesis and evaluation of binding affinity for α-synuclein fibrils using a fluorescence assay

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Abstract: Accumulation of misfolded, fibrillar α-synuclein (α-syn) in Lewy bodies and Lewy neurites is the pathological hallmark of Parkinson's Disease (PD). Ligands with high affinity for α-syn fibrils could be utilized as imaging agents to improve the diagnosis of PD and to monitor disease progression. To identify ligands that have high binding potency toward aggregated α-syn, we synthesized a series of phenothiazine derivatives and assessed their binding affinity to recombinant α-syn fibrils using a fluorescence assay. The binding affinity of phenothiazine compounds for fibrils was determined based on competitive binding with the fluorescent compound Thioflavin T (ThioT), after determination of the K_d for ThioT binding in saturation binding studies. In the ThioT competitive binding assay, increasing concentrations of phenothiazine compounds were added to binding reactions containing ThioT and α-syn fibrils. Among 16 new analogues, the competitive binding results indicate that compound **11b** has high affinity to α-syn fibrils (K_i = 32.10 ± 1.25 nM) and compounds **11d**, **16a** and**16b** have moderate affinity to α-syn fibrils ($K_i \approx$ 50 to 100 nM). Several of these compounds are suitable for synthesis of radiolabeled ligands to further evaluate binding affinity and selectivity, including

[^{11}C]**11b,** [^{18}F]**16a, and [^{125}I]16b.** Further optimization of the structure of these analogues may yield compounds with high affinity and selectivity for aggregated α-syn. A high affinity synuclein imaging ligand suitable for quantifying the distribution of fibrillar α-syn *in vivo* could improve diagnostic accuracy and could also improve the understanding of disease progression in PD.

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Poster

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Michael J. Fox Foundation

Title: Binding of the alpha-synuclein radioligand Synuclein Imaging Ligand 23 (SIL23) and related analogues reflects the level of fibrillar α-synuclein in Parkinson's Disease brain tissue

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Abstract: Accumulation of misfolded, fibrillar α-synuclein (α-syn) in Lewy bodies and Lewy neurites is the pathological hallmark of Parkinson's disease (PD). Ligands with a high affinity for α-syn fibrils could be utilized as imaging agents to improve the accuracy of clinical diagnosis of PD, which may become increasingly valuable as disease modifying therapies are developed. Additionally, quantifying the distribution of pathological α-syn *in vivo* could improve understanding and assessment of disease progression, including the onset of dementia in PD patients. We previously identified a group of phenothiazine derivatives that bind α-syn fibrils. To further characterize the potential utility of this compound class as radiotracers, we synthesized the ^{125}I-labeled phenothiazine analogue Synuclein Imaging Ligand (SIL23) and characterized its affinity and specificity for the pathological form of α-syn. Radioligand binding studies demonstrated that SIL23 has moderately high binding affinity for fibrils prepared from recombinant α-syn (K_d 140 nM, B_{max} 5 pmol/nmol α-syn monomer) and that it has relative selectivity for α-syn fibrils over other amyloid fibrils, with 5 fold lower affinity for $A\beta_{1-42}$ fibrils

and 2 fold lower affinity for tau fibrils. An additional phenothiazine analogue, SIL26, has a K_i of 15 nM, with more than 5 fold selectivity for both Aβ and tau fibrils. Binding studies also demonstrated that a 140 nM SIL23 binding site is present in the insoluble fraction from PD postmortem brain tissue but not control tissue and that the density of SIL23 binding sites is comparable to binding site densities for amyloid Aβ tracers in Alzheimer's disease. These results define a radioligand binding site on α-syn fibrils that is specifically present in PD brain tissue and that can be feasibly targeted to quantify pathological α-syn accumulation *in vivo*. Further optimization of the binding affinity and selectivity of SIL23 analogues could yield a radiotracer that can be used to improve diagnostic accuracy and monitor disease progression in PD, and could facilitate the evaluation of new therapeutic approaches.

Disclosures: D. Bagchi: None. **M. Udan-Johns:** None. **L. Yu:** None. **R.H. Mach:** None. **Z. Tu:** None. **P.T. Kotzbauer:** None.

Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

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SFRH/BD/25515/2005

Title: Interplay between oxidative stress and alpha-synuclein phosphorylation in Parkinson's disease cell models

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Abstract: Alpha-synuclein (alpha-syn) is an abundant neuronal protein that has been linked to neurodegeneration in both sporadic and familial forms of Parkinson's disease (PD). Mutations, duplications and triplications in the gene encoding alpha-syn are responsible for earlier forms of PD. Moreover, aggregated alpha-syn, which is a major component of Lewy bodies, alpha-syn post-translational modifications, mitochondrial dysfunction and oxidative stress have also been pointed as key pathogenic events of this disorder. In this study we analysed the link between

oxidative stress and alpha-syn phosphorylation at Ser129 in SH-SY5Y neuroblastoma cell lines overexpressing wild-type (WT) and mutant A53T alpha-syn, either transiently transfected (in the case of WT versus A53T alpha-syn, linked to GFP) or conditionally expressing (in the case of WT protein only) in a system regulated by doxycycline (-Dox). Oxidative stress induced with ferrous iron ($FeSO_4$) was shown to increase the production of reactive oxygen species (ROS), namely hydroperoxides and superoxide anion. In transiently transfected cells, iron-induced ROS was correlated with the formation of ubiquitin-labelled alpha-syn aggregates, mitochondrial depolarization and phosphorylation of alpha-syn at Ser129. Furthermore, we reported a higher susceptibility of mutant A53T alpha-syn expressing cells. In SH-SY5Y cells conditionally overexpressing alpha-syn increased ROS was observed under basal conditions in cells overexpressing alpha-syn, compared to cells expressing the endogenous protein (+Dox), which was exacerbated by iron exposure. Analysis of cellular antioxidants activity revealed a decrease in superoxide dismutase (SOD)1 under basal conditions in -Dox cells (in total and mitochondrial fractions), whereas SOD2 activity was increased in mitochondria upon exposure to iron, an effect that was significant in -Dox cells. Moreover, GSH and GSSG levels diminished in -Dox cells under basal conditions, in total or mitochondrial fractions, which may explain the increase in ROS. Accordingly, the levels of glutamate-cysteine ligase catalytic subunit (GCLc) were also decreased in -Dox cells, accompanied by a decrease in the activity of enzymes of glutathione synthesis. Interestingly, preliminary data support an increase in alpha-syn phosphorylation at Ser129 in -Dox cells. Overall, data suggest that overexpression of alpha-syn may be involved in modifying the susceptibility to oxidative stress and the antioxidant response, defining subsequent alpha-syn aggregation and phosphorylation in agreement with the pathological events described in PD.

Disclosures: R. Perfeito: None. **M. Ribeiro:** None. **T.F. Outeiro:** None. **L. Stefanis:** None. **A.C. Rego:** None.

Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

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Parkinson Soc of Canada

Title: Effects of S129 phosphorylation on alpha-synuclein self-assembly and membrane binding

Authors: *F. SAMUEL, V. NG, P. E. FRASER, C. B. COLE, P. E. FRASER, A. TANDON
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Abstract: α-Synuclein (α-syn) is a highly abundant presynaptic protein that exists as membrane-bound and freely-diffusible cytosolic. In normal brains, less than 5% of α-syn is phosphorylated at serine residue 129 (pS129), whereas nearly 90% of the α-syn in Parkinson disease Lewy bodies (LB) is phosphorylated at S129. To further clarify the relationship between S129 phosphorylation and the biophysical and biological properties of α-syn, we generated recombinant α-syn with and without pS129. To do this, we used *E. coli* to express human α-syn with and without polo-like-kinase 2 (PLK2), a major kinase responsible for S129 phosphorylation. Mass spectrometric and western blot analyses revealed complete S129 phosphorylation when α-syn and PLK2 were co-expressed versus the absence of pS129 when α-syn was expressed alone. Structural properties of non-phosphorylated and phosphorylated forms of α-syn were then assessed by circular dichroism spectra and electron microscopy. Our results suggest that serine phosphorylation accelerated the conversion of wild-type α-syn secondary structure from random coil to beta sheet, as well as fibril formation. Moreover, the presence of PD-linked mutations, A53T and A30P, further increased the self-assembly of the pS129 α-syn. We also assessed whether pS129 affected the binding of α-syn to synaptosome membranes. Although, S129 phosphorylation had little effect on the WT α-syn membrane binding, it appeared to increase association of A30P α-syn and decrease that of A53T α-syn. Overall, our results indicate that aberrant S129 phosphorylation modifies α-syn structure and its self-assembly, and differentially impacts α-syn mutant binding to presynaptic membranes.

Disclosures: F. Samuel: None. **V. Ng:** None. **P.E. Fraser:** None. **C.B. Cole:** None. **P.E. Fraser:** None. **A. Tandon:** None.

Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

Location: Hall F-J

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Program#/Poster#:855.14/F36

Topic: C.03. Parkinson’s Disease

Title: Studies of *in vivo* and *in vitro* seeding of α-synuclein pathology.

Authors: A. SACINO, C. CEBALLOS-DIAZ, A. ROSARIO, P. E. CRUZ, J. PINGEL, J. LEWIS, B. GIASSON, *T. E. GOLDE
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Abstract: α-Synuclein is normally a soluble presynaptic protein, but it also has an intrinsic ability to aggregate and form 10-15nm amyloidgenic fibrils, which are the major component of Lewy bodies and Lewy neurites. These Lewy pathologies are a defining hallmark of Parkinson's disease (PD) and several recent findings suggest that α-synuclein aggregate formation may proceed through a seeded prion-like mechanism, but this has not been formally proven as other alternative mechanisms may also explain these observations. Furthermore, aggregation of α-synuclein may propagate in vivo through glia. α-Synuclein fibrils can be taken up by cultured cells leading to the seeded formation of cytoplasmic inclusions, similar to those seen in PD. In primary neuronal culture, we have been able to seed the formation of α-synuclein inclusions, similar to Lewy body pathology, using in vitro fibrilized protein. However, inclusion formation in our primary cultures occurs mainly in astrocytes and is associated with changes in astrocytic morphology and cytokine secretion profiles. Similarly, preformed α-synuclein fibrils can also induce the formation of α-synuclein inclusions in the brain of mice. These inclusions are predominantly found in neurons and can be induced in various areas of the brain. Fibrillar α-synuclein-induced brain pathology is associated with massive astrogliosis and microgliosis. Collectively, these findings show that α-synuclein is capable of inducing its own inclusion formation, and that the process is associated with an immune response.

Disclosures: A. Sacino: None. **C. Ceballos-Diaz:** None. **A. Rosario:** None. **P.E. Cruz:** None. **J. Pingel:** None. **J. Lewis:** None. **B. Giasson:** None. **T.E. Golde:** None.

Poster

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Topic: C.03. Parkinson's Disease

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Title: AMPK over-activation leads to accumulation of alpha-synuclein oligomers and decrease of neurites

Authors: *P. JIANG, M. GAN, A. EBRAHIM, M. CASTANEDES-CASEY, D. DICKSON, S.-H. YEN
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Abstract: Parkinson disease (PD) is an age-related neurodegenerative disorder characterized pathologically by abnormal deposition of filamentous α-synuclein (α-syn) as Lewy bodies and Lewy neurites. Increased α-syn expression can occur in brains of aging human and neurotoxin (e.g. MPTP) treated mice. Since previous studies have shown increased brain lactate levels in aging brains, in PD affected subjects when compared to age-matched controls, and in mice treated with MPTP, we tested the effect of lactate exposure on α-syn in a cell based-study. We demonstrated that (i) lactate treatment led to α-syn accumulation and oligomerization in a time- and concentration-dependent manner, (ii) such alterations were mediated via adenosine-monophosphate activated protein kinase (AMPK) and associated with increases of cytoplasmic phosphorylated AMPK levels, (iii) AMPK activation facilitated α-syn accumulation and phosphorylation at Ser129, (iv) lactate treatment or overexpression of active AMPK decreased α-syn turnover, and (v) Lewy body-bearing neurons displayed abnormal cytoplasmic distribution of phosphorylated AMPK, which normally is located in nuclei. Together, our results suggest that chronic α-syn accumulation in neurons induced by lactate-triggered AMPK activation in aging brains may be a novel mechanism underlying α-synucleionpathies in PD and related disorders.

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Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

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Topic: C.03. Parkinson’s Disease

Title: AAV-alpha-synuclein-induced overexpression as rat model to investigate olfactory dysfunction and disease modification in Parkinson’s disease

Authors: *P. VOEHRINGER, B. FERGER
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Abstract: Parkinson's disease (PD) is a common progressive neurodegenerative movement disorder, which is characterized by a severe loss of nigrostriatal dopaminergic neurons and accumulation of aggregated alpha-synuclein (alpha-syn) in neurons of the central and peripheral nervous system. Moreover, olfactory dysfunction is a frequent non-motor symptom of PD and is considered as a potential biomarker for preclinical PD even before onset of motor impairment. Here, we used the AAV-alpha-syn-WT overexpression model to mimic motor and non-motor symptoms of PD in rats. Nigral injection of AAV-alpha-syn-WT resulted in severe motor

disturbances accompanied by dopamine (DA) depletion in the striatum and loss of tyrosine hydroxylase (TH) positive neurons in the substantia nigra pars compacta (SNpc). Additionally, by injecting AAV-alpha-syn-WT into the bulbus olfactorius of rats, we were able to induce olfactory impairments measured by a buried food pellet test.
In conclusion, we showed that the AAV-alpha-syn rat model is suitable for the recapitulation not only of motor impairments but also of early symptoms in the pathogenesis of PD such as olfactory dysfunction. Treatment of olfactory dysfunction in a rodent animal model of PD could be important to identify novel mechanisms for disease modification and biomarker studies in PD.

Disclosures: P. Voehringer: None. **B. Ferger:** Employment; Boehringer Ingelheim Pharma GmbH & Co. KG.

Poster

855. Parkinson's Disease: Alpha-Synuclein Mechanisms and Behavior

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Topic: C.03. Parkinson’s Disease

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Title: Rab protein expression mediates alpha-synuclein oligomer formation and cytotoxicity

Authors: *N. R. MCFARLAND[1], T. SAHARA[1], L. KIBUUKA[2]
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Abstract: Abnormal accumulation and deposition of α-synuclein (αsyn) have been implicated in intracellular stress, vesicle trafficking deficits, and cytotoxicity, suggesting a pathogenic mechanism for Parkinsonism. Recent data in several PD models, including yeast, primary cultures, C. elegans, and Drosophila, demonstrate that expression of specific Rab proteins ameliorate toxicity and trafficking deficits as a result of αsyn over-expression, suggesting that abnormal accumulation of αsyn may interfere with normal Rab protein function. Rab proteins may also play a role in protecting against αsyn toxicity and vesicle accumulation. We hypothesized that one potential role of Rabs may be to prevent abnormal αsyn accumulation and formation of “toxic” oligomeric species. We thus
examined the effects of specific Rab proteins, including mouse Rab1, its human paralog Rab8A, and Rab 3A, on αsyn dependent toxicity and oligomer formation in an H4 neuroglioma cell model. Flag-tagged Rab protein sequences were cloned into a pcDNA vector. We then transiently co-transfected H4 cells with human wt αsyn and Rabs, and measured cytoxicity at 36-

48 hours. To examine the effects of Rab proteins on αsyn oligomer formation and toxicity, we utilized a protein complementation assay employing constructs with αsyn fused to N and C-terminal fragments of the Gaussian luciferase molecule (or Venus-YFP). Luciferase fragments alone are not active, but when brought together by αsyn interactions reconstitute their bioluminescent activity. Rab proteins co-expressed with Syn-luciferase N and C-terminal fragments significantly (p=.024) reduced luciferase activity compared to empty vector control. Both Rab1 and Rab8A had greater, but non-significant, effects compared to Rab3A. Consistent with these findings, native Western blot analysis of cell extracts also showed a reduction in high-molecular weight αsyn species, suggesting expression of these Rabs decreases αSyn interactions and high-molecular weight oligomer species. Size-exclusion data similarly shows a shift toward smaller αsyn species, and provide further support that specific Rab proteins reduce αsyn oligomer species. Samples run under fully denatured conditions indicated no change in total αsyn expression as a result of Rabs. These data suggest that Rabs alter αsyn oligomer, rather than total αsyn levels, either through enhanced degradation or reduction in αsyn-αsyn interactions. These findings provide evidence that Rab proteins may be important mediators of αsyn accumulation and toxicity through possible reduction of toxic oligomeric species.

Disclosures: N.R. McFarland: None. **T. Sahara:** None. **L. Kibuuka:** None.

Poster

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Title: Blockade of type 5 metabotropic glutamate receptors protects the serotonergic system from degeneration in MPTP-treated monkeys

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Abstract: Pathologic, biochemical, and imaging studies indicate a significant degree of degeneration of the serotonin (5-HT) system in Parkinson's disease (PD), which may contribute to some of the non-motor symptoms of the disease. However, reports of 5-HT neurons in toxin-based models of PD have been inconsistent. Differences in toxin use and administration regimen, animal species, and quantitative assessment of neuronal loss may account for these inconsistencies. The goal of the present study was to assess the state of the 5-HT system in a monkey model that mimics the slow and progressive degeneration that occurs in idiopathic PD. Adult Rhesus monkeys were treated with weekly injections of MPTP (n=3, 0.2-0.5 mg/kg, i.m.) for 21 weeks, leading to progressive development of parkinsonian symptoms. Postmortem 5-HT immunohistochemistry and stereological cell count revealed ~80% loss of 5-HT terminals in the striatum and ~35% loss of neuronal cell bodies in the dorsal raphe ($P<0.001$) in MPTP-treated monkeys compared with control. Furthermore, taking into consideration the functional expression of metabotropic glutamate receptor 5 (mGluR5) in 5-HT cells (Bradbury et al., 2003, Neuropharmacology 44: 562), we sought to determine whether treatment with an mGluR5 antagonist could protect 5-HT neurons from degeneration in MPTP-treated monkeys. To address this issue, the highly specific mGluR5 antagonist,3-[(2-methyl-1,3-thiazol-4-yl) ethynyl] pyridine (MTEP) was administered daily (10 mg/kg i.m.) in 4 additional MPTP-treated monkeys. Densitometry measurements revealed a significant preservation of the striatal 5-HT innervation in MPTP/MTEP-treated monkeys (~10% loss from controls) compared with MPTP/vehicle-treated animals (~80% loss from controls). Stereological cell counts of 5-HT-positive neurons in the dorsal raphe are in progress to determine if MTEP also protects 5-HT cell bodies from degeneration in this nonhuman primate model of PD. Together with our recent findings (Masilamoni et al., 2011, Brain 134:2057), the available data suggest that MTEP protects against degeneration of dopaminergic, noradrenergic and 5-HT cells in MPTP-treated monkeys, adding to the potential relevance of mGluR5 antagonists as neuroprotective agents in PD.

Disclosures: G.M. Jeyaraj: None. **A.P. Weinkle:** None. **S. Jenkins:** None. **J.F. Pare:** None. **T. Wichmann:** None. **Y. Smith:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Topic: C.03. Parkinson's Disease

Support: University of Helsinki research foundation, Young scientist research grant

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Support from S. Juselius Foundation

Title: Overexpression of endogenous GDNF in natively expressing cells increases cell numbers and dopamine levels in the nigrostriatal system

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Abstract: Glial cell line-Derived Neurotrophic Factor (GDNF) protects and promotes the survival of embryonic dopamine (DA) neurons in vitro, whereas exogenous and viral vector-induced overexpression of GDNF have been shown to promote survival of midbrain DAergic neurons in vivo against neurotoxic insults. However, the role of endogenous GDNF signaling in the normal function and maintenance of nigrostriatal DAergic neurons remains unclear. To study the effect of increased endogenous GDNF in the development and function of nigrostriatal dopaminergic system, we created a knock-in, hypermorphic mouse model that is overexpressing physiological GDNF in natively GDNF-producing cells under physiological promoter. Heterozygous (hetz) and homozygous (homoz) hypermorphic mice have 30 % and 70 % increased brain GDNF levels, respectively, compared to wt. Hetz mice are viable until at least 17 months of age, whereas homoz mice die by P10, because of kidney failure. We estimated the number of dopaminergic cells in substantia nigra pars compacta with stereological microscopy in the age groups of 7.5 days and 3 months by using TH- and VMAT2-stainings. We also measured the striatal tissue levels of DA and its metabolites with HPLC in the age groups of 7.5 days, 4 months and 17 months. Finally we measured amphetamine (1 mg/kg) induced locomotor activity, and striatal DA response to local amphetamine stimulation (100 µM for 1 hour) using in vivo microdialysis in freely moving animals. We found a 15 % increase in DAergic cell number at the age of P7.5 in both hetz and homoz hypermorphic mice. This increase was retained until 3 months between wt and hetz mice. Also the striatal levels of DA were increased (20 %) at P7.5 equally in both genotypes. This increase also persisted until adulthood and old age. Amphetamine-induced locomotor activity and striatal DA response were augmented by about 30 % in hetz mice as compared to wt. In conclusion, our results demonstrate that already a modest increase in the expression of endogenous GDNF in the naturally GDNF expressing cells functionally enhances the nigrostriatal DAergic system in vivo with persistent changes until old age.

Disclosures: J.J. Kopra: None. **J. Andressoo:** None. **J. Mijatovic:** None. **C. Amberg:** None. **N. Pulkkinen:** None. **P. Piepponen:** None. **M. Saarma:** None.

Poster

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Topic: C.03. Parkinson’s Disease

Support: FAPESP

CAPES-BRAZIL

CNPq

NAPNA-USP

Title: BDNF receptor is involved in the beneficial effects of exercise in hemi-parkinsonians rats.

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Abstract: Parkinson disease (PD) is the focus of intense research of possible strategies for its treatment and prevention. Physical exercise is known to produce beneficial effects to the nervous system, and, in most cases, brain-derived neurotrophic factor (BDNF) appears to be involved in such effects. Our aim was to investigate the role of BDNF in the treadmill exercise-induced histological/neurochemical and behavioral changes in a rat model of unilateral PD induced by striatal injection of 6-hydroxydopamine (6-OHDA). Adult male Wistar rats were divided into four groups: saline (SAL), sedentary (SED), exercised for 30 days before and 30 days after the induction of PD (EXB+EXA), and exercised for 30 days after the induction of PD (EX30A). These groups were further divided into two subgroups each, where half of the rats received K252a (K; a BDNF receptor blocker). To analyze their behavior, the rats were placed in an open field. The animals were placed on a treadmill 3 days/week at the speed of 10m/min during 40min. One month after 6-OHDA injections, the brains were collected for immunohistochemistry and immunoblotting assays, where we measured tyrosine hydroxylase (TH) in the substantia nigra pars compacta (SNc) and the striatum (CPu). BDNF was analyzed by immunoblotting in the groups without K. Statistical analyses were performed using one-way

ANOVA with the Tukey post hoc test.
The exercised groups exhibited reduced damage to dopaminergic cells, in terms of a higher postlesion TH expression. When we added K, TH expression decreased substantially.
There was a decrease of TH immunostaining in the SNc of SED animals without K, when compared to the SAL group (41%, $p<0.01$). The decrease also occurred in the groups receiving 6-OHDA and K (SED - 68%, $p<0.001$; EXB+EXA - 73%, $p<0.001$; EXA - 42%, $p<0.01$). We observed decrease in the immunoblots in the same groups: SED (53%, $p<0.001$), SED-K (42%, $p<0.01$), EXB+EXA-K (38%, $p<0.05$), e EXA-K (47%, $p<0.05$). The CPu provided data similar to those found in the SNc in all cases.
BDNF decreased in the SNc in the SED group (54%, $p<0.05$), which was the group that presented the lowest TH expression. The open field test revealed that the animals whose TH levels decreased explored more actively the environment in the second exposure, suggesting a memory deficit. The trained animals that received K showed the worst results (EXA+EXD - 38%, $p<0.05$; EXD - 32%, $p<0.05$). SAL and SED groups did not change after K injection.
Our data reveal that physical exercise is capable of reducing the damage induced by 6-OHDA, and that BDNF receptors are involved in that effect. Supported by FAPESP, CAPES, NAPNA - USP and CNPq (Brazil).

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Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: LSRB-DRDO EPB200/ 2009

Title: Neuroprotective effect of Naringenin in Rotenone induced model of Parkinson’s disease

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Abstract: Parkinson's disease (PD) is a multifactoral age related neurodegenerative disorder eliciting death of dopaminergic neurons in the substantia nigra. Investigations have been done using experimental models of PD to understand the pathogenesis of the disease, especially, those produced by inducing neurotoxins like rotenone. Rotenone is linked to the development of PD as it interferes with the electron transport system in mitochondria. It is a well-known inhibitor of mitochondrial complex I. An increasing large body of research on Parkinson's disease (PD) has been focused on understanding the mechanisms behind the potential neuro protection offered by bioflavonoids. Rotenone induced rodent model of PD was generated, which was found to have debilitating phenotype and neurodegeneration. This study was aimed at investigating whether the natural phenolic compound naringenin can be neuro protective in the rotenone induced model of PD. Naringenin being powerful antioxidants also possess neuro protective and anti-ageing properties. Rotenone induced neurodegeneration and loss in motor skills was observed in the rodent model of PD. The histopathological analysis and immunofluorescence study of proteins of interest revealed betterment after oral administration of naringenin to the rotenone induced PD models. The drug significantly attenuated neurodegeneration in different regions of the rotenone treated PD rat brain. A modest increase in neuronal survivability was also found. In conclusion, treatment with bioflavonoid naringenin conferred neuro protection against rotenone induced neurotoxicity in PD rat model.
Keywords: Parkinson's disease, Bioflavonoids, Rotenone, Neuroprotection
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Conflict of interest: None

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Poster

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Topic: C.03. Parkinson's Disease

Support: Oakland University - Beaumont Multidisciplinary Grant

donation from family of Norman Merollis

Title: Serum alpha synuclein and anti-alpha synuclein antibodies in healthy subjects and individuals with Parkinson's disease, atypical parkinsonism, or rapid eye movement sleep behavior disorder

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Abstract: α-synuclein is thought to play a prominent role in the pathogenesis of Parkinson's disease (PD) because it is the major protein in Lewy bodies, and because gene mutations and deletions increasing its expression and aggregation are associated with early-onset, autosomal dominant PD. There are conflicting reports as to the status of serum and plasma α-synuclein and anti-α-synuclein antibodies in PD, and the association between the levels of these proteins is unknown. The objectives of this study were therefore (1) to compare, by ELISA measurement, the concentrations of serum α-synuclein and anti-α-synuclein antibodies between subjects with PD (both idiopathic PD [n = 14] and atypical parkinsonism [n = 11]), rapid eye movement sleep behavior disorder (RBD; n = 10), and healthy controls (n = 9), and (2) to determine the strength and direction of association between serum α-synuclein and anti-α-synuclein antibody levels. Results: Mean concentrations for specific anti-α-synuclein antibodies (i.e., after subtraction of nonspecific binding of serum to BSA-coated wells) ranged from 0.97 to 1.46 μg/ml, and mean α-synuclein concentrations ranged from 6.92 - 10.51 ng/ml. Analysis on log-transformed data found no significant differences between groups for either concentration. Mean nonspecific binding of serum samples ranged from 28% to 41% of total binding to α-synuclein. No relationship was evident between serum anti-α-synuclein antibody or α-synuclein levels and subject age among all subjects; however, in subjects with idiopathic PD or atypical parkinsonism, specific antibody concentrations above 1.5 μg/ml were associated with shorter disease duration. Power analysis indicated that a two-sided t test on log - transformed data at the .05 level with 87 samples per group achieves 80% probability to detect a 50% increase in mean anti-α-synuclein antibody levels; similarly, 283 samples per group achieves 80% power to detect a 25% increase. Conclusions: This study found no evidence for differences in serum levels of anti-α-synuclein antibodies or α-synuclein between subjects with idiopathic or atypical PD, RBD, and healthy controls. Measurements of these proteins in peripheral blood are therefore not likely to be useful as biomarkers for early diagnosis of PD. The functional significance of anti-α-synuclein antibodies in normal subjects and individuals with PD requires further clarification. (Funding: Oakland University - Beaumont Multidisciplinary Grant; donation from the family of Norman Merollis)

Disclosures: D.A. Loeffler: None. **L. Smith:** None. **M. Coffey:** None. **A. Klaver:** None. **M. Schiess:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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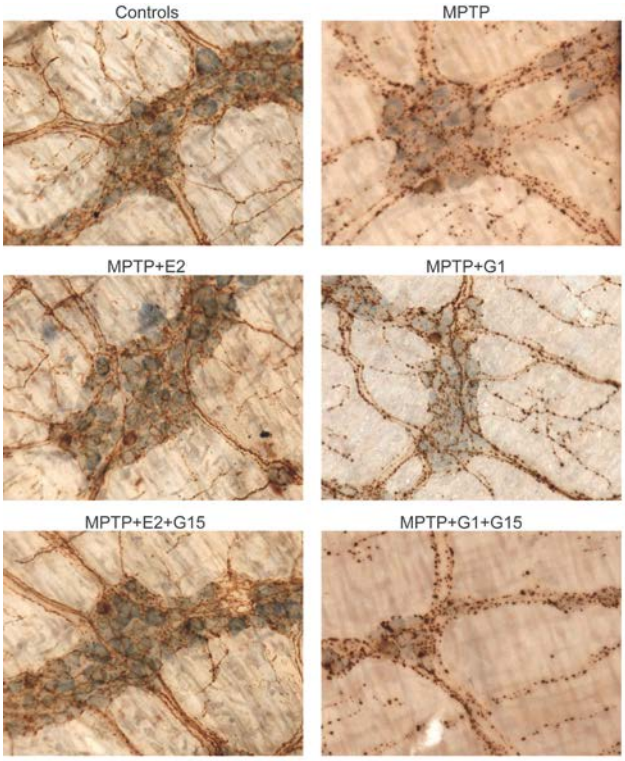
Topic: C.03. Parkinson's Disease

Title: Neuroprotective role of estradiol in MPTP-induced myenteric neuron degeneration

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Abstract: Introduction: Patients suffering from Parkinson's disease (PD) frequently display non-motor symptoms like delayed gastric emptying, constipations and defecatory dysfunctions. These gastrointestinal dysfunctions are associated with the degeneration of dopaminergic (DA) neurons in the myenteric nervous system. Importantly, the immune system plays a critical role in the MPTP-induced degeneration of DA neurons in the myenteric plexus (MP) in mice. Of great interest, the hormone estradiol (E2) has shown immunomodulating properties in cell culture and is also well known to be neuroprotective in the central nervous system in animal models of PD. Since estrogen receptors (ERs) activation is associated with an increase of thrombosis, stroke and breast cancer risk, we decided to investigate the role of the new G-Protein coupled estrogen receptor-1 (GPER1) in the effect of E2 on MPTP toxicity.
Objectives: We aim to study the therapeutic potential of E2 and the activation of GPER1 in the MP of a PD mouse model.
Methods: Various treatments were performed in male mice with MPTP combined with E2, the GPER1 agonist G1 as well as its antagonist G15. MPs from distal ileum were microdissected and then processed for immunohistochemistry. Macrophage density and neuronal survival were assessed by stereology.
Results/Discussion: Treatment with MPTP induced 80% loss in tyrosine hydroxylase (TH)-positive neurons in the MP as well as a significant loss in TH-positive fiber density. This degeneration was accompanied with a 50% significant increase in macrophage density within the MP, suggesting that a strong immune response takes place locally. MPTP-treated mice receiving E2, G1 or E2+G15 showed no loss of TH neurons in the MP, while the G1+G15 treatment was inefficient to decrease the MPTP-induced DA neuronal death. In presence of E2 or G1, the immune response was also abolished. Altogether, these results suggest that the estrogen receptors alpha and beta as well as GPER1 are involved in the protection of DA neurons in the MP, and

could be potential therapeutic targets in gastrointestinal dysfunctions in PD.



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Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: NSERC grant to MM

Title: Quercetin and sesamin protect dopaminergic cells from MPP^+-induced neuroinflammation in a microglial-neuronal co-culture system.

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Abstract: Growing evidence suggests that a majority of cases of Parkinson's disease (PD) are associated with microglia activation with resultant elevation of various inflammatory mediators and neuroinflammation. Cytokines have been proposed as candidates that mediate the apoptotic cell death of dopaminergic neurons implicated in PD. In this study, we investigated the effect of two natural molecules, quercetin and sesamin, on neuroinflammation induced by the parkinsonian toxin 1-methyl-4-phenylpyridinium (MPP^+) in a glial-neuronal co-culture system. Firstly, we established that quercetin or sesamin defend microglial cells from the MPP^+-induced increase of mRNA or protein levels of three proinflammatory cytokines (IL-6, IL-1alpha and TNFalpha) as revealed by RT-qPCR and ELISA assay, respectively. Quercetin or sesamin also decrease MPP^+-induced oxidative stress in microglial cells by reducing iNOS protein expression as well as mitochondrial superoxide radicals. We then measured neuronal cell death and apoptosis after MPP^+ activation of microglia, in a microglial-neuronal co-culture system. Our results demonstrated that quercetin or sesamin could rescue neuronal PC12 cells from apoptotic death induced by MPP^+ activation of microglial cells.
Altogether our results demonstrate that the phytoestrogen quercetin and the lignan sesamin diminishes MPP^+-induced microglial activation and suggest that these molecules may be regarded as potent natural anti-inflammatory compounds.

Disclosures: J. Renaud: None. **J. Bournival:** None. **M. Plouffe:** None. **A. Arel-Dubeau:** None. **M. Martinoli:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Topic: C.03. Parkinson's Disease

Support: the National Natural Science Foundation of China (Grants No. 30901800)

the Priority Academic Program Development of Jiangsu Higher Education Institutions

Title: α7 nicotinic acetylcholine receptor-mediated neuroprotection against dopaminergic neuron loss in an MPTP mouse model via inhibition of astrocyte activation

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Abstract: Background: Although evidence suggests that the prevalence of Parkinson's disease (PD) is lower in smokers compared to nonsmokers, the mechanisms of nicotine-induced neuroprotection remain unclear. α7 nicotinic acetylcholine receptor (α7-nAChR) stimulation appears to be a critical mechanism underlying the anti-inflammatory potential of cholinergic agonists in immune cells, including astrocytes. The inhibition of astrocyte activation has been proposed as a novel strategy for the treatment of neurodegenerative disorders such as PD. The objective of the present study was to determine whether nicotine-induced neuroprotection in an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model occurs via α7-nAChR-mediated inhibition of astrocytes.
Methods: Both in vivo (MPTP) and in vitro (1-methyl-4-phenylpyridinium ion, MPP^+; lipopolysaccharide, LPS) models of PD were used to investigate the role(s) and the possible mechanism(s) whereby α7-nAChRs protect against dopaminergic neuron loss. Multiple experimental approaches, including behavioral tests, immunochemistry and stereology experiments, astrocyte cell cultures, reverse transcription-polymerase chain reaction, laser scanning confocal microscopy, TNF-α assays and Western blot, were employed to elucidate the mechanisms of α7-nAChR-mediated neuroprotection.
Results: Systemic administration of nicotine alleviated MPTP-induced behavioral symptoms, improved motor coordination, protected against dopaminergic neuron loss and the activation of astrocytes and microglia in the substantia nigra. The protective effects of nicotine were abolished by administration of the α7-nAChR-selective antagonist methyllycaconitine (MLA). In mouse primary cultured astrocytes, pretreatment with nicotine suppressed MPP^+- or LPS-induced astrocyte activation evidenced by both decreased production of TNF-α and inhibition of Erk1/2 and p38 activation in astrocytes, and these effects also were reversed by MLA.
Conclusion: Taken together, our results suggest that α7-nAChR-mediated inhibition of astrocyte activation is an important mechanism underlying the protective effects of nicotine.

Disclosures: H. Jun: None. **Y. Liu:** None. **J. Wu:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Topic: C.03. Parkinson's Disease

Support: Research Grand for Longevity Science (21A-13, 23A-2) from the Ministry of Health, Labour and Welfare, Japan

Title: Induction of GDNF and neurotrophins by rasagiline and selegiline

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Abstract: Rasagiline and selegiline, inhibitors of type B monoamine oxidase (MAO-B), protect neuronal cells in animal and cellular models of neurodegenerative disorders. Molecular mechanism behind their neuroprotective action has been clarified to be the regulation of mitochondrial death signal pathway and the induction of pro-protective anti-apoptotic Bcl-2 and neurotrophic factors (NTFs). Type A MAO (MAO-A) was found to mediate the induction of Bcl-2 by these MAO-B inhibitors in MAO-A-expressing neuroblastoma SH-SY5Y cells. Rasagiline and selegiline increased the mRNA and protein levels of glial cell line-derived neurotrophic factor (GDNF) and neurotrophins [brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin 3 (NT-3)] in SH-SY5Y cells. Rasagiline increased GDNF more markedly than neurotrophins, and vice versa selegiline increased neurotrophins. The distinct induction of GDNF and neurotrophins by MAO inhibitors was also confirmed in non-human primates. Rasagiline was systematically administered in Japanese monkeys (n=4) for 4 weeks by daily subcutaneous injection, and the cerebrospinal fluid (CSF) was taken once a week. The NTF levels were quantified by the EIA assay. Rasagiline at 0.25 mg/day increased GDNF significantly, followed by BDNF, NGF and NT-3. On the contrary, selegiline increased BDNF in the CSF from parkinsonian patients treated with selegiline. The role of MAO-A and MAO-B in the induction of neurotrophins was investigated in wild SH-SY5Y cells and MAO-B-expressing glioblastoma U118MG cells. The effects of these MAO-B inhibitors on mRNA levels of neurotrophins were measured by use of real-time RT-PCR method. Rasagiline increased BDNF mRNA in wild SH-SY5Y cells, which was suppressed by treatment with siRNA against MAO-A, whereas selegiline increased BDNF mRNA in wild and siRNA treated cells. Selegiline and rasagiline induced BDNF mRNA in U118MG cells, and selegiline was more potent than rasagiline. These results suggest that NTF gene induction of rasagiline may depend on MAO-A, whereas selegiline may not in SH-SY5Y cells. In U118MG cells, these MAO-B inhibitors induce prosurvival NTFs through different signal pathway. The distinct signal pathway activated by rasagiline and selegiline may account for the preferential induction of GDNF and neurotrophins in animal and human experiments. The molecular mechanisms of the signal pathways should be further investigated to clarify the selective induction of these two NTF classes by rasagiline and selegiline.

Disclosures: M. Naoi: None. **K. Inaba-Hasegawa:** None. **M. Shamoto-Nagai:** None. **W. Maruyama:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: Michael J. Fox Foundation for Parkinson's Research

NIH-NCRR000168/OD011103

MedGenesis Therapeutix Inc

Title: Intranasal GDNF for Parkinson's disease: Next steps in preclinical development

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Abstract: We have previously shown that glial cell line-derived neurotrophic factor (GDNF) given by the nasal route of administration provides significant protection of substantia nigra (SN) dopamine neurons in the rat 6-hydroxydopamine (6-OHDA) model of Parkinson’s disease (PD) (Migliore et al., Soc. Neurosci. Abstr. #247.21, 2008 and #828.21, 2009). The next step in preclinical development is to evaluate the neuroprotective efficacy of intranasal GDNF in a primate model of PD. We have chosen the MPTP-lesioned marmoset model and selected an MPTP dose regimen which produces a partial lesion of the SN dopamine neurons, i.e. 2 mg/kg s.c. on day 1 and 1 mg/kg s.c. on days 2 and 3. We established in a pilot study that this regimen causes an approximately 50% loss of SN dopamine neurons and 65% decrease in tyrosine hydroxylase (TH) immunostaining density in the SN relative to that of unlesioned controls. To test the neuroprotective efficacy of intranasal GDNF, marmosets were anesthetized with ketamine and placed in a supine position with their noses upright. Doses were administered intranasally in five 10 µl aliquots at 5 min intervals alternating sides using a BD Accuspray device. GDNF (100 µg in 50 µl phosphate-buffered saline; n=9) or an equivalent volume of

saline (n=9) was given intranasally on 5 consecutive days. The first intranasal dose was given 1 day prior to the start of MPTP administration, and subsequent doses were given 1 hr prior to each of the 3 daily doses of MPTP, and 1 day after the last dose of MPTP. The animals were allowed to recover for 2 months prior to sacrifice. Lesion severity was assessed using both behavioral and neurochemical measures. Behavioral tests, including locomotor activity monitoring and performance on an object retrieval task, were performed for 2 to 8 wks prior to MPTP, respectively, and for the 2 months after MPTP. Motor disability scoring was performed biweekly after MPTP using a rating system devised for marmosets by Jenner and co-workers (Iravani et al, Eur. J. Neurosci. 21: 841, 2005). Neurochemical evidence of lesion severity was assessed by tyrosine hydroxylase (TH) immunohistochemistry (IH). Dopamine cell counts in the SN, and TH immunostaining densities in the SN and striatum of each animal, were used as measures of relative neuroprotection in the GDNF-treated animals.
The study concludes in September 2012. Results will be presented.

Disclosures: G.C. Newsome: None. **L. Wachtman:** None. **M.M. Migliore:** None. **A. Aly:** None. **N. Silva:** None. **S. Westmoreland:** None. **J. Rowlett:** None. **B.L. Waszczak:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:856.11/G4

Topic: C.03. Parkinson’s Disease

Support: JPB Foundation

NIH Grant NS059806

Title: Unsubstituted Phenothiazine is protective in the rotenone model of Parkinson’s disease

Authors: *V. TAPIAS[1], P. G. MASTROBERARDINO[2], X. HU[1], J. NELSON[1], T. SEW[1], J. T. GREENAMYRE[1,3]
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Abstract: Selective degeneration of dopaminergic (DA) neurons in the substantia nigra underlies the cardinal motor impairments of Parkinson’s disease (PD). Although the molecular mechanisms of pathogenesis remain unknown, there is substantial evidence that mitochondrial dysfunction and oxidative stress play key roles in the pathogenesis of PD. Inhibition of complex I enhances reactive oxygen species (ROS) formation; then, a feed-forward cycle ensues in which

local ROS production can damage complex I, increasing ROS production and leading to deleterious oxidation of lipids, proteins, and nucleic acids. Oxidative stress and mitochondrial dysfunction are early events in DA cell death evoked by rotenone, a pesticide implicated in human PD that crosses biological membranes. Rotenone had a detrimental effect, reducing the number of DA neurons and leading to shrinkage of neuronal processes as well as causing oxidative stress, both in *in vitro* cell systems and in animals. The aim of the current study was to evaluate the neuroprotective potential of several antioxidant drugs (which remain under-explored in the PD field) in the rotenone model *in vitro* and *in vivo*. Our *in vitro* drug screening revealed a neuroprotective role of various dosages of different drugs in primary ventral midbrain cultures, with a significant preservation of the number of DA neurons, neurite outgrowth, and protein thiol oxidation. Among the candidates, phenothiazine (PTZ) - an aromatic imine - was selected for the subsequent *in vivo* study for diverse reasons: PTZ exerts significant effects at nanomolar concentration, an EC_{50} value 100-10,000-fold lower than most other antioxidants, and may penetrate the BBB more effectively. Furthermore, PTZ has been shown to lack any significant DA receptor binding activity. Our data showed that post-treatment with PTZ (20 mg/kg/day BID) robustly protected against rotenone-induced nigrostriatal lesion in rats.

Disclosures: V. Tapias: None. **P.G. Mastroberardino:** None. **X. Hu:** None. **J. Nelson:** None. **T. Sew:** None. **J.T. Greenamyre:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:856.12/G5

Topic: C.03. Parkinson’s Disease

Support: Midwestern University Intramural Funds

Title: Nicotine increases lifespan and rescues motor and olfactory deficits in parkin heterozygote loss-of-function *Drosophila* melanogaster

Authors: R. P. CHAMBERS, G. B. CALL, *T. JONES, L. M. BUHLMAN
Midwestern Univ., GLENDALE, AZ

Abstract: Parkinson’s disease (PD) is the most common neuromotor degenerative disease, affecting around 13 out of 100,000 people in the US, can exist in either sporadic or familial forms (Van Den Eeden *et al*., 2003). *Drosophila melanogaster* is a particularly attractive model for familial PD, as park25/park25 mutants exhibit many of the pathologies found in PD patients, including mitochondrial pathology, motor deficits and decreased lifespan. Progressive motor

deficits found in park25/park25 *D. melanogaster* are thought to result from mitochondrial pathology that causes indirect flight muscle degeneration (Greene *et al*., 2003). We have found that the heterozygote parkin loss-of-function *D. melanogaster* (park+/park25) have decreased lifespan, motor function and olfaction when compared with the park+/park+ (w1118). Epidemiological studies suggest that tobacco smokers are dose-dependently less likely to develop PD (Hernan *et al*., 2001; Grandinetti *et a*l., 1994; Rajput *et al*., 1987); subsequent *in vitro* and *in vivo* studies show that nicotine is protective in models of sporadic PD (reviewed in Quik *et al*., 2009). Lack effective PD therapeutic treatment could be due to the fact that extensive neurodegeneration has occurred by the time motor deficiencies appear and therapy is implemented. Evidence suggests that earlier therapeutic intervention could be more effective in halting or slowing disease progression (Jeyarasasingam *et al*., 2002) and prolonging patient quality of life. Olfactory deficits are one of the initial symptoms of PD, and we have determined that park+/ park25 also have olfactory deficits that precede motor deficits. Literature addressing the potential protection by nicotine in parkin loss-of-function models spans limited concentrations and selected time points in the organism's lifespan. Park+/ park25 were exposed to a range of nicotine concentrations from eclosion until behavioral assays were performed on days 5, 10 15 and 20. Our data show that at several concentrations and days of administration, nicotine can protect against the various deficits in this model of familial PD. Patients with inherited forms of PD can be easily identified via genetic testing. Our results suggest that early nicotinic therapeutic intervention in parkin-loss-of-function PD warrants further investigation.

Disclosures: R.P. Chambers: None. **G.B. Call:** None. **T. Jones:** None. **L.M. Buhlman:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:856.13/G6

Topic: C.03. Parkinson's Disease

Title: Δ9-Tetrahydrocannabinol and pioglitazone are protective in a cell culture model of Parkinson's disease through divergent PPARγ dependent mechanisms

Authors: *M.-L. ZEISSLER, C. O. HANEMANN, J. P. ZAJICEK, C. B. CARROLL
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Abstract: Introduction: Cannabinoids such as Δ9-tetrahydrocannabinol (Δ9-THC) are neuroprotective in animal and cell culture models of Parkinson's disease (PD). In a PD cell culture model we recently demonstrated that Δ9-THC is neuroprotective through activation of

the nuclear receptor peroxisomal proliferator-activated receptor γ (PPARγ) and activation by specific agonists rosiglitazone and pioglitazone, has been found neuroprotective by others. Here we investigate downstream mechanisms of PPARγ receptor activation by Δ9-THC and pioglitazone.
Methods: SH-SY5Y neuroblastoma cells were differentiated with retinoic acid and exposed to the mitochondrial complex 1 inhibitor 1-methyl-4-phenylpyridine (MPP+) a neurotoxin known to induce a parkinsonian phenotype in man. Δ9-THC and pioglitazone were co-administered with the minimum concentration of the specific PPARγ antagonist T0070907 able to block the protective effect of each compound respectively for 48 hours. The production of reactive oxygen species was then measured and proteins were extracted for Western blotting.
Results: Although others have shown enhanced expression of the nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor alpha (IκBα) to be involved in PPARγ mediated neuroprotection against MPP+ we found that the expression of this protein was not significantly changed by Δ9-THC or pioglitazone. However, Δ9-THC resulted in significant inhibition of MPP+ induced oxidative stress which could be completely reversed with the PPARγ antagonist T0070907 whereas pioglitazone induced reduction in oxidative stress did not seem to be PPARγ dependent. Expression of the anti-oxidant enzymes catalase and superoxide dismutase 1 (SOD1) which are both known to have a PPARγ response element (PPRE) within their promoter region, remained unchanged by both pioglitazone and Δ9-THC. However, both pioglitazone and Δ9-THC were able to restore MPP+ induced downregulation of peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1-α), a protein involved in oxidative stress response and mitochondrial biogenesis, to the level of untreated control. This effect was inhibited by T0070907 in the case of Δ9-THC but not pioglitazone.
Conclusions: Even though Δ9-THC and pioglitazone are both protective against MPP+ in a PPARγ dependent manner they appear to activate different downstream mechanisms.

Disclosures: M. Zeissler: None. **C.O. Hanemann:** None. **J.P. Zajicek:** None. **C.B. Carroll:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Program#/Poster#:856.14/G7

Topic: C.03. Parkinson’s Disease

Support: NIH/NIGMS (2 SO6 GM08016-39)

OTKAK72592, MTA Momentum Program

Title: Protective effects of PACAP against salsolinol-induced toxicity in SH-SY5Y cells: Implications for Parkinson's disease

Authors: *D. O. BROWN[1], D. REGLODI[2], Y. TIZABI[1]
[1]Howard Univ. Col. of Medicine, Dept. of Pharmacol., Washington, DC; [2]Dept. of Anat., PTE-MTA Momentum PACAP Res. Group, Univ. of Pecs, Pecs, Hungary

Abstract: Pituitary adenylate cyclase-activating polypeptide (PACAP) is an endogenous neuropeptide with various cytoprotective functions including neuroprotection. Exogenous administration of PACAP has been shown to reduce damage induced by ischemia, trauma or toxic substances. Moreover, mice deficient in PACAP are more vulnerable to damaging insults. In this study we sought to determine whether PACAP may also be protective against salsolinol-induced toxicity in SH-SY5Y cells. Salsolinol (SALS) is an endogenous dopamine metabolite with selective toxicity to nigral dopaminergic neurons, which are directly implicated in Parkinson's disease (PD). SH-SY5Y cells, derived from human neuroblastoma cells express high level of dopaminergic activity and are used extensively as a model to study these neurons. Exposure of SH-SY5Y cells to 400 uM SALS for 24 h resulted in approximately 40% toxicity as determined by MTT assay. Pretreatment with PACAP (1-38) dose-dependently attenuated SALS-induced toxicity with maximal protection (approx. 62%) by 200 nM PACAP. The effects of PACAP in turn were dose-dependently blocked by PACAP antagonist (PACAP 6-38) with full antagonism at 2 uM. PACAP (6-38) is a strong blocker of PAC1 receptor. Neither PACAP (1-38) nor PACAP antagonist (6-38) had any effect of their own on cellular viability. These results suggest protective effects of PACAP in a cellular model of PD. Moreover, the effects of PACAP appear to be mediated through PAC1 receptor. Hence, PACAP or PAC1 agonists could be of therapeutic benefit in PD.

Disclosures: D.O. Brown: None. **D. Reglodi:** None. **Y. Tizabi:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:856.15/G8

Topic: C.03. Parkinson's Disease

Support: NIMH Grant 1RO1MH087986

Title: The efficacy of neuroprotective molecules in blocking MPTP-mediated apoptosis of mature dopaminergic neurons in a model of Parkinson's disease

Authors: *J. N. DRAWBRIDGE[1], H. J. DE JESUS CORTES[2], P. XU[1], S. ESTILL[1], P. HUNTINGTON[3], J. NAIDOO[1], N. S. WILLIAMS[1], J. READY[1], S. MCKNIGHT[1], A. PIEPER[3]
[1]Biochem., [2]Basic Sci. Grad. Program, [3]Psychiatry, UT Southwestern Med. Ctr., Dallas, TX

Abstract: We previously reported the *in vivo* discovery of P7C3, an aminopropyl carbazole endowed with proneurogenic and neuroprotective properties in newborn neural precursor cells in the subgranular zone of the dentate gyrus. We now show that P7C3 additionally displays neuroprotective efficacy in mature neurons as well. P7C3 blocks MPTP-mediated cell death of dopaminergic neurons in the substantia nigra of adult mice, as well as in dopaminergic neurons of *Ceanorhabditis elegans*. In both *in vivo* paradigms of toxicity, the P7C3 analog P7C3A20 blocks cell death with greater potency and efficacy than P7C3. This parallels the relative potency and efficacy of these molecules in blocking apoptosis of newborn neural precursor cells of the dentate gyrus. The chemical scaffold represented by P7C3 and P7C3A20 may serve as a basis for developing new pharmacologic agents for treating patients with Parkinson's disease.

Disclosures: J.N. Drawbridge: None. **H.J. De Jesus Cortes:** None. **P. Xu:** None. **S. Estill:** None. **P. Huntington:** None. **J. Naidoo:** None. **N.S. Williams:** None. **J. Ready:** None. **S. McKnight:** None. **A. Pieper:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS059806

JPB Foundation

Michael J. Fox Foundation

Title: Characterization of a novel peroxidase (PXDNL) and its interaction with the molecular chaperone HSP90.

Authors: *E. K. HOFFMAN[1,2], J. T. GREENAMYRE[2]
[1]Univ. Pittsburgh, PITTSBURGH, PA; [2]Neurol., Univ. Pittsburgh, Pittsburgh, PA

Abstract: Parkinson's disease (PD) is the second most common age-related neurodegenerative disease with pathological characteristics including reduced activity of mitochondrial complex I and loss of dopaminergic neurons in the substantia nigra. Despite the fact that the etiology of PD

has been the focus of extensive investigation, the genetic and biochemical pathways leading to the onset and progression of PD remain poorly understood. The environmental toxin rotenone may increase the risk of PD in humans, and has been used to model the disease in rodents. It is believed that rotenone's toxicity is due to inhibition of mitochondrial complex I, leading to increased oxidative stress and reduced ATP production. Recent evidence from our laboratory has demonstrated that overexpression of a heme peroxidase, PXDNL (Peroxidasin homolog Drosophila-like), is associated with increased resistance to rotenone and hydrogen peroxide toxicity. In the present study we characterize the interaction of PXDNL protein with the molecular chaperone HSP90 in the human neuroblastoma cell line SH-SY5Y. Co-immunoprecipitation studies identified HSP90 as a binding protein interacting with PXDNL. Treatment of cells with rotenone resulted in transcriptional up-regulation of both PXDNL and HSP90. This change in gene expression may represent a novel neuroprotective mechanism initiated in response to toxic stress. We hypothesize that the interaction of PXDNL with HSP90 is a uniquely attractive target for therapeutic manipulation in PD.

Disclosures: E.K. Hoffman: None. **J.T. Greenamyre:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Program#/Poster#:856.17/G10

Topic: C.03. Parkinson's Disease

Support: NIHR01NS041674

NIHR21NS049623

Title: Cooperation between prenatal MPTP and postnatal SAM in the reduction of striatal dopamine, DOPAC and 3-MT in C57BL mice.

Authors: *G. MUTHIAN, M. SMITH, V. MACKAY, B. GRIFFIN, C. CHARLTON
Neurosci. & Pharmacol., Meharry Med. Col., NASHVILLE, TN

Abstract: Parkinson's disease (PD) is marked by the loss of dopamine (DA) neurons in the substantia nigra and DA depletion in the striatum. The cause is unknown. Our lab demonstrated that S-adenosy-L-methionine (SAM), the product of ATP and methionine, caused PD-like changes in rodents. SAM is the biological methyl donor, is ubiquitous and its activities are increased in aging. SAM avidly reacts with and destroys DA and L-dopa, therefore, it may contribute to the reduction of DA seen in PD. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

(MPTP) is also a toxin to DA neurons, so MPTP and SAM were used to test the hypothesis that a **sensitization stage and a precipitating stage** underlie idiopathic PD. To induce the **sensitization stage**, pregnant C57BL/6J mice were treated with MPTP (10mg/kg/dy) during gestation days 8-12 to target the emerging fetal nigrostriatal DA neurons. For the **precipitating stage**, the 3-months old offspring were administered dosage of SAM into the lateral ventricle, to simulate an age-related change; since methylation is increased during aging and SAM depletes DA and methylates and down-regulates DA receptors. Both the prenatal MPTP (MPTP+PBS) and postnatal SAM (PBS+SAM) treatments reduced DA and its metabolites. As compared to the control (PBS-PBS) offspring prenatal MPTP (MPTP+PBS) reduced DA by 32.1%. In the PBS control icv SAM of 0.125µmol, 0.25µmol and 0.5µmol reduced DA by 46.7, 67.2 and 75.1%. In the prenatal MPTP offspring the same dosage of SAM reduced DA by 72.8, 76.9 and 92.0%. Similar changes were noted for DOPAC and 3-MT. The study showed that prenatal exposure to the toxin, MPTP, caused sub-threshold harm to the striatal dopaminergic system and that a naturally occurring metabolite, SAM, that is increased during aging had an additive effect in reducing DA to the threshold at which PD-symptoms are seen, thus SAM may be considered a precipitating factor. SAM is produced by all cells, controls multiple reactions and may be one of the metabolites that allow aging to be a major risk factor for PD and for other degenerative disorders.

Disclosures: G. Muthian: None. **M. Smith:** None. **V. Mackay:** None. **B. Griffin:** None. **C. Charlton:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant NS74443

NIH Grant ES10586

Title: Rationally designed PKD1 activator protects against neurodegeneration in pre-clinical models of Parkinson’s disease

Authors: *A. G. KANTHASAMY, A. ASAITHAMBI, A. GHOSH, M. AY, H. JIN, M. NEAL, K. MULLIN, V. ANANTHARAM, A. KANTHASAMY
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Abstract: Oxidative stress has been implicated as a major pathological mechanism in the degenerative processes underlying Parkinson's disease (PD). The key signal transduction and regulatory networks involved during the degenerative process in PD are currently being explored for identification of novel neuroprotective strategies. We recently discovered that the activation of Protein Kinase D1 (PKD1) acts as a novel compensatory mechanism in PD models, and positive modulation of PKD1 can offer neuroprotection against oxidative damage of dopaminergic neurons (Asaithambi et al., 2011. Mol Neurodegener. 6:43). Consistent with these findings, herein we show that positive modulation of PKD1 activity by using constitutively active PKD1 or pharmacological PKD1 activator protected against MPP+ and 6-OHDA-induced toxicity in cell culture PD models, whereas pharmacological inhibition of PKD1 activity with the small molecule inhibitor kbNB 142-70 exacerbated the neurotoxicity. To extend our translational strategy, we rationally designed and synthesized PKD1 activator peptides and further characterized their neuroprotective role in pre-clinical models of PD. Following the extensive screening, the peptide AK-P4 was identified to activate PKD1 specifically, and protected against MPP+ and 6-OHDA-induced neurotoxicity cell culture models of PD. Importantly, intravenous administration of AK-P4 tagged with TAT sequence (AK-P4T) significantly activated PKD1 in mice. The neuroprotective effects of AK-P4T were tested in the MPTP-induced PD model. Co-treatment with AK-P4T significantly protected against MPTP-induced behavioral deficits, neurotransmitter depletion and histological cell loss. Collectively, our results demonstrate that rationally designed PKD1 activator peptide AK-P4T protected against neurodegeneration in the pre-clinical models of PD. These results suggest that positive modulation of PKD1 using AK-P4T peptide shows promise as a potential therapeutic agent against PD.

Disclosures: A.G. Kanthasamy: None. **A. Asaithambi:** None. **A. Ghosh:** None. **M. Ay:** None. **H. Jin:** None. **M. Neal:** None. **K. Mullin:** None. **V. Anantharam:** None. **A. Kanthasamy:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Program#/Poster#:856.19/G12

Topic: C.03. Parkinson's Disease

Support: AG000959-07

AG000945-03

Title: Aldehyde dehydrogenase 1 determines the regional selectivity of nigral neuron loss in Parkinson disease

Authors: *G. LIU
NIA, NIH, BETHESDA, MD

Abstract: While a preferential degeneration of nigral dopaminergic (DA) neurons is well recognized in Parkinson disease (PD), less is known about the regional selectivity of DA neuron loss within the substantia nigra pas compacta (SNpc). This differential susceptibility of nigral DA neurons in PD indicates the existence of diverse subpopulations of DA neurons in the SNpc, which may possess the molecular clues critical for understanding the cause of nigral neurodegeneration in this disease. Here we show that aldehyde dehydrogenase 1 (ALDH1A1), an enzyme involved in oxidation of dopamine-derived aldehyde intermediates, contributes to the regionally selective loss of nigral DA neurons in PD. ALDH1A1 is selectively expressed by DA neurons located in the ventrolateral (VL) tier of SNpc in the rodent brain, which may divide the nigral DA neurons into two subpopulations along the dorsomedial (DM) to VL axis. Interestingly, we found in a line of PD-related mutant α-synuclein transgenic mice, the progressive loss of DA neurons mainly occurred in the DM tier of SNpc that lacks the expression of ALDH1A1. Furthermore, genetic ablation of ALDH1A1 exacerbated the degeneration of nigral DA neurons in these mice, supporting a protective function of ALDH1A1 against α-synuclein-induced nigral DA neuron loss. Consistent with our findings in the mouse model, ALDH1A1 also displayed a conserved topographic distribution in the ventral tier of human SNpc. In particular, a similar preferential loss of ALDH1A1-lacking dorsal nigral neurons was evident in the SNpc of patients with sporadic PD. Therefore, our findings establish ALDH1A1 as a key molecular determinant of the regional vulnerability of nigral DA neurons in PD, suggesting that ALDH1A1 may protect against nigral DA neurodegeneration by preventing the cytotoxic accumulation of α-synuclein aggregates.

Disclosures: G. Liu: Research Grant; the intramural research programs of National Institute on Aging.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

Location: Hall F-J

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Program#/Poster#:856.20/G13

Topic: C.03. Parkinson’s Disease

Support: PAPIIT 220111

PAPCA–Iztacala UNAM- No. 30-2010-2011

Title: L-Dopa/melatonin treatment effect on neurogenesis in substantia nigra compacta in rats lesioned with 6-OHDA.

Authors: M. MORENO RIVERA[1], C. TORRES ESQUIVEL[1], R. GARCÍA RUÍZ[1], A. GUTIÉRREZ VALDEZ[1], J. SANCHEZ BETANCOURT[1], T. IBARRA GUTIÉRREZ[1], J. RAMOS JIMENEZ[2], M. AVILA COSTA[1], *V. ANAYA-MARTINEZ[1]
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Abstract: Parkinson disease (PD) is a neurodegenerative disorder of unknown etiology characterized by the loss of dopaminergic neurons in the substantia nigra compacta (SNc), conventional therapy with L-Dopa produces adverse effects after the prolonged period of administration probably due to the generation of free radicals. Melatonin has endogenous antioxidant actions. Previously we concluded that the co-administration of some antioxidants like melatonin with L-Dopa, helps to reverse some alterations induced by L-Dopa treatment. Researches show that adult rat SNc progenitor cells have the capability of differentiation into neurons under adequate environmental stimuli. Thus, we proved the L-DOPA effect, separately and with the co-administration L-dopa/Melatonin number neurogenesis in the SNc of rats lesioned with 6-OHDA. Our results show a greater number of TH/BrdU positive neurons in the L-Dopa/Melatonin treated group compared to 6-OHDA group and animals treated only with L-Dopa or Melatonin. We concluded that L-Dopa/Melatonin co-administration could promote the generation of new dopaminergic neurons in the SNc probably by reducing the oxidative environment

Disclosures: M. Moreno Rivera: None. **C. Torres Esquivel:** None. **R. García Ruíz:** None. **A. Gutiérrez Valdez:** None. **J. Sanchez Betancourt:** None. **T. Ibarra Gutiérrez:** None. **J. Ramos Jimenez:** None. **M. Avila Costa:** None. **V. Anaya-Martinez:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Program#/Poster#:856.21/G14

Topic: C.03. Parkinson’s Disease

Support: DOD W8IXWH-10-1-539

Title: Glyceryl-tris-3-hydroxybutyrate protects dopaminergic neurons in a MPTP model of Parkinson's Disease

Authors: *J. BLESA[1], V. JACKSON-LEWIS[1], N. BOAZ[2], S. HASHIM[3], S. PRZEDBORSKI[1]
[1]Columbia Univ., New York, NY; [2]Eastman Chem. Co., Kingsport, TN; [3]Dept. of Med., St. Luke-Roosvelt, New York, NY

Abstract: Parkinson's Disease (PD) is the second most neurodegenerative disease after Alzheimer Disease. It is a sporadic condition and several lines of evidence suggest that a defect in oxidative phosphorylation contributes to its pathology. Previously, we have shown that the administration of D-B-Hydroxybutyrate (DBHB) mitigates tyrosine hydroxylase positive (TH+) neuronal loss in the substantia nigra pars compacta of mice caused by mitochondrial poisons such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). DBHB is a ketone body produced by hepatocytes and astrocytes and is an alternative source of energy in the brain. We noted that this beneficial effect is mediated by the stimulation of ATP production in mitochondria. At this point, the long-term effects of the chronic use of DBHB are not known. One major limitation to the clinical use of DBHB resides with its extremely short half-life, hence, requiring its infusion via Alzet pumps to reach effective steady-state levels in the blood. Because of the short half-life of DBHB, an analogue of the DBHB, Glyceryl-tris-3-hydroxybutyrate (3GHB), was synthesized. This compound has a pharmacokinetic suitability for single-dose daily administration. We, therefore, have tested 3GHB to gauge the effectiveness of its "extended" neuroprotective action, both in *in vivo* and *in vitro* studies in comparison to DBHB. We show that 3GHB protects SNpc dopaminergic neurons against MPTP in a dose dependent manner and prevents the development of abnormalities in mice. Also, we have preliminary data suggesting that this protection may be via inhibition of histone deacetylase activity (HDAC). This new analog may represent a promising and valuable therapeuthic strategy for a range of pathological conditions affecting the brain, such as Parkinson's Disease or Alzheimer's Disease.

Disclosures: J. Blesa: None. **V. Jackson-Lewis:** None. **N. Boaz:** None. **S. Hashim:** None. **S. Przedborski:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Topic: C.03. Parkinson's Disease

Support: MJFF

Title: Cav1.3 LTCC selective inhibition by 1-(3-chlorophenethyl)-3-cyclopentylpyrimidine-2,4,6-trione in ameliorating PD motor deficits

Authors: *G. COOPER, JR[1], D. J. SURMEIER[2], R. B. SILVERMAN[3], J. N. GUZMAN[2], D. J. GALTIERI[2], S. KANG[3]
[1]Chem., Northwestern Univ. -- Dept. of Chem., Evanston, IL; [2]Physiol., Northwestern Univ., Chicago, IL; [3]Chem., Northwestern Univ., Evanston, IL

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease, in which the primary motor deficits are the result of selective degeneration of mesencephalic dopaminergic neurons (DA) in the substantia nigra pars compacta (SNc). We have shown that SNc DA neuronal physiology engages a low-threshold, high-conductance, long-lasting voltage-dependent calcium channel with a Cav1.3 pore-forming subunit. Cav1.3 L-type calcium channels (LTCCs) are engaged throughout the pacemaking cycle of adult SNc DA neurons, allowing extraordinary amounts of calcium into SNc DA neurons -- manifested as phasic dendritic calcium oscillations. As a consequence of increased intracellular free calcium, mitochondrial oxidative phosphorylation in SNc DA neurons increases to enable ATP-dependent calcium buffering mechanisms. These active buffering mechanisms are metabolically expensive -- leading to increased reactive oxygen species production. Inhibiting Cav1.3 LTCCs non-selectively via 1,4-dihyropyridines (DHPs) attenuates these dendritic calcium oscillations, relieves mitochondrial oxidant stress, and is neuroprotective in PD animal models. DHPs are a class of FDA approved anti-arrhythmic and anti-hypertension small organic molecules, which have nanomolar affinities to all neuronal LTCCs. Thus, chronic treatment of DHPs for PD preventive purposes might have unintended physiological consequences. Using whole-cell voltage-clamp electrophysiology, we present the first Cav1.3 LTCC isoform selective small molecule 1-(3-chlorophenethyl)-3-cyclopentylpyrimidine-2,4,6-trione (PYT), which selectively inhibits Cav1.3 LTCCs in a voltage-dependent and reversible manner. In addition, using two-photon laser scanning microscopy (2PLSM), we show PYT attenuates dendritic calcium oscillations and reduces mitochondrial oxidant stress. In conclusion, PYT represents a novel pharmacological tool in calcium channel physiology and potential therapeutic measure in slowing the progression of SNc DA neuronal degeneration in PD.

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Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Topic: C.03. Parkinson's Disease

Support: Hong Kong Baptist University Research Committee Mini-Area of Excellence project 2009 (RC/09-10/03)

General Research Fund, Hong Kong Research Grants Council (HKBU 262107)

Title: Expression of BDNF in reactive astrocytes provides neuroprotection to SH-SY5Y cells against 6-OHDA toxicity *in vitro*

Authors: *Y. TSANG[1], *Z. X. BIAN[2], K. YUNG[2]
[1]Dept. of Biology, Hong Kong Baptist Univ., Hong Kong, China; [2]Biol., Hong Kong Baptist Univ., Hong Kong, China

Abstract: Brain-derived neurotrophic factor (BDNF) has been shown to enhance the survival of dopaminergic neurons and to protect them against the neurotoxic effects of 6-hydroxydopamine (6-OHDA), a specific neurotoxin to dopaminergic neurons (Bove *et al*., 2005). Nestin is an embryonic protein and is a marker for neural stem cells. Nestin is not found in mature astrocytes. In our previous studies using rodent models of Parkinson's disease, a few nestin-immunoreactive reactive astrocytes were found to appear and express BDNF after the onset of Parkinson's disease (Chung *et al*., 2008). In order to investigate the neuroprotective roles of BDNF in reactive astrocytes, primary cell cultures of rat astrocytes were employed in the present study. By immunocytochemistry and Western blot analysis, a vast proportion of astrocytes in culture were found to be nestin-immunoreactive. In addition, most of these reactive astrocytes were found to express BDNF immunoreactivity. Reactive astrocytes were treated with BDNF (5, 50, 100, 200 ηg/ml). The levels of BDNF expression were found to be further enhanced and up-regulation of p-MEK and p-Erk1/2 in astrocytes were found by Western blot analysis. Moreover, when BDNF treated reactive astrocytes were co-cultured with neuroblastoma SH-SY5Y cells, activation of Akt and MAPK pathway were found by Western blot analysis. Cells in co-cultures were then treated with 6-OHDA (40μM), significant reductions of cell death of SH-SY5Y cells were found. The present results as a whole indicate that astrocytes *in vitro* display characterizations of reactive astrocytes and express high levels of BDNF. Administration of BDNF can stimulate further expression of BDNF in reactive astrocytes through activation of the MAPK pathway.

Disclosures: Y. Tsang: None. **Z.X. Bian:** None. **K. Yung:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:856.24/G17

Topic: C.03. Parkinson's Disease

Title: Neuro-protective effect of oxytocin on rotenone-induced Parkinson's disease in rats

Authors: *O. ERBAS, F. OLTULU, D. TASKIRAN
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Abstract: Aim: Our study was undertaken to investigate the neuroprotective effects (if any) of oxytocin (OT) on rotenone-induced Parkinson's disease (PD) in rats. OT is essentially associated with uterine contraction during parturition and milk ejection reflex. Although several studies have implicated the role of OT in other behavioral, and neuroprotective processes involving the anti-inflammatory, anti-oxidative and anti-apoptotic pathways, there is insufficient data with regard to the latter features of OT in neurodegenerative models such as Parkinson's disease (PD).
Material-Methods: Eighteen adult male Sprague-Dawley rats were stereotaxically injected with rotenone (an abundantly used pesticide and insecticide; 3 μg/ μl in DMSO) and vehicle (DMSO) into the left substantia nigra pars compacta and ventral tegmental area, and the expression of PD was verified by apomorphine-induced rotational test 10 days post-infusion. The valid PD rats were randomly divided into two groups: Group 1 (n= 6) and Group 2 (n=6) were administered saline (1 ml/kg/day, i.p.) and OT (160 μgr/kg/day, i.p.) through 20 days, respectively. The effects of OT treatment were evaluated by behavioral, immunohistochemical and electrophysiological analysis.
Results: Apomorphine-induced stereotypic rotations in PD rats were significantly inhibited by OT treatment ($p<0.05$). EEG analysis showed that OT treatment significantly reversed PD-induced changes ($p<0.0001$). In addition, immuno-histochemical data clearly demonstrated suppression of i-NOS, Bax, caspase-3, caspase-8, and elevation of Bcl-2 and tyrosine hydroxylase immune-expression in OT-treated rats.
Conclusion: Our findings suggest that OT may have neuroprotective effect on in vivo PD model. The underlying mechanisms might be involved with the anti-apoptotic effects and/or anti-inflammatory effects of OT. Next, we foresee enlightening the sites and mechanisms of action relevant to OT in this model.

Disclosures: O. Erbas: None. **F. Oltulu:** None. **D. Taskiran:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

Location: Hall F-J

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Program#/Poster#:856.25/G18

Topic: C.03. Parkinson's Disease

Support: CHRP

NIH

Title: PAOPA, an allosteric modulator of dopamine D2 receptor, enhances the therapeutic effects of levodopa in the *C. elegans* model: Implications for adjunctive therapy for Parkinson's disease.

Authors: A. H. SIDDIQI[1], B. P. GUPTA[2], P. R. SELVAGANAPATHY[3], R. JOHNSON[4], *R. K. MISHRA[5]
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Abstract: Parkinson's disease (PD) is a complex neurodegenerative disorder characterized by the selective and progressive degeneration of dopaminergic neurons within the substantia nigra. Although medications such as amantadine, monoamine oxidase B inhibitors and catechol-O-methyl inhibitors are effective in treating the disease, with time, every patient requires Levodopa treatment. However, despite being considered the gold standard treatment of PD, chronic use of levodopa leads to dyskinesia and wearing off or on-off phenomena. Therefore, there is a need to investigate efficacious therapeutics that not only treat the symptoms of PD but also have limited debilitating side effects. The aim of the present study was to screen potential therapeutics for PD in model genetic organisms like Caenorhabditis elegans (C. elegans). Worms show degeneration of dopaminergic neurons and movement deficits suggestive of PD when exposed to neurotoxins such as 6-Hydroxydopamine (6-OHDA). Conventional behavioral assays can be very subjective and labor intensive because they involve manually counting the nematodes and observing their movement on a plate. Recently, a novel microfluidics-electrotaxis system has been developed that allows for the high throughput study of behavioral deficits in C. elegans. This assay relies on the feature of C. elegans sensory neurons to mediate attraction towards the cathode in a DC electric field. Previous results have revealed the effectiveness of this system in studying PD, as the neurotoxin 6-OHDA-treated worms were defective in electrotactic behavior, showing a reduced swimming speed. The results of the current study demonstrate that co-administration of the neuroprotective compound Levodopa and Carbidopa are protective against 6-OHDA toxicity, and this protection was further enhanced when administered with PAOPA ((3(R)-[(2(S)-pyrrolidinylcarbonyl) amino] -2-oxo-1- pyrrolidineacetamide)), the confirmationally constrained analog of Pro-Leu-glycinamide. PAOPA is a specific allosteric modulator for the dopamine D2 receptor, which potentiates the effects of dopamine in the system. PAOPA has been shown to cross the blood brain barrier and have very low toxicity. This provides support for a potential role of PAOPA as an adjunctive therapy for the treatment of PD and to reduce the necessary dose

of levodopa thus minimizing the risk of levodopa induced diskinesia. This work was supported by CHRP grant from CIHR-NSERC and NIH (USA).

Disclosures: A.H. Siddiqi: None. **B.P. Gupta:** None. **P.R. Selvaganapathy:** None. **R. Johnson:** None. **R.K. Mishra:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Program#/Poster#:856.26/G19

Topic: C.03. Parkinson’s Disease

Support: NAT-VIEP BUAP-2010-2012

Ministerio Español de Educación y Ciencia SAF2009-13626 to J.A.

Title: The C-terminal domain of tetanus toxin has neuroprotective and restorative effects that leads improvement of the motor behavior and decrease the striatal neurodegeneration of rats with 6-hydroxydopamine

Authors: *A. SÁNCHEZ GONZÁLEZ[1], L. MENDIETA[1], P. DE LA CRUZ[1], V. PALAFOX[1], M. HERRANDO[2], J. AGUILERA[2], J. GUEVARA[3], D. LIMÓN[1]
[1]Lab. De Neurofarmacología/Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; [2]Inst. de Neurociènces, Departament de Bioquímica i de Biología Mol., Univ. Autònoma de Barcelona, Barcelona, Spain; [3]Dept. de Bioquímica, Facultad de Medicina, Univerdad Nacional Autónoma de México, México DF, Mexico

Abstract: The C-terminal domain of tetanus toxin (Hc-TeTx) is a peptide that lacks the toxicity of the whole neuromuscular toxin and has a neuroprotector activity against dopaminergic damage with MPP^+ in vitro and in vivo. The trophic effects of Hc-TeTx are related to its ability to active tyrosine kinase receptor’s pathways, which are crucial for survival process. The aim of our study was examined the neuroprotective and restorative effects in the nigrostriatal system by the intramuscular injection of the Hc-TeTx fragment in hemiparkinsonian rats. Animals were administered with vehicle (ISS) or Hc-TeTx (20 µg/kg) in the gastrocnemius muscle for three consecutive days pre- or post- the dopaminergic lesion with 6-hydroxydopamine (6-OHDA). Previous to the surgery the rats were administered ketamine/xylazine (55/5 mg/Kg i.p.) to underwent the intrastriatal injection of 6-OHDA (16 µg / 2µL) or vehicle (2µL of ascorbic acid at 0.01%) by stereotactic surgery. Therefore, we obtain groups with vehicle and groups with 6-OHDA, of which we obtain pre- or post-Hc-TeTx treatment groups. Animals with pre-Hc-TeTx

treatment were tested for rotational behavior with methamphetamine (5 mg/kg s.c.) after 2 weeks from lesion and the animals with post-Hc-TeTx treatment were tested for rotational behavior 8 days after surgery. For all groups, the motor asymmetry were tested every 7 days over a month in the cylinder test and then the neurodegenerative process in the nigrostriatal system was examined by the aminocupric-silver staining and tyrosine hydroxylase immunoreactivity (TH-IR). Our results showed that the pre-treatment with Hc-TeTx prevents the motor asymmetry by 6-OHDA during rotational behavior by methamphetamine (24 turns/h) and in the cylinder test (~70% of use of both forelimbs), in contrast with the 6-OHDA group (500 turns/h and ~22 %, respectively). In addition, the pre-Hc-TeTx hemiparkinsonian rats showed a major TH-IR in the striatum and SNpc respect to 6-OHDA group and we found minor argyrophilic-structures as a marker of neurodegeneration in the ipsilateral striatum of these animals after one month. The hemiparkinsonian rats with post-Hc-TeTx treatment showed a constant motor asymmetry (~60%) compared with its control 6-OHDA group (~30%). Moreover, the ipsilateral striatum of post-Hc-TeTx group has a lower number of argyrophilic-estructures and a major TH-IR in striatum and the SNpc compared to the 6-OHDA group. In summary, the intramuscular injection of Hc-TeTx has the ability to delay the progression of motor asymmetry, decrease the neurodegenerative process in striatum and prevent the progression of dopaminergic neurons death before and after 6-OHDA lesion.

Disclosures: A. Sánchez González: None. **L. Mendieta:** None. **P. De la Cruz:** None. **V. Palafox:** None. **M. Herrando:** None. **J. Aguilera:** None. **J. Guevara:** None. **D. Limón:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:856.27/G20

Topic: C.03. Parkinson’s Disease

Title: The neuroprotective efficacies of dichloroacetate with potent bioantioxidant polyphenols, resveratrol and curcumin, against neurotoxic-induced Parkinson's disease in neuroblastoma cells *in vitro*

Authors: *A. BETTICA, N. Y. YOUSAF, T. ZELEKE, M. GEBREMICHAEL
Manhattanville Col., Purchase, NY

Abstract: Accumulation of reactive oxygen species (ROS), due to increased oxidative stress, may lead to neuronal cell loss with strong associations to neurodegenerative disorders, such as Parkinson's disease (PD). Dichloroacetate (DCA), a small, readily available sodium salt, has

been shown in cancer cells to restore damaged mitochondrial function. Many polyphenolic compounds have been shown to be strong antioxidants and possible neuroprotective agents. In animal studies, resveratrol, an active constituent of red wine extracts, was shown to reduce beta-amyloid plaques by its possible copper-binding mechanisms. Curcumin, from the ginger family, acts as a scavenger and chelator, which also reduces plaques and can directly inhibit MPP+ toxicity in several neuronal cell lines. To determine the neuroprotective efficacies of DCA and potent bioantioxidants with relationship to the oxidative stress-induced *in vitro* PD model, pretreatments of neuroblastoma cells with these individual and combination agents against the neurotoxic insults, MPP+ and 6-OHDA, were undertaken. To formulate dose-response curves, the cells were pretreated in 96-well plates with each of the serially-diluted agents added for 24 hours before a potent dose of MPP+, a mitochondrial complex I inhibitor, or 6-OHDa, a potential ROS generator. For posttreatment studies, the cells were exposed to each insult first for 24 hours, followed by the antioxidants for 24 hours. Cotreatments with each agent and the neurotoxic insults were performed to elucidate possible preconditioning or gene activation versus actual mechanisms of action. Combination treatments were determined through
single concentration-dependent effective dosages. The MTT mitochondrial and Neutral Red lysosomal assay were used to assess cell viability/cell proliferation of triplicate data sets. All single treatment studies showed significant neuroprotection over a range of concentrations. DCA seemed to be the most effective in protecting the cells against MPP+ than against 6-OHDa. DCA lowered the effective dose for curcumin, however the pretreatment combination studies favored DCA with resveratrol over curcumin. Biphasic dose-response curves and poor availability of the polyphenols need to be carefully considered. If the polyphenols prove to be neuroprotective agents either singly or in combination, their bioavailability could be improved with attachment of phosphatidylcholine or linkage to a phytosome. But it is the multiple beneficial effects of these constituents, with little toxic side effects, that offers great potential for the prevention or treatment of Parkinson's disease and other neurodegenerative disorders.

Disclosures: A. Bettica: None. **N.Y. Yousaf:** None. **T. Zeleke:** None. **M. Gebremichael:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Program#/Poster#:856.28/G21

Topic: C.03. Parkinson’s Disease

Support: Charles and Mary Latham Foundation

Title: Antiapoptotic effects of 2,3-dichloro-1,4 naphthoquinone derivatives on salsolinol-induced toxicity in human shsy-5y cells.

Authors: S. T. WHITE[1], *R. L. COPELAND, Jr[2]
[1]Pharmacol., Howard Univ., Washington, DC; [2]Howard Univ. Col. Med., Washington, DC

Abstract: The cause and underlying mechanism of chronic cell death of the neural tissues in Parkinson's disease (PD) remains elusive. Salsolinol, (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) is an endogenous catechol isoquinoline detected in the brain, the cerebrospinal fluid and the urine. Elevated levels of salsolinol (SAL) have been detected in patients with idiopathic PD, which have also been proposed as biological markers of PD as they may result from an altered metabolism of dopamine. Several studies indicated that SAL is toxic to dopaminergic neurons in vitro as well as in vivo. Previous studies have shown that 2,3-dichloro-1,4 naphthoquinone (2,3DCNQ) derivatives possess a wide variety of biological responses including antibacterial, antifungal, anti-inflammatory, antiviral and anticancer. However, these compounds are known to have biphasic effects and at low nM concentrations these compounds have neuroprotective activity. This study was designed to evaluate the neuroprotective effect of 2,3DCNQ derivatives against SAL-induced cytotoxicity in the human neuroblastoma cell line, SH-SY5Y, by inhibition of apoptosis. Exposure of SH-SY5Y cells to 0.2 and 0.5 mM SAL for 24 hours resulted in 40 to 70 % cell death respectively as determined by WST-8 cell proliferation assay. Cells pretreated for 60 minutes with 15 nM 2,3DCNQ resulted in attenuation of salsolinol-induced cytotoxicity. Data was further supported by cell flow cytometry analysis which showed the highest number of early plus late apoptotic cells in SAL treated group exposed for 24 h. The 2,3DCNQ pretreatment reduced the apoptotic cell death. Western Blot analysis showed increased Akt activation. In nM concentrations, 2,3DCNQ appears to be specific for the antiapoptotic pathway, particularly PI3/Akt. This study suggests that 2,3DCNQ protect SH-SY5Y cells from SAL-mediated cytotoxicity by suppressing apoptosis. Since PD is a slowly progressing neurodegenerative disease, associated with excitotoxicity and apoptosis, therapeutic strategies exhibiting anti-apoptotic potential could be developed as a possible target to treat PD.

Disclosures: S.T. White: None. **R.L. Copeland:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Topic: C.03. Parkinson's Disease

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NIH Grant 5P30EY13080

NEI 1R01EY022349

Title: RAAV-mediated shrna knock-down of gd3 synthase (gd3s) reverses “executive” deficits in parkin null mice

Authors: *E. O. AKANO[1,2], M. P. MCDONALD[2,3], T. S. REX[4]
[1]Program in Neurosci., [2]Dept. of Neurol., [3]Anat. and Neurobio., [4]Dept. of Opthalmology, Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: The purpose of this study was to address two unmet needs in Parkinson’s disease therapeutics. The current therapies do not address the cognitive manifestations of the disease, and more importantly, they are not neuroprotective. The goal of the present project was to characterize the cognitive deficits in parkin null mice and to determine whether knocking down GD3 synthase (GD3S) would reverse these and other deficits exhibited by the knockouts. Inhibition of the gene (St8sia1) that codes for the ganglioside biosynthetic enzyme GD3S increases levels of GM1 ganglioside while inhibiting the expression of the pro-apoptotic GD3 ganglioside. GM1 has neuroprotective and noötropic properties, inhibits the fibrillization of α-synuclein in vitro, and partially protects nigrostriatal neurons in MPTP-treated mice. We trained knockouts and age-matched wild-type littermate controls on a 3-hole serial reaction time task commonly used to assess sustained attention, but modified to include indices of impulsive behavior. At baseline, using 1-s cue duration, there was no significant difference in accuracy (hits) across genotype, although reaction time and hopper latencies were slower in the parkin null mice compared to controls. On sessions with pseudo-randomized shorter cue durations (0.125-1.0 s), the parkin knockouts had significantly fewer hits and significantly slower reaction times than wild-type controls. During a probe session using variable inter-trial intervals, the parkin null mice had fewer hits, slower reaction time, and increased premature responses, indicating impaired attention and increased impulsivity. At the age of 13 months, the experimental subjects had intrastriatal injections of shRNA targeting GD3S or a scrambled control (scrRNA). Injection of GD3S.shRNA reversed the deficits in the reaction time task using variable cue durations. This study shows that parkin null mice can be used to study “executive” deficits characteristic of Parkinson’s disease and that knockdown of GD3S warrants further investigation as a potential therapeutic agent for Parkinson’s disease.

Disclosures: E.O. Akano: None. **M.P. McDonald:** None. **T.S. Rex:** None.

Poster

856. Parkinson's Disease: Neuroprotective Mechanisms

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Program#/Poster#:856.30/G23

Topic: C.03. Parkinson's Disease

Support: The Japanese governmental Grant-in-Aid for Scientific Research, KAKENHI (22590129)

Title: Oxicam non-steroidal anti-inflammatory drugs ameliorate motor dysfunction and dopaminergic degeneration by maintaining Akt-signaling in a mouse Parkinson's disease model.

Authors: *Y. TASAKI[1], J. YAMAMOTO[1], T. OHKUBO[1], T. NODA[1], T. OMURA[1], T. ONO[1], N. KIMURA[1], M. SUNO[2,1], T. SAKAGUCHI[1], K. MATSUBARA[3,1]
[1]Hosp. Pharmacy&Pharmacology, Asahikawa Med. Univ., Asahikawa, Japan; [2]Pharmaceut. Care & Hlth. Sci., Okayama Univ., Okayama, Japan; [3]Pharm., Kyoto Univ. Hosp., Kyoto, Japan

Abstract: Oxicam-bearing non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to be neuroprotective against 1-methyl-4-phenyl pyridinium in human neuroblastoma SH-SY5Y cells in our previous research. The mechanism of neuroprotection by oxicam NSAIDs was via maintenance of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway independent of cyclooxygenase (COX) inhibition. The aim of this study was to establish this novel effect of oxicam NSAIDs on a mouse Parkinson's disease (PD) model using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Male C57BL/6 mice, which received MPTP (30 mg/kg/day; s.c.) for 5 consecutive days (chronic model) with 10-day follow-up saline administrations, showed significant motor dysfunction in the pole test due to reduced tyrosine hydroxylase (TH) protein levels in the brain on day 16 after MPTP/saline treatment. Daily coadministrations of meloxicam (MLX) (10 mg/kg/day; i.p.) and MPTP for the first 5 days and follow-up 10 days with MLX administrations alone (MPTP/MLX treatment) significantly ameliorated MPTP-induced behavioral abnormalities in mice. Concomitant decreases of TH protein levels in the striatum and midbrain of MPTP/MLX-treated mice were not only significantly ($p<0.01$ and $p<0.05$, respectively) ameliorated but phosphorylated Akt (pAkt473) expression in the midbrain was also significantly ($p<0.01$) increased in the midbrain when compared with MPTP/saline-treated mice. In addition, preliminary study showed that another oxicam NSAID, piroxicam, ameliorated motor dysfunction in the chronic model of Parkinson's disease. These results suggest that oxicam NSAIDs attenuated dopaminergic neuronal death in the experimental MPTP-PD model by maintenance of the Akt-signaling. Oxicam NSAIDs may serve as potential drugs for PD treatment via a novel mechanism of action.
Keywords: Neuroprotection; Akt; MPTP

Disclosures: Y. Tasaki: None. **J. Yamamoto:** None. **T. Ohkubo:** None. **T. Noda:** None. **T. Omura:** None. **T. Ono:** None. **N. Kimura:** None. **M. Suno:** None. **T. Sakaguchi:** None. **K. Matsubara:** None.

Poster

857. Huntington's Disease: Other

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant #U01NS063953

Title: Efficacy of the HDAC1/3-targeting inhibitor, 4b, in a Huntington's disease mouse model: Involvement of the ubiquitination pathway.

Authors: H. JIA, R. KAST, *E. A. THOMAS
Dept Mol Biol, The Scripps Res. Inst., LA JOLLA, CA

Abstract: We previously demonstrated that the HDAC inhibitor, 4b, which preferentially targets HDACs 1 and 3, ameliorates Huntington's disease (HD)-related phenotypes in transgenic Drosophila and R6/2 transgenic mice. In the current study, we investigated the behavioral and biological effects of 4b at three different doses in another mouse model of HD, N171-82Q transgenic mice, and further explored potential molecular mechanisms of 4b action. Using an intermittent drug administration, we found that 4b significantly prevented body weight loss, improved several parameters of motor function and ameliorated Htt-elicited cognitive decline in HD N171-82Q transgenic mice. Pathways analysis of microarray data from the brains of 4b-treated mice revealed gene networks involving post-translational modification, including protein phosphorylation and ubiquitination pathways, associated with 4b drug treatment. Focusing on over 20 genes in these pathways, we measured expression changes in the striatum and cortex elicited by 4b at different durations of treatment using real-time qPCR analysis. We found that several genes showed significant differential regulation by 4b at different treatment time points. These included Ubiquitin-conjugating enzyme E2K (Ube2K; a.k.a. Hip2), Ubiquilin 2 (Ubqln2), Ubiquitin-conjugating enzyme E2E 3 (Ube2e3), Ubiquitin specific peptidase 28 (Usp28), BCL2/adenovirus E1B interacting protein 3 (Bnip3) and Autophagy-related 4C (Atg4c), among others. We propose that one mechanism of 4b action is associated with modulation of the ubiquitin-proteasomal and/or autophagy pathways, which might affect accumulation of the huntingtin protein.

Disclosures: H. Jia: None. **R. Kast:** None. **E.A. Thomas:** None.

Poster

857. Huntington's Disease: Other

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Program#/Poster#:857.02/G25

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR

CHDI

Title: Effect of cellular stress on Huntingtin promoter function

Authors: *R. DE SOUZA, B. R. LEAVITT
Leavitt Lab., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Huntington's Disease (HD) is a neurodegenerative disorder caused by a trinucleotide repeat expansion in exon 1 of the huntingtin gene (HTT). Despite ongoing research, the function of wild-type HTT remains largely unknown. In particular, we know very little about the pathways that regulate HTT expression at a transcriptional level. Previous studies have largely focused on identifying putative transcription factor (TF) binding sites, however the effect of modulation of these TFs on HTT expression has not been explored. By understanding pathways which modulate HTT expression we can gain a better understanding of the role of HTT in normal cellular function as well as insight into the mechanisms by which mutant HTT causes HD. Previous work done by our lab has indicated that cellular stress may play a role in HTT promoter function. I have created several HEK293 cell lines which stably express HTT promoter-luciferase constructs. I have used these cell lines to test the effects of a particular type of cellular stress, hypoxia, on HTT promoter function. My preliminary results indicate that hypoxia does in fact alter HTT promoter function. In addition to hypoxia studies I will be using these cells in the future to create a high-throughput screening system to identify potential therapeutic compounds that could be used to reduce HTT expression in HD patients.

Disclosures: R. De Souza: Employment; University of British Columbia. Research Grant; Canadian Institue of Health Research, Cure Huntington Disease Initiative. **B.R. Leavitt:** Employment; University of British Columbia. Research Grant; Cure Huntington Disease Initiative.

Poster

857. Huntington's Disease: Other

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:857.03/G26

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Allele-specific silencing of mutant huntingtin

Authors: *N. H. SKOTTE[1], A. L. SOUTHWELL[1], C. DOTY[1], J. CARROLL[2], S. WARBY[3], S. GREENLEE[4], A. T. WATT[4], S. M. FREIER[4], G. HUNG[4], W. YANG[5], F. BENNETT[4], M. R. HAYDEN[1]
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Abstract: The pathogenesis of HD is complicated and multiple pathways are compromised. Therefore attacking the problem at its root by silencing mutant huntingtin (htt) seems to be a promising therapeutic strategy to prevent subsequent pathology and delay or prevent the onset of HD. However, wild type htt is essential for brain function and therefore selectively silencing mutant and sparing wild type htt is ideal, especially when considering the fact that treatment will mostly begin early and continue throughout life.
Recent studies show that the majority of HD patients may be treatable by individualized allele-specific mutant htt silencing by targeting HD associated single nucleotide polymorphism (HD-SNPs) that are significantly enriched in the mutant HTT gene. Combinatorial analysis suggests that a panel of anti-sense oligonucleotides (ASOs) targeting as few as 3 of these HD-SNPs would provide a therapeutic option for up to 85% of the HD population. ASOs silence gene expression post-transcriptionally and can discriminate between SNP alleles anywhere in the pre-mRNA, including introns, providing flexibility to oligo design.
To generate a suitable mouse line for both in vitro and in vivo testing of ASOs, we have cross bred BACHD, YAC18 and Hdh-/-mice. The resulting humanized line, Hu97/18, is the first murine model of HD that fully genetically recapitulates human HD. We have generated a large number of ASOs targeting HD-SNP. Using primary neuronal cultures from the Hu97/18 mouse, we are currently screening these ASOs to identify those that are both potent and selective. In addition, to improve triage we have developed an assay to evaluate toxicity by measuring the cleavage of spectrin, a caspase and calpain substrate, which indicates the initiation of apoptosis. This assay allows us to exclude toxic ASOs before in vivo testing of the most promising candidates. Finally, we show that our lead ASOs can potently and selectively silence mutant htt expression in primary Hu97/18 neuronal cultures without toxicity. These results support the feasibility of an ASO mediated allele-specific mutant htt silencing strategy.
Following successful delivery and toxicity studies in mice, allele-specific mutant htt silencing may represent the most targeted therapeutic strategy for the treatment of HD and could be rapidly translated into an analogous approach for humans.

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Poster

857. Huntington's Disease: Other

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Program#/Poster#:857.04/G27

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Optineurin reduces mutant huntingtin aggregation

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Abstract: Huntington’s disease (HD) is a neurodegenerative disease characterized by progressive cognitive, psychiatric and motor deficits which correlate with neuronal loss in the cortex and striatum. It is caused by an expansion of CAG repeat in the *Htt* gene encoding huntingtin (htt). While there are palliative therapies of HD, an effective disease-modifying treatment is still lacking. In brain of HD patients, inclusion bodies (IBs) formed by mutant htt (mhtt) are a pathologic hallmark, and correlated with onset and severity of HD. Therefore, diminishing the formation of IBs may serve as an important therapeutic strategy. Here, we demonstrated that optineurin (OPTN), colocalized with mhtt IBs both in cell models and R6/2 transgenic mice. Overexpression of OPTN reduced the number of mhtt IBs, but OPTN with the ubiquitin binding domain deleted or mutated did not. Consistently, knockdown of OPTN increased the number of mhtt IBs. Altogether, our results indicate that OPTN modulates formation or degradation of mhtt IBs.

Disclosures: W. Shen: None. **Y. Chern:** None. **B. Tu:** None.

Poster

857. Huntington's Disease: Other

Location: Hall F-J

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Grant Early Discovery Initiative

NIH Grant R01NS064138

Title: Inhibition of mutant huntingtin expression using phosphorodiamidate morpholino oligomers (PMOs)

Authors: *X. SUN, M. BHAT, Z. CORDNER, J. L. PRUITT, R. L. MARGOLIS, D. D. RUDNICKI
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Abstract: Huntington's Disease (HD) is a neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the *huntingtin* (*HTT*) gene. While the majority of evidence supports the idea that the repeat is neurotoxic at the protein level, there is growing evidence that *HTT* RNA itself is neurotoxic and that the gene antisense to *HTT* (*HTTAS*) may also be involved in disease pathogenesis. Use of antisense oligonucleotides (ASOs) to suppress HTT expression is an attractive therapeutic strategy that could address both protein and RNA toxicity. ASOs targeted to the CAG repeat region are of special interest due to their ability, at least in part, to discriminate between normal and expanded *HTT*. Compared to other types of ASOs, phosphorodiamidate morpholino ASOs (PMOs) are very stable, highly soluble and non-toxic to cells even at high concentration. We therefore designed PMOs targeted to either the *HTT* CAG repeat or to regions outside of the repeat and tested their ability to suppress HTT expression. Control and HD patient-derived fibroblasts with 44, 77 or 109 CAG repeats were treated for 48 hours with repeat-targeting (CTG)n PMOs of different lengths or with PMOs targeting sequence outside of the repeat region, and mutant and normal HTT protein expression was measured. (CTG)n PMOs preferentially suppressed mutant HTT protein, dependent both on the length of CAG repeat in the HD fibroblasts and the length of the PMOs. As predicted, PMOs targeted to regions 5' of the *HTT* repeat reduced normal and expanded HTT protein expression without any effect on protein and RNA expression of endogenous control genes. These preliminary results demonstrate the potential of PMO therapy. While targeting the HD repeat region results in allele specificity, targeting non-repeat regions minimizes off-target effects. We are currently testing other PMOs in HD cell models, and the effect of PMOs in mouse models of HD.

Disclosures: X. Sun: None. **M. Bhat:** None. **Z. Cordner:** None. **J.L. Pruitt:** None. **R.L. Margolis:** None. **D.D. Rudnicki:** None.

Poster

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Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:857.06/G29

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIRM

NIH

Title: Transcriptional dysregulation in Huntington's Disease induced pluripotent stem cells

Authors: M. CASALE, *A. R. KING, S. WINOKUR, J. STOCKSDALE, N. JACKSON, M. VAWTER, P. DONOVAN, L. LOCK, L. THOMPSON
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Abstract: Understanding the underlying mechanisms that contribute to Huntington's disease (HD) and the identification of molecular signatures as indicators for pathogenesis are of importance to the development of preventative therapies. Human induced pluripotent stem cells (iPSC) offer an opportunity to use a genetically relevant representation of disease in culture with the expanded CAG repeat expressed within the normal endogenous locus. Transcriptional dysregulation in the brain appears to represent an early and central mechanism in HD pathogenesis. As part of an HD iPS Consortium, we have demonstrated that differentiated HD lines show CAG expansion-associated gene expression patterns in HD versus control neural stem cells (NSC) using microarray techniques. Hierarchical clustering of the most significant genes showed a clear separation of HD and control NSC datasets. Of the statistically significant gene changes, key pathways were identified using Ingenuity Pathway Analysis (IPA) and the results showed patterns of altered expression consistent with known HD changes in specific genes and proteins. In addition, this analysis provided identification of novel genes, functional categories and pathways impacted by the mutant full-length Huntingtin (Htt) protein. Several key pathways emerged, including altered G protein coupled receptor networks. Further, alterations in the innate immune response and glucocorticoid signaling were observed, providing evidence of an early inflammatory phenotype. Evidence was also found for the activation of adaptive immunity, including the extravasation of cytotoxic T-Cells across the blood brain barrier. Inflammation in the brain is a well-established feature of neurodegenerative diseases, including HD. Early detection of inflammatory cytokines IL-6, IL-8, and TNF-α in CSF and brain has been observed from HD mouse models and in CSF from HD patients (Bjorkqvist et al., 2008). Such increases may have either neuroprotective or detrimental effects. The glucocorticoid receptor (GR) pathway, which plays a major role in anti-inflammatory responses, has also been implicated in HD. Specifically, the serum and glucocorticoid activated kinase (SGK) phosphorylates Htt on S421 in a manner which is neuroprotective (Rangone et al., 2004). Although these proposed

mechanisms are likely playing a role in HD pathogenesis, our current understanding of immune responses and neuroinflammation in HD is limited, therefore iPS cells provide a powerful system to delineate the distinct roles of these processes through stages of neuronal development and in specific cell lineages.

Disclosures: M. Casale: None. **A.R. King:** None. **S. Winokur:** None. **J. Stocksdale:** None. **N. Jackson:** None. **M. Vawter:** None. **P. Donovan:** None. **L. Lock:** None. **L. Thompson:** None.

Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR MOP-10268

CIHR MOP-74465

NSERC

Title: Somatostatin receptors-mediated inhibition of nmda currents and dissociation of nmdars complex in striatum: Implication in excitotoxicit

Authors: P. S. RAJPUT[1], B. YANG[2], B. R. SASTRY[2], *U. KUMAR[1]
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Abstract: Overactivation of N-methyl-D-aspartate receptors (NMDARs) in heteromeric complexes play a crucial role in excitotoxicity that is associated with several neurodegenerative diseases. Recently, intense efforts have been made to minimize the risk of NMDA induced excitotoxicity. In the present study we used the combination of morphological, biochemical and electrophysiological techniques to characterize the role of somatostatin receptor 1 and 5 (SSTR1 and SSTR5) on NMDA induced changes in rat brain stratum. Here we show that the activation of SSTR 1 and 5 disrupt NMDARs complex formation and modulate signaling pathways associated with neuronal degeneration in the striatum. SSTR1 and SSTR5-specific agonist in combination exert pronounced inhibitory effect on NMDA currents and decreases the expression of PSD-95, calpain and enhanced CREB phosphorylation indicating the activation of synaptic NMDA receptor which correlates with dissociation of NMDARs complex as well as inhibition of NMDA

current upon SSTR activation. SSTR1 and SSTR5 agonist suppress downstream signaling pathways and modulate DARPP-32 phosphorylation at Thr34 and Th75 position. In conclusion, our results establish SSTR1/5 as a promising therapeutic strategy in dissociation of NMDARs heteromeric complex and abrogating cell death promoting signaling in striatum.

Disclosures: P.S. Rajput: None. **B. Yang:** None. **B.R. Sastry:** None. **U. Kumar:** None.

Poster

857. Huntington's Disease: Other

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NINDS 5R01NS055903-03

CHDI Foundation

Title: Behavior assessments in children at risk for huntington's disease

Authors: *J. LEE[1], A. CONRAD[1], J. S. PAULSEN[1], E. EPPING[1], L. C. RICHMAN[2], P. NOPOULOS[1]
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Abstract: Huntington's disease (HD) is an autosomal dominant disease caused by an expansion of greater or equal to 39 CAG repeats on the gene encoding for the huntingtin protein (*HTT*). Although the primary neuropathology is that of neurodegeneration of the striatum, mutant huntingtin (*mHTT*), which is present throughout the life span, may also affect normal brain development. In the current study, the effect of *mHTT* on neural development was examined by evaluating the behavior of children (7-18 years of age) at risk for HD (no juvenile HD included). Parental ratings on two standardized questionnaires, Pediatric Behavior Scale (PBS) and Behavior Rating Inventory of Executive Function (BRIEF), were assessed in order to evaluate problems in behavior and behavior regulation of the participants. Behavioral measurements of children tested as gene-expanded (n=29, CAG repeats $\geq$ 39) or gene non-expanded (n=30, CAG repeats $\leq$ 39) as a result of presymptomatic gene assessment (for research purposes only) were compared to those of a large number of healthy children matched for age and sex. The results showed that gene-expanded children, but not gene non-expanded children, had significantly more disruption in behavior when compared to healthy peers. Gene-expanded children showed significantly more problems in measures of Opposition, Explosiveness, Impulsivity, Hyperactivity and Inattention within PBS. Specific indices of executive function in which gene-

expanded children showed significantly more disruption include: Shifting, Initiation, Working Memory, Planning/Organizing and Monitoring as measured by BRIEF. The significant increase, as compared to healthy controls, in disrupted behavior in children who are estimated to be decades ahead of HD diagnosis may reflect early influence of *mHTT* on normal neural developmental process. Investigation of subtle but persistent effects of *mHTT* on gene-carriers may further our understanding of the pathogenesis of HD and thereby lead to the development of effective early intervention.

Disclosures: J. Lee: None. **A. Conrad:** None. **J.S. Paulsen:** None. **E. Epping:** None. **L.C. Richman:** None. **P. Nopoulos:** None.

Poster

857. Huntington's Disease: Other

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Medical Research Council (UK)

CHDI (USA)

Title: Astrocyte conditioned medium enhances neuronal maturation of iPS derived cultures: Role of GABAA evoked Ca2+ influx.

Authors: *D. J. RUSHTON[1], H.-E. TSENG[1], V. B. MATTIS[2], C. N. SVENDSEN[2], N. D. ALLEN[1], P. J. KEMP[1]
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Abstract: Astrocytes promote synaptogenesis *in vitro*, a development which may be crucial to generating appropriate stem cell derived models of neurodegenerative diseases. However, the mechanisms by which they exert such effects are currently unknown.
We investigated the influence of mouse striatal astrocyte conditioned medium during the time course of differentiation and maturation of human iPS-derived neuroprogenitors, as described in [1], using whole-cell patch-clamp and Ca^{2+} imaging. Although the proportion of cells able to fire an action potential in response to depolarization was unaffected, the conditioned medium evoked: i) significant hyperpolarization of the membrane at rest; ii) upregulation of L-, N- and R-type Ca^{2+} channel function, and; iii) a dramatic increase in the proportion of spontaneously active cells.

Increasing the extracellular [Ca^{2+}] from 0.6 to 1.8 mM resulted in accelerated neurogenesis and mimicked the enhanced maturity seen with astrocyte conditioned medium. These effects were blocked using specific inhibition of N- and L- type Ca^{2+} channels or $GABA_A$ receptors. Taken together, these data demonstrate a clear role for $GABA_A$-evoked depolarization, and consequent voltage-gated Ca^{2+} influx, in the process of neuronal maturation, and suggest that astrocytes may promote synaptogenesis, at least in part, via increased gain of the GABA excitation pathway.

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Mattis *et al.* Cell Stem Cell, In the press.

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Poster

857. Huntington's Disease: Other

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:857.10/G33

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Histological analysis of brain region changes indicated by whole brain *Ex vivo* 3D diffusion tensor MRI with tract-based spatial statistics in R6/2 mouse model of Huntington's disease

Authors: A. SIERRA[1], K. LEHTIMÄKI[2], T. HEIKKINEN[2], T. LAITINEN[2], J. PUOLIVÄLI[2], D. HOWLAND[3], O. KONTKANEN[2], *S. HÄTINEN[2], O. GRÖHN[1], J. YRJÄNHEIKKI[2], L. PARK[4]
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Abstract: White matter alterations occur at early stage in Huntington's disease (HD), an autosomal neurodegenerative disorder characterized by severe motor, behavioral and cognitive symptoms, and degeneration in basal-ganglia-thalamo-cortical circuits may play important role in clinical HD. In R6/2 mouse model of HD, we have previously shown extensive diffusion tensor imaging (DTI) changes at the age of 12 wks using *ex vivo* high-resolution 3D DTI with group-level whole brain statistical analysis method, tract based spatial statistics (TBSS). We showed decreased fractional anisotropy (FA) bilaterally in lateral septal areas, external capsule,

corpus callosum and in the dentate gyrus, as well as in various different bilateral thalamic and hypothalamic nuclei e.g. parafascicular, lateral posterior and mediodorsal thalamic and dorsomedial hypothalamic nuclei.
In this study, we performed extensive histological assessment of R6/2 and WT mice at 12 wks to corroborate the DTI findings for microstructural white matter pathologies. Nissl and gold chloride stainings were used as basic stainings to evaluate cytostructural and myelin alterations, and amyloid precursor protein (APP) immunohistochemistry was used to assess axonal damage. Myelin was assayed with Myelin Basic Protein (MBP) immunohistochemistry. Systematic analysis of MBP sections from wild-type and transgenic mice in anatomical areas displaying changes in FA after the TBSS analysis did not indicate significant wide-spread white matter damage difference in transgenic animals. Similarly, APP and Nissl staining analyses did not reveal major structural changes.
Generally, myelin has been considered to be one of the major tissue components contributing to restricted water diffusion in tissue. However, these data suggests that myelin sheath may not be the main contribution to the changes in water diffusion in transgenic animals, and diffusion of water may also be restricted by structures such as neuronal cell bodies, dendrites or glia. Moreover, TBSS analysis of R6/2 mice changes were also in grey matter structures with low content of myelinated fibers, such as the lateral septum, paraventricular thalamic nuclei, mediodorsal thalamic nucleus or dentate gyrus. In summary, these findings suggest that DTI changes are not related to axonal damage or wide-spread
degradation of white matter but may be associated with other microstructural alterations which need further investigation.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS036233

NIH Grant NS041669

Title: Early postnatal deficiency of huntingtin-associated protein 1 leads to depressive behavior in adult mice

Authors: *J. XIANG[1], H. YANG[2], S. LI[1], X.-J. LI[1]
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Abstract: Huntingtin-associated protein 1 (Hap1) is an intriguing neuronal protein that interacts with several disease-causing proteins such as huntingtin and Ahi1, whose mutations lead to Huntington's disease and Joubert syndrome, respectively. Germline knockout (KO) of Hap1 in mice results in early postnatal death due to depressed feeding behavior. Hampered by this early lethality and lack of animals surviving into adulthood, the role of Hap1 in the adult mice remains to be investigated. To gain insight into this question, we utilized tamoxifen-inducible Cre-Loxp system to generate an inducible Hap1 KO mouse model in which Hap1 can be depleted significantly after tamoxifen injection. This model allows us to deplete Hap1 at different ages and therefore test the role of Hap1 at various stages of development and in the adults. By inducing Hap1 KO at early postnatal days, we observed growth retardation, a phenotype that is similar to that of germline Hap1 KO mice. Interestingly, these mice when grown to adults exhibited depressive behavior as examined by forced swimming and tail suspension tests, a phenomenon that is consistent with the previously reported observation in Ahi1 KO mice. Moreover, this phenotype can be recapitulated in adult camk2a-Hap1 conditional KO mice, in which Hap1 deletion occurs postnatally, and is most prominent in the cortex and hippocampus. The depressive behavior can be rescued when these mice were given antidepressant drug imipramine. To further examine the mechanism underlying this phenomenon, we tested hippocampal neurogenesis after Hap1 depletion, and found that early Hap1 KO resulted in a reduction in BrdU incorporation in the dentate gyrus. The deficit in neurogenesis was not seen when Hap1 was depleted at a later developmental stage but could be detected in camk2a-Hap1 KO mice. This study suggests that Hap1 is required for postnatal neurogenesis, a mechanism that may underlie depressive phenotypes of Hap1 KO mice.

Disclosures: J. Xiang: None. **H. Yang:** None. **S. Li:** None. **X. Li:** None.

Poster

857. Huntington's Disease: Other

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R01 AR41928

CHDI Foundation

Title: Neurochemical correlates of disease progression in subjects with pre-manifest and early Huntington disease: a ^{1}H magnetic resonance spectroscopy study

Authors: J. M. PADOWSKI[1], K. E. WEAVER[1], T. RICHARDS[1], K. E. CONLEY[1], *N. M. KLEINHANS[1], E. H. AYLWARD[2]
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Abstract: Objective. Atrophy of the caudate nucleus occurs in Huntington disease (HD), and reduced caudate volume precedes onset of clinically-observable motor, cognitive and psychiatric dysfunction by at least a decade. Animal models and postmortem studies suggest a preferential loss of neurons relative to other cell types in the caudate during HD. This study was undertaken to test whether (1) neurochemical changes are related to reduced caudate volume in HD, and (2) the specific neurochemical changes support the hypothesis that a disproportionate loss of neurons occurs in the pre-manifest to early stage of HD.
Methods. Single voxel proton magnetic resonance spectroscopy (^{1}H MRS) at 3T was used to determine neurochemical concentrations in the left caudate of 10 subjects with pre-manifest to early HD and 10 age- and gender-matched controls. Concentrations of N-acetyl aspartate (NAA), creatine, choline, myo-inositol, glutamate and glutamate + glutamine (GLX) were quantitated relative to unsuppressed water signal with LCModel software. MRI was used to determine the volume of the left caudate in all subjects.
Results. We identified a series of neurochemicals, detectable by ^{1}H MRS, that evidenced reduced concentration in association with smaller caudate volume in subjects with the HD gene. Concentrations of NAA ($p<0.01$), creatine ($p<0.05$), choline ($p<0.01$), GLX ($p<0.05$), and glutamate ($p<0.05$) were significantly correlated with caudate volume in HD gene-carrying subjects. NAA and creatine also correlated with disease burden (a function of CAG repeats and age; $p<0.05$). No difference in caudate neurochemical concentrations between controls and HD gene-carrying subjects was detected (*t*-test).
Conclusions. The new insight provided by these results is that a decrease in concentration of a suite of neurochemicals accompanies loss of caudate nucleus volume. The relationship in HD gene-positive subjects between decreased caudate volume and decreased mass of NAA (a neuronal marker) per unit caudate volume suggests that neurons indeed may be lost disproportionately, consistent with animal models of HD and postmortem studies demonstrating spiny neuron loss in the caudate during HD progression. The relationship between decreased concentrations of creatine, choline, GLX and glutamate and decreased caudate volume in subjects with the HD gene suggests that additional qualitative changes in cellular composition occur during HD progression. The results of this study indicate that ^{1}H MRS can be used to

explore fundamental relationships between neurochemical and brain structural changes in subjects with pre-manifest to early HD.

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Poster

857. Huntington's Disease: Other

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NINDS

Title: Cag expansion-dependent cell toxicity in hd ips cells: darpp32 positive vs darpp32 negative cells, and rescue of toxicity

Authors: S. S. AKIMOV[1], N. ARBEZ[1], T. RATOVITSKI[1], E. CHIGHLADZE[1], E. WATKIN[1], M. JIANG[1], V. MATTIS[3], L. THOMPSON[4], C. SVENDSEN[3], H. SONG[2], K. YE[5], W. DUAN[1], *C. A. ROSS[1]
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Abstract: Huntington’s disease (HD) is a genetic neurodegenerative disorder caused by a trinucleotide repeat expansion mutation in exon 1 of the *HTT* gene encoding protein huntingtin. Dramatic loss of medium spiny neurons is typically observed in the striatum of HD patients, with more moderate loss of other neurons. Recently, as a part of the HD iPSC Consortium, we generated and characterized human iPS cell lines with an allelic series of HD mutations. We have demonstrated HD specific phenotypes such as increased toxicity in HD (109 and 180 CAG) versus control (21 and 33CAG) iPS cells under oxidative stress and autophagy inhibition stress in cells differentiated towards neurons positive for markers of striatal medium spiny neurons, including DARPP32 and BCL11B. We have also shown that maintenance of differentiated cells in neural induction medium alone (without BDNF) for 48 hours resulted in increased toxicity in a CAG expansion dependent manner. Here, we report that BDNF and 7,8-dihydroxyflavone (7,8-DHF), a small molecule reported to activate the high affinity BDNF receptor (TrkB) can rescue BDNF withdrawal induced toxicity in the HD iPSC model. We demonstrate 7,8-DHF dose-dependent decrease of cell death in differentiated HD iPS cells. BDNF withdrawal induced toxicity is significantly higher in the DARPP32-positive differentiated HD iPSC population

versus DARPP32-negative cell population. However, we also did find an increase of cell toxicity in DARPP32-negative cells in a CAG expansion dependent manner, indicating that cells other than DARPP32 positive neurons can be affected by the *HTT* mutation. We have also continued identification of markers associated with expression of mutant Htt using iTRAQ (isobaric tags for relative and absolute quantification), a method of quantitative proteomics analysis. We observed a polyQ length-dependent increase in expression of several neuronal proteins, such as FABP7 and CRMP1, and a decrease in expression of the RNA-binding protein FUS/TLS, and confirmed by Western Blotting. Further validation of these and other candidate proteins emerging from proteomics studies will involve RNAi silencing and toxicity assays and analysis of HD iPS cells differentiated into striatal medium spiny neurons.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Medtronic, Inc.

Title: Six-month partial suppression of wild-type Huntingtin does not cause observable ill effects or marked pathology in the adult rhesus striatum

Authors: R. GRONDIN[1], M. D. KAYTOR[2], E. N. BURRIGHT[2], Y. AI[1], P. T. NELSON[3], D. THAKKER[2], J. HEISEL[2], M. WEATHERSPOON[2], J. L. BLUM[2], Z. ZHANG[1], *W. F. KAEMMERER[4]
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Abstract: Huntington’s disease (HD) is caused by expression of a mutant form of huntingtin (Htt) protein. One possible treatment for HD may be to reduce expression of mutant Htt in the brain via RNA interference. Unless the therapeutic molecule is designed to be allele-specific, both wildtype and mutant protein will be suppressed by RNA interference treatment. Whether Htt performs essential functions in the adult brain is unclear. Here, we report that the adult primate brain can tolerate moderately reduced levels of wildtype Htt protein for an extended

period of time. A serotype 2 adeno-associated viral vector encoding for a short hairpin RNA (shRNA) targeting rhesus Htt mRNA (active vector) was bilaterally injected into the striatum of four adult rhesus monkeys. Four other animals received a comparable vector encoding a scrambled control shRNA (control vector). General health and behavior were monitored for six months at which point brain tissues were sampled and assessed blindly for Htt mRNA and protein knockdown as well as for neuropathology. Reduction in wildtype Htt mRNA levels averaging about 30% was measured by qPCR in the striatum of active vector recipients six months post-injection relative to controls. Reduction in Htt protein levels was also observed in these animals relative to controls, with an average protein reduction in the putamen of about 45% measured by Western blot analysis. As with control vector recipients, no adverse effects were observed behaviorally, and no neurodegeneration was found on histological examination of active vector recipients. Our results suggest that long-term partial suppression of wildtype Htt may be safe. Thus, if a comparable level of suppression of mutant Htt is beneficial, then partial suppression of both wildtype and mutant Htt may result in a net benefit in heterozygous HD patients.

Disclosures: **R. Grondin:** Research Grant; University of Kentucky. **M.D. Kaytor:** Employment; Medtronic. **E.N. Burright:** Employment; Medtronic. **Y. Ai:** None. **P.T. Nelson:** None. **D. Thakker:** Employment; Medtronic. **J. Heisel:** Employment; Medtronic. **M. Weatherspoon:** Employment; Medtronic. **J.L. Blum:** Employment; Medtronic. **Z. Zhang:** None. **W.F. Kaemmerer:** Employment; Medtronic.

Poster

857. Huntington's Disease: Other

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Support: CHDI Foundation

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TG2-01160

Taube-Koret Center

Hellman Family Foundation

Title: Rapid neural differentiation and high-content analysis of Huntington's disease iPS cells reveals CAG repeat length-associated phenotypes.

Authors: *J. KAYE[1], B. THOMPSON[2], V. MATTIS[3], C. SMITH[2], D. RUSHTON[2], C. SVENDSEN[3], S. FINKBEINER[1,4], P. KEMP[2], N. ALLEN[2]
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Abstract: A major issue for modeling adult onset diseases such as Huntington's disease (HD) using induced pluripotent (iPS) cells concerns the age and maturity of in vitro generated neurons that may be required to observe disease- associated phenotypes. A necessity for complex and extended neural differentiation and culture protocols also has implications for efficient assay development. Here we have used a simple and rapid differentiation protocol in which iPS cell-derived neural stem cells are plated and differentiated over a 2-4 week period. In this time frame neurons express markers of neuronal maturation such as MAP-2, Synaptophysin and striatal markers such as CALRETININ, CALBINDIN and express functional neuronal properties including evoked and spontaneous action potential generation. Importantly, these cultures are suitable for high content phenotypic screens.
Using this protocol, we differentiated HD iPS cell lines and established assays to investigate disease relevant phenotypes. Firstly, we have used an automated imaging system that follows individual mature neurons and tracks cell survival over time. Our imaging techniques determine multiple cellular parameters that are analyzed with powerful statistical approaches that confer high sensitivity (Arrasate et al., 2004; Arrasate and Finkbeiner, 2005). Secondly we have used fura-2 ratiometric Ca2+-imaging to examine Ca2+ dyshomeostasis in neurons in response to glutamate stimulation across cell populations on a cell by cell basis.
Significantly, results show that CAG-associated phenotypes manifest in rapidly differentiated neurons and do not necessarily require long and elaborate differentiation protocols to be used to be relevant to HD. We are extending our assays to study mechanisms of disease and investigate strategies for neuroprotection. We are evaluating the effects of several trophic factors and autophagy inducers on survival and will report our results.

Disclosures: J. Kaye: None. **B. Thompson:** None. **V. Mattis:** None. **C. Smith:** None. **D. Rushton:** None. **C. Svendsen:** None. **S. Finkbeiner:** None. **P. Kemp:** None. **N. Allen:** None.

Poster

857. Huntington's Disease: Other

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:857.16/G39

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: European Community's Seventh Framework Programme (FP7/2007-2013) grant agreement n261358 (PADDINGTON)

Title: Mutant huntingtin in immune cells tracks Huntington's disease progression

Authors: *R. ANDRE[1], A. WEISS[2], U. TRAEGER[1], S. GRUENINGER[2], R. FARMER[3], C. LANDLES[4], R. I. SCAHILL[1], D. MACDONALD[5], C. FROST[3], G. P. BATES[4], G. BILBE[2], R. KUHN[2], E. WILD[1], S. J. TABRIZI[1]
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Abstract: Huntington's disease (HD) is a fatal, inherited neurodegenerative disorder caused by an expanded CAG repeat in the gene encoding huntingtin (*HTT*). Mutant HTT expression in the central nervous system (CNS) is the primary pathogenic factor for the development of HD, with increasing expression levels associated with disease severity and toxicity. However, the importance of the peripheral immune system in neurodegenerative disease is becoming increasingly recognized. Peripheral immune system dysfunction in HD includes increases in innate immune proteins in patient plasma, many of which track with disease progression.
Inflammatory cytokines and chemokines are elevated in the plasma of HD patients, the origin of which appears to be hyper-reactive monocytes carrying the HD mutation. The hyper-reactivity observed in monocytes is reflected by a similar pattern in HD microglia and microglial activation is seen before symptom onset in HD mutation carriers. These effects appear to be due to the cell intrinsic expression of mutant huntingtin (mHTT), the effects of which on intracellular signalling pathways are being investigated by expressing and knocking-down both mutant and wild-type HTT. Therapeutic approaches to lower mutant mHTT levels are expected to proceed to human trials soon, but non-invasive quantification of mHTT is not currently possible. HTT levels in peripheral immune cells have not been quantified before. A recently described time-resolved Förster resonance energy transfer (TR-FRET) immunoassay was used to quantify mutant and total HTT protein levels in HD

patient leukocytes. Mean mHTT levels in monocytes, T cells and B cells differed significantly between HD patients and controls, and between pre-manifest mutation carriers and those with clinical onset. Monocyte and T cell mHTT levels were significantly associated with disease burden scores and caudate atrophy rates in HD patients. Mutant HTT N-terminal fragments detected in HD monocytes may explain the progressive increase in mHTT levels in these cells. These findings indicate that quantification of mHTT in peripheral immune cells by TR-FRET holds significant promise as a non-invasive disease biomarker. More broadly, peripheral immune
dysfunction may be pathogenically important in HD and could offer a window onto relevant CNS events.

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Poster

857. Huntington's Disease: Other

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant RC2-NS069422

Hereditary Disease Foundation Fellowship

Title: A novel *in vitro* and *in vivo* system to study Huntington's disease using iPS cells

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Abstract: Huntington's disease (HD) is an autosomal dominant disorder caused by expansion of polyglutamine (CAG) repeats in the huntingtin gene leading to loss of striatal and cortical neurons and deficits in coordinated movements and cognition. Transgenic rodent models are valuable for testing cell death mechanisms and therapeutic intervention. However, mouse and rat models may not fully represent the human condition. Therefore, we, in collaboration with the HD iPSc consortium, have recently reported a new in vitro model of HD based on the generation of induced pluripotent stem cells (iPSCs) from subjects with between 60 and 180 CAG repeats and controls. CAG repeat expansion had no obvious effects on reprogramming, neural stem cell

(NSC) production, or the generation of neurons. However, HD and control iPS cell lines showed differential risk of cell death, specifically upon BDNF withdrawal.
We therefore wanted to use this novel HD model to study the roles of BDNF in HD iPSCs. Specifically, we wanted to elucidate the mechanisms of cell death in for continued validation of this iPSC HD model and with the hope of gaining important insight into HD for future therapeutic targets. Additionally, by transplanting striatal neurons differentiated from HD iPS cells into the striatum of neonatal mice, cells will integrate and mature, allowing us to study them in vivo. This will also allow us to study non-cell autonomous effects of HD and generate a novel "humanized" HD mouse model.

Disclosures: V.B. Mattis: None. **J.A. Kaye:** None. **S. Finkbeiner:** None. **C.N. Svendsen:** None.

Poster

857. Huntington's Disease: Other

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Program#/Poster#:857.18/G41

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Biomarker assays for Huntington's Disease: Detection of human and mouse huntingtin proteins using the meso scale discovery platform

Authors: M. A. TESSARI[1], I. BOOGAARD[1], M. SMITH[2], K. PULLI[1], A. SZYNOL[1], F. ALBERTUS[1], S. GRIFFIOEN[1], M. B. A. C. LAMERS[2], C. ESSRICH[1], G. MCALLISTER[2], I. MUNOZ-SANJUAN[3], *D. F. FISCHER[4], D. MACDONALD[3]
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Abstract: Huntington's disease (HD) is caused by an expansion of a CAG triplet repeat in the huntingtin (Htt) gene, resulting in expression of an expanded poly-glutamine (polyQ) tract in the N-terminal region of the Htt protein. RNAi technology aiming to reduce the amount of Htt protein has shown considerable potential for the treatment of HD. Therefore, robust biomarker assay read-outs for expanded and non expanded Htt protein levels will be essential for both pre-clinical and clinical validation of such novel therapies.
BioFocus, in collaboration with the CHDI Foundation, is developing a panel of ELISA-based Meso Scale Discovery (MSD) electrochemiluminescence assays to enable the detection of non-expanded and polyQ-expanded Htt in cellular, rodent, and clinical biosamples. Here we describe three of these assays: 1) a pan human Htt assay that detects non-expanded and polyQ-expanded

Htt proteins, 2) a polyQ-expanded human Htt assay for mutant Htt protein, and 3) a mouse/rat Htt specific assay that detects endogenous Htt proteins in disease relevant rodent models. These assays are highly sensitive (detecting fmols of protein) and robust as they are able to measure Htt levels in a variety of complex biological samples. As a proof-of-concept, we will show examples of Htt protein detection in brain tissue of transgenic rodent HD models and quantification of both transgenic and endogenous proteins utilizing purified recombinant Htt proteins as a standard. These assays represent an essential and versatile tool to assess the efficacy of Htt suppression therapeutic strategies and will contribute to the validation and development of such novel therapies for HD.

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Poster

858. Motoneuron Disease Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:858.01/G42

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: The C9orf72 ALS mutation causes both increased expression and aberrant splicing of the endothelin-B receptor, and its ligand endothelin-1 is increased in CNS tissue from ALS patients and mutant mice.

Authors: *L. W. OSTROW[1], C. DONNELLY[1], Y. LI[1], S. VIDENSKY[1], P. TIENARI[2], N. J. MARAGAKIS[1], B. J. TRAYNOR[3], J. D. ROTHSTEIN[1]

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Abstract: Endothelin-1 (ET-1, gene symbol EDN1) and the endothelin-B receptor (ET_B, gene symbol EDNRB) are upregulated in reactive astrocytes. ET_B receptor expression is also increased on spinal motor neurons in animal models of peripheral nerve and spinal cord injury. Astrocyte reactivity/dysfunction is implicated in ALS pathogenesis, and ET-1 expression is upregulated in the motor cortex of human autopsy specimens from sporadic ALS patients. The recently discovered *C9orf72* mutation is found in >30% of familial ALS cases, and has been reported in 4-10% of sporadic ALS cases. The function of the C9orf72 protein is unknown. Like other non-coding repeat expansion mutations, C9orf72 mutation based toxicity may be due to aberrant RNA splicing. We have generated fibroblast cell lines from ALS patients with the C9orf72 expansion and normal controls. ET_B receptor expression is increased > 2-fold in fibroblast cell lines derived from C9orf72 patients compared to normal controls, and the EDNRB gene shows significant aberrant exon splicing. The increased expression and aberrant splicing pattern recapitulate published results on ET_B receptor gene expression and splicing abnormalities described in motor-neuron enriched mRNA from human ALS autopsy spinal cord tissue. Furthermore, our preliminary data suggests C9orf72 siRNA can knock down ET_B receptor gene expression in the fibroblasts. Additionally, we have demonstrated by ELISA that the ET-1 peptide is increased in spinal cord tissue from SOD1 ALS mutant mice compared to wild-type littermates, and in human ALS autopsy tissue from CNS regions involved in ALS. In aggregate, these studies in SOD1 ALS mice and human ALS patient-derived cell lines and autopsy tissues suggest that alterations in the endothelin system could contribute to ALS pathogenesis. Further investigations into this pathway and its relevance to motor neuron toxicity are now warranted.

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Poster

858. Motoneuron Disease Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:858.02/G43

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Characterization of the ALS-linked C9ORF72 gene in NSC34 cells and patient lymphoblasts

Authors: *X. WEN, W. TAN, A. BOGUSH, P. PASINELLI, D. TROTTI
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Abstract: Amyotrophic lateral sclerosis (ALS), a progressively fatal neurodegenerative disease of upper and lower motor neurons, has recently been the subject of major advances, especially in relation to frontotemporal dementia (FTD) subtypes and shared pathogenic mechanisms. Two independent groups have identified an expansion of GGGGCC hexanucleotide repeats (G4C2) located in the non-coding region of the first intron of the C9ORF72 gene as the most common cause of ALS and FTD identified to date. Based on the initial analysis of this region, normal individuals usually carry up to 23 repeated G4C2 repeats, whereas affected individuals carry repeat expansions that may be up to 10 Kb, containing over 1,000 repeats. C9ORF72 encodes a highly conserved alternatively spliced 481 amino acid protein (full length) and a much shorter 222 amino acid transcript, both of unknown biological function. Here, we have successfully characterized antibodies, shRNA, siRNA and plasmids for studying the function and localization of C9ORF72. We also generated stable knock-down cell lines for C9ORF72 to study its cellular functions. Preliminary results suggest that this protein is up-regulated in differentiated NSC-34 cells and possibly involved in stress response. However, by stably knocking down C9ORF72 in NSC-34 cells, these cells do not display any significant morphological differences compared to controls when differentiated into motor neurons. By using immunofluorescence and subcellular fractionation, we show that C9ORF72 is a cytosolic protein with some perinuclear localization pattern, possibly associated with ER or stress granule. Immunoprecipitation results also reveal its ability to oligomerize with itself and across isoforms, which may suggest its ability to form protein complex with RNA or other proteins. Using the antibodies that we characterized with the knock-down NSC34 cells, we also demonstrated that in lymphoblast cell line of a C9ORF72 expanded repeat carrier, the protein levels of C9ORF72 isoform a is decreased compared to the control lymphoblast cell line. Experiments are undergoing to further characterize the cellular functions of C9ORF72 and its potential role in ALS.

Disclosures: X. Wen: None. **W. Tan:** None. **A. Bogush:** None. **P. Pasinelli:** None. **D. Trotti:** None.

Poster

858. Motoneuron Disease Mechanisms

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Program#/Poster#:858.03/G44

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: P2ALS

Title: Analysis of the C9ORF72 hexanucleotide repeats size in ALS patient derived fibroblasts, iPS cells and postmortem brain tissues

Authors: *P.-W. ZHANG[1], C. DONNELLY[1], R. SATTLER[1,2], L. OSTROW[1], U. BALASUBRAMANIAN[1], N. MARAGAKIS[1], J. ROTHSTEIN[1,2]
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease with familial transmission in about 10% of the cases. Mutations in SOD1 encoding the cooper/zinc superoxide dismutase, TARDBP (TAR DNA-binding protein 43) and FUS (fused in sarcoma) are responsible for 20%-30% of familial ALS disease. Rare mutations in other genes like ANG, VAPB, DAO, OPTN, VCP and UBQLN2 have been reported. Recent studies have identified expanded GGGGCC hexanucleotide repeats in the C9ORF72 gene in ALS, frontotemporal dementia (FTD) and ALS-FTD. This novel mutation is currently the most common genetic cause of familial ALS (23%-47%) as well as sporadic ALS (4%-21%) in the USA, Belgium and Finland. Characterization of the role of repeat length and tissue distribution effects of variation in repeat expansion size in the C9ORF7 gene is suggested to be crucial for the understanding of the mechanisms and pathways of ALS pathology.
Here we report data on the repeat size in the C9ORF72 gene in different cell types and tissue samples obtained from ALS patients. Using an optimized Southern Blotting approach, the size and number of repeats were determined in fibroblasts and iPS cell lines derived from ALS patients with C9ORF72 repeat mutations. All of the repeats were over 3 kb long and one repeat number was found to contain over 1,000 repeats. Interestingly, the size and number of repeats in the iPS cell lines were different from the fibroblasts derived from the same patients. We also examined the dynamic changes in the repeat size as cell lines underwent increasing passages. Finally we analyzed repeat lengths in multiple different brain regions from more than six C9ORF72 autopsies.

Disclosures: P. Zhang: None. **C. Donnelly:** None. **R. Sattler:** None. **L. Ostrow:** None. **U. Balasubramanian:** None. **N. Maragakis:** None. **J. Rothstein:** None.

Poster

858. Motoneuron Disease Mechanisms

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Program#/Poster#:858.04/G45

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R00AG032362

Title: PIKK dependent phosphorylation induces nuclear-cytoplasmic translocation of FUS

Authors: *Q. DENG[1,2], K. HUDSON[1], G. TAYLOR[1], D. DUONG[2,3], N. T. SEYFRIED[2,3], D. ITO[5], T. KUKAR[1,4,2]
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Abstract: Mutations in TARDBP (TDP-43) and the Fused in sarcoma (FUS) gene have been identified that cause inherited and sporadic forms of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). TDP-43 and FUS are both DNA/RNA-binding proteins with striking structural and functional similarities. Both TDP-43 and FUS are predominantly found in the cell nucleus. In disease, however, TDP-43 and FUS have been observed to redistribute to the cytoplasm and accumulate in neuronal cytoplasmic inclusions. Post-translational modifications (PTMs) including cleavage, ubiquitination, and phosphorylation have been described as markers of pathological changes in TDP-43 proteinopathies. To date, similar PTMs of FUS have not been described in cases with pathological FUS changes. In the course of studying the cell biology of FUS, we have discovered that FUS undergoes phosphorylation. A combined approach using small molecule kinase inhibitors and phospho-specific antibodies revealed that FUS is phosphorylated at serine and threonine sites by DNA-dependent protein kinase and Ataxia telangiectasia mutated (ATM) kinase. We examined a series of truncated FUS mutants and narrowed down the N-terminus as the primary site of phosphorylation. Mass spectrometry experiments are underway to map the precise sites of FUS phosphorylation. Experiments in cells suggest that phosphorylation of FUS plays a critical role in regulating import and export of FUS between the nucleus and cytoplasm. Because accumulation of FUS in neuronal cytoplasmic inclusions is a pathological hallmark of some ALS and FTD cases, our data suggests that deregulation of FUS phosphorylation may play an important role in disease pathogenesis. Furthermore, FUS phosphorylation could be a viable drug target for ALS and FTD therapies.

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Poster

858. Motoneuron Disease Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:858.05/G46

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Expression of truncated FUS/TLS protein triggers proteinopathy in cultured cells and transgenic mice

Authors: T. A. SHELKOVNIKOVA[1,2], N. CONNOR-ROBSON[1], A. DEIKIN[3], O. PETERS[1], H. ROBINSON[1], E. R. SADCHIKOVA[3], V. BUCHMAN[1], *N. N. NINKINA[1,2]
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Abstract: Pathological inclusions formed by aggregated FUS or TDP-43 proteins have been recently identified as characteristic features for a number of neurodegenerative conditions. These DNA/RNA-binding proteins share
striking structural similarity, though the sequence of domains in FUS is
inverted to those in TDP-43. It is well established that ~25 kDa highly
aggregate-prone C-terminal fragments of TDP-43 are common components of pathological protein aggregates. We hypothesized that expression of a structurally similar fragment of FUS, i.e. the C-terminally truncated form, comprising intact prion-like domain but disrupted RNA-binding region and lacking nuclear localization signal (NLS), might also yield robust proteinopathy in vivo. In cell culture studies we demonstrated that truncated human FUS is highly aggregate-prone and forms aggresome-like structures. This is in contrast to other modified forms of FUS localizing in the cytoplasm due to the lack of functional NLS that become recruited into stress granules. To assess if expression of C-terminally truncated FUS would trigger proteinopathy in vivo, we used a Thy1 promoter expression cassette to generate transgenic mice. Mice expressing truncated human FUS in the nervous system developed early onset and fast progressing motor dysfunction that caused severe disability and premature death. Abundant FUS-positive inclusions were detected in lower motor neurons of symptomatic transgenic mice, some of which were ubiquitinated. FUS proteinopathy was accompanied by neuroinflammatory changes, neurofilament disturbances, loss of nerve fibers and muscle denervation. The mice demonstrated decreased numbers of lower motor neurons both in spinal cord and selected motor nuclei of brainstem. Results of our studies demonstrate that expression of truncated FUS loosing both nuclear localization and RNA-binding ability triggers proteinopathy in cultured cells and in mice. Pathological changes developed in the nervous system of transgenic mice recapitulate many characteristic features of human ALS pathology. These models will be useful for getting further insights into mechanisms and consequences of FUS proteinopathy in humans and testing novel therapeutic interventions.

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Poster

858. Motoneuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH-NINDS 1RC1NS068391

ALS Therapy Alliance

CWRU-NCRM pilot award

Title: ALS mutants of FUS/TLS may influence the function of a neuroprotective long non-coding RNA

Authors: J. LAWSON[1], B. ZHANG[2], F. JAHANIANI[2], L. GUNAWARDANE[2], *L. J. HAYWARD[1], S. VALADKHAN[2]
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Abstract: Mutations in FUS/TLS, a protein with a diverse range of functions in cellular RNA metabolism, are associated with familial cases of amyotrophic lateral sclerosis (ALS), suggesting that disturbances in RNA processing may contribute to the development of this disease. We have studied the function of a long non-coding RNA (lncRNA-1) which is almost exclusively expressed in neurons, including anterior horn neurons. lncRNA-1 is induced in response to a variety of stressful stimuli including oxidative and ER stress in neuronal cells. To determine if the stress-induced rise in the level of lncRNA-1 has a functional significance, we analyzed the impact of forced overexpression and knock-down of lncRNA-1 in neuronal cells. Interestingly, the level of lncRNA-1 expression correlated directly with cellular survival after stress. To determine the mechanism through which lncRNA-1 performs its pro-survival function in neurons, we purified lncRNA-1 along with its interacting proteins and identified the co-purified proteins using mass spectrometry. Interestingly, we identified FUS as a major interacting partner of lncRNA-1, suggesting that FUS may influence the biogenesis and/or function of lncRNA-1. To investigate this possibility further, we induced the expression of human ALS-associated FUS mutants in an HEK293 cell system and measured the effects on lncRNA-1 expression. Our preliminary results indicate that ALS mutants of FUS, but not wild type FUS, decrease the cellular level of lncRNA-1, suggesting that perturbation of the FUS-lncRNA-1 interaction or lncRNA-1 function could be relevant to neuronal vulnerability in one form of ALS. Further

study of the specific mechanism(s) by which FUS may affect lncRNA-1 function may provide novel insights into the impact of this or other lncRNAs in the pathogenesis of ALS.

Disclosures: J. Lawson: None. **B. Zhang:** None. **F. Jahaniani:** None. **L. Gunawardane:** None. **L.J. Hayward:** None. **S. Valadkhan:** None.

Poster

858. Motoneuron Disease Mechanisms

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Program#/Poster#:858.07/H2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH NINDS- RO1 NS051488

Farber Family Foundation

Title: Non-cell autonomous fus/tls mediated toxicity in als.

Authors: *A. T. KIA, A. BOGUSH, D. TROTTI, P. PASINELLI
Jefferson Hosp. of Neurosci., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease primarily characterized by degeneration and loss of motor neurons. Although most forms of ALS are sporadic (sALS), ~10% of patients suffer from a familial form (fALS). Mutations in several genes have been linked to fALS including fused in sarcoma (FUS), which accounts for ~5% of fALS cases. FUS is a DNA/RNA binding protein involved in multiple levels of gene expression including: DNA repair, RNA splicing, transcription, and transport of mRNA from the nucleus to distal destinations. While motor neurons are the primary target, ALS is a non-cell autonomous disease in which non-neuronal cells such as astrocytes and microglia contribute to motor neuron degeneration. In particular, in vitro systems demonstrated that astrocytes expressing SOD1 mutations are toxic to normal motor neurons when co-cultured. To determine whether FUS-expressing astrocytes are similarly toxic to motor neurons, we set up a co-culture system in which motor neurons are plated on astrocytes harboring mutant and wild-type FUS respectively. In our system, we determine motor neuron toxicity by measuring neurite outgrowth for each neuron as well as cumulative neurite sizes for each experimental population. To begin to address the mechanisms of FUS-astrocyte toxicity on motor neurons, we used conditioned media (CM) from wild-type or mutant FUS-expressing astrocytes to treat primary motor neurons. Our in vitro data indicate that similar to mutant SOD1 astrocytes, FUS expressing astrocytes contribute to motor neuron death.

Disclosures: A.T. Kia: None. **A. Bogush:** None. **D. Trotti:** None. **P. Pasinelli:** None.

Poster

858. Motoneuron Disease Mechanisms

Location: Hall F-J

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Program#/Poster#:858.08/H3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Creation of a screening platform to study the pathogenesis of ALS-FUS

Authors: N. WILMANS[1], E. BOGAERT[1], W. SCHEVENEELS[1], E. STORKEBAUM[3], P. CALLAERTS[2], W. ROBBERECHT[1,4], *L. M. VAN DEN BOSCH[5]
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Abstract: Background: Mutations in the Fused in Sarcoma (FUS) gene have been identified as a cause of FALS and classified as ALS-FUS. Most of the mutations cluster in the C-terminal part of the protein, which harbors a non-canonical nuclear localization signal. In the Belgian familial ALS patients we identified the R521H and the P525L mutations.
Objectives: We aimed to create small animal models to study the pathogenic mechanism of ALS-FUS.
Methods: Human FUS cDNA was incorporated into the *Drosophila* genome using site-directed integration. In zebrafish embryos transient overexpression of human FUS was obtained by injection of human FUS RNA and motor-axonal morphology investigated.
Results and Discussion: Overexpression of human FUS in the fly results in a larval and adult onset phenotype. Hatching problems have been observed in flies expressing human FUS during embryogenesis. Adult onset expression leads to severely reduced life span and motor performance defects. However, no differences were observed between wt and mutant FUS overexpressing flies.Transient expression of human FUS proteins in zebrafish embryos resulted in shortening and increased branching of motor axons. Also in this organism no significant difference was observed between wt and mutant FUS expressing zebrafish embryos.
To address the toxicity arising from human wt FUS, we deleted all functional domains from the wt protein and addressed toxicity in both animal models.
Conclusions: We have created two small animal models to study ALS-FUS pathogenesis. The *Drosophila* model will be used as a screening platform, to gain insight into the pathogenic mechanism and to identify disease-modifying genes. Potential hits will be validated

in the zebrafish model.

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Poster

858. Motoneuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Advancing a Healthier Wisconsin (ADE)

NIH R21 HD060899 (ADE)

Title: Building cellular circuits *in vitro* using iPSCs for neurodegenerative disease modeling

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Abstract: Defects in neuromuscular junction (NMJ) stability are seen in animal models of spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS) and these defects may play a role in motor neuron loss and muscle atrophy. In vivo examination of their physiological and biochemical properties is valuable, but examining NMJs in human cultures may provide additional insight into disease progression. Using SMA disease-specific induced pluripotent stem cells (iPSCs), we have generated cultures containing a mix of both neural and non-neural cell types. Upon further examination, we found HB9+/SMI32+ motor neurons along with Pax3+ and Pax7+ skeletal muscle progenitors and Myosin Heavy Chain (MHC)+ mature skeletal tissue. In order to optimize culture conditions for skeletal muscle, we dissociated and plated progenitor spheres in the presence of 2% B27, which significantly increased expression of skeletal muscle markers and produced more mature multi-nucleated and striated muscle cells. To date, two independent SMA patient lines and one control iPSC line have produced Pax3+, Pax7+ and MHC+ skeletal muscle cells. Further characterization showed upregulation of the appropriate skeletal muscle transcription factors Myf5, MyoD, and Myogenin in a temporal fashion. Pax3 expression has been seen in all lines; however, a significantly smaller proportion of cells express Pax7, which appears to be more indicative of MyoD, Myogenin, and MHC induction, and

ultimately skeletal muscle production. As such, further optimization is necessary for increasing Pax7 induction. Interestingly, recent reports have shown sustained neuregulin-1 (NRG-1) expression from neural crest cells maintains Pax7+ expression during embryonic development. Although we find HNK+ neural crest progenitors in our sphere cultures, they may be unable to significantly increase the expansion of Pax7+ cells. Therefore, we are incorporating exogenous NRG-1 along with additional factors into our culture conditions, such as insulin, EGF, and BSA, in order to improve efficiency of generating Pax7+ cells and subsequent sustainability of MHC+ skeletal muscle cells. Finally, in order to fully utilize this system, we are generating co-cultures of disease specific and control iPSCs to establish NMJs in vitro. Importantly, these co-cultures identify SMI32/ChAT+ motor neurons as well as synaptic markers synapsin, SV2, and fluorescent conjugated a-bungarotoxin. Establishing culture techniques that consistently generate patient specific iPSC derived skeletal muscle that can be used to form and maintain functional NMJs will be a powerful tool for studying a wide range of neuromuscular diseases.

Disclosures: J.V. McGivern: None. **T. Hosoyama:** None. **B.M. Heins:** None. **M. Suzuki:** None. **A.D. Ebert:** None.

Poster

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P2 ALS Consortium

The Wenner-Gren Foundation, Sweden

The Foundation BLANCEFLOR Boncompagni-Ludovisi, née Bildt

Title: A dual color screen using stem cell-derived motor neurons to model amyotrophic lateral sclerosis (ALS)

Authors: *S. J. THAMS[1,2], K. KANNING[2], H. LI[2], C. E. HENDERSON[3], H. WICHTERLE[4]
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurological disease causing selective degeneration of upper and lower motor neurons. 90% of all cases are sporadic (sALS) and 10%

are familial (fALS) with identified mutations. One of the major genes mutated in fALS is superoxide dismutase 1 (SOD1). Multiple point mutations in SOD1 have been identified and many of these recapitulate human disease when overexpressed in rodents. In vitro models using transgenic neurons would enable mechanistic studies of motor neuron degeneration. However, ALS relevant phenotypes are difficult to reproduce in short term cell culture system, likely reflecting the late onset nature of the disease. One approach to address this problem is to screen for stressors that can selectively accelerate pathological changes linked to mutations causing ALS.

The aim of this project was to set up a cell-based assay suitable for high-throughput drug screening, aimed at identifying stressors acting in synergy with SOD1 mutations. Such an assay requires a large amount of motor neurons harvested or generated in a reproducible and time efficient manner. We therefore took advantage of a previously described protocol for differentiating embryonic stem cells (ES) into motor neurons (Wichterle et al. 2002). We derived a series of ES cell lines from blastocysts carrying one of two fluorescent reporters (eGFP or tagRFP) under the control of the motor neuron specific Hb9 promoter; together with either the wild type (WT) or ALS causing mutated form (G93A) of human SOD1. The screen for biological stressors provoking or accelerating cell autonomous phenotypes linked to mutations in SOD1 was performed on mixed cultures containing GFP positive SOD mutant and RFP expressing WT ES cell derived motor neurons. Survival of WT (RFP) and ALS (GFP) motor neurons was monitored using a live imaging platform and the analysis was carried out with unbiased automated image software. After validating the robustness of the assay, we used the setup to screen a library of 1300 small molecule compounds. The goal was to identify compounds preferentially reduce the survival of mutant motor neurons, thereby revealing potentially new mechanisms for selective motor neuron degeneration in ALS.

From this primary screen we identified 10 stressors that promoted selective loss of ALS motor neurons. We are currently validating these findings in secondary screens, mapping pathways regulated by the compounds, and testing whether the compounds also elicit preferential degeneration of human ALS motor neurons derived from patient induced pluripotent stem cells.

Disclosures: S.J. Thams: None. **K. Kanning:** None. **H. Li:** None. **C.E. Henderson:** None. **H. Wichterle:** None.

Poster

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HHMI (ALH)

Title: Electrophysiological properties of spinal motoneurons from transgenic ALS mice recorded *in vitro*

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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that primarily affects lower and upper motoneurons. In the G93A-SOD1 transgenic mouse model of ALS, one of the earliest detected differences from wild-type animals is enhanced expression of a persistent inward current (PIC) in spinal motoneurons (Kuo et al., J Physiol 563: 843-54, 2005; Quinlan et al., J Physiol 589: 2245-60, 2011). Using our recently developed in vitro adult spinal slice model (Carp et al, J Neurophysiol 100: 474-81, 2008), we studied spinal motoneuron intrinsic properties from a higher copy strain of G85R-YFP-SOD1 transgenic mice (Wang et al., PNAS 106: 1392-7, 2009) that becomes paralyzed by 5-6 months, in order to determine if enhanced PIC expression is a common feature of mutant SOD1 mice.
Four transgenic and four wild type mice (3 months) were anesthetized and perfused transcardially with cold low-Na+ artificial cerebrospinal fluid (aCSF). After dorsal laminectomy, the lumbosacral spinal cord was rapidly excised and transferred to a cold low-Na aCSF bath. The L3-S1 spinal cord was isolated, embedded in agar, and sliced transversely in 300-µm sections in cold low-Na aCSF with a vibrating microtome. Slices were incubated in 30% polyethylene glycol for 60 s and then in normal-Na aCSF at 34-36°C for one hr, and were maintained at 28-30°C.
We recorded intracellularly from 14 mutant and 13 wild-type motoneurons with action potentials (APs) ≥60 mV. During repetitive firing elicited by triangular current ramp injection, the differences between derecruitment and recruitment current and firing rate were more negative (i.e., lower current and firing rate at derecruitment than at recruitment) in mutant than in wild type motoneurons ($p=0.05$ and $p=0.01$ by t-test, respectively). There were no differences between mutant and wild type motoneurons in input resistance or current threshold (rheobase) for eliciting a single action potential.
The results suggest that G85R mice, like G93A mice, have enhanced PICs, despite differences between the two mutants in metallation and misfolding of the mutant SOD1 protein. Thus, they support the hypothesis (Quinlan, Integr Comp Biol 51: 913-25, 2011) that enhanced PIC

expression contributes to metabolic stress, and thereby increases motoneuron vulnerability in SOD1-mutant mice.

Disclosures: J.S. Carp: None. **B.K. LaPallo:** None. **J.R. Wolpaw:** None. **A.L. Horwich:** None. **X.Y. Chen:** None. **J.R. Wolpaw:** None.

Poster

858. Motoneuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: FAPESP

CNPq

Title: Futher analyses of the role of astrocytes on motor neuron death in an *in vitro* model of Amyotrophic Lateral Sclerosis

Authors: *J. M. SCORISA, T. DUOBLES, G. P. OLIVEIRA, J. R. MAXIMINO, G. CHADI
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Abstract: The Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder characterized by a selective death of motor neurons. The transgenic mouse expressing human superoxide dismutase 1 (SOD1) gene with a G93A mutation (SOD1G93A) is the most accepted experimental model to study disease mechanisms. Recent studies indicate that astrocytes contribute to the ALS disease, however the molecular mechanisms are not yet established. Moreover, in vitro models describing toxic influence of astrocytes to motor neurons are based upon astrocytes collected from newborn mouse and also post morten material from spinal cord of ALS patients. Here we investigated the role of astrocyte in promoting motor neuron toxicity at pre-symptomatic phase of ALS.
Astrocyte cell cultures were obtained from spinal cord of post natal day 1 (P1) and 60 (P60) SOD1G93A (TG) and wild type (WT) mice. Motor neuron cell culture was performed from spinal cord of P1 neonatal of both genotypes. Firstly, the TG and WT motor neurons were treated with conditionated medium of TG and WT astrocyte cultures (n=3wells) and neuronal medium was used as a control. Secondly, TG and WT astrocytes were co-cultured with TG and WT motor neurons. Neurite length of motor neurons was measured by a stereological method at the first and fifth days of culture and neuronal death was quantified using a Flouro JadeC marker. Conditioned medium experiment demonstrated that TG astrocytes derived from P1 and P60 led

to a less neurite growth of TG cultured spinal cord motor neurons [124% (P1) and 90.5% (P60, $p<0.05$)] compared to WT [188% (P1) and 125% (P60)]. Co-cultured experiments have shown a higher neuronal death of TG cultured spinal cord motor neurons [50.6% (P1, $p<0.05$) and 53.43% (P60, $p<0.05$) compared to WT [10.9%(P1) and 20.4% (P60) mice. The results also showed that the above described events were more pronounced considering the astrocyte cultures of P60 TG than neonatal TG ($p<0.05$)
In conclusion, the astroglial toxicity to motor neurons in the ALS seems to be more pronounced at the period close to pre-symptomatic phase thus influencing the disease onset.

Disclosures: J.M. Scorisa: None. **T. Duobles:** None. **G.P. Oliveira:** None. **J.R. Maximino:** None. **G. Chadi:** None.

Poster

858. Motoneuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Guanabenz reduces proteasome dysfunction induced by tunicamycin in primary cell lines from mouse models of als

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Abstract: 2,6-dichlorobenzylidenaminoguanidine (Guanabenz, GA), an a2 adrenergic receptor agonist, is a well studied antihypertensive drug with more recently identified neuroprotective properties. Mechanisms proposed for GA's protective effect include, binding to the ribosome to inhibit it's protein folding activity and binding to and inhibiting a phosphatase, PPP1R15A/GADD34, which mediates translation initiation factor 2 (eIF2a) -dependent protein translation. Defects in protein folding and proteasomal function have been hypothesized to cause cell death, dysfunction and symptoms in amyotrophic lateral sclerosis (ALS). To establish a model of neurodegeneration, we treated primary cells from wild type and ALS mouse models with the proteasome dysfunction inducer, tunicamycin. Tunicamycin induced a dose dependent reduction in cell viability as measured by WST-1 assay. Pre-treating cells with 10uM guanabenz blocked 25% of the cell death induced by 0.1-0.3 uM tunicamycin as measured by WST-1 assay. Tunicamycin treatment also caused robust changes in unfolded protein response (UPR) and apoptosis mediator’s gene expression as determined by RT-qPCR assay. Guanabenz

concentrations of 10-30 uM caused a 25% reduction in UPR genes CHOP and Bip and an approximately 25% increase in Bcl2 gene expression compared to basal levels. Tunicamycin (0.1-0.3 uM) -induced changes in gene expression were altered to the same extent by 10 uM guanabenz as they were in response to GA treatment alone. We conclude guanabenz may exert a protective effect by mediating cellular response to defects in proteasome function and the unfolded protein response. The current studies inform us of dosing parameters to be used for in vivo mouse study design. These data also suggest that guanabenz may have a beneficial effect in the G93A SOD1 mouse model for ALS which is characterized by defects in proteasomal function.

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Poster

858. Motoneuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: The activity of anti-aggregant and pro-degradative molecules varies with the polyQ size in cell models of spinal and bulbar muscular atrophy (SBMA)

Authors: *A. POLETTI, E. GIORGETTI, P. RUSMINI, V. CRIPPA, A. BONCORAGLIO, R. CRISTOFANI, E. BOLZONI
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Abstract: The CAG/PolyQ-related diseases comprise nine different neurodegenerative disorders in which neuronal death is triggered by a mutant protein with an elongated polyglutamine stretch (polyQ). To cause disease, the polyQ size has to reach a specific threshold (tipically 35-40 Gln), but disease severity increases proportionally to polyQ size. Shorter (Q-35-Q60) polyQs associate with higher age-of-onset, milder symptoms and slower progression rate than longer polyQs (Q61-Q120 and over). Several factors are capable to counteract aggregation and induce degradation of mutant polyQ proteins, but their power against polyQ of different size might differ. We thus studied the effects of some compounds/proteins on a CAG/polyQ disease, the SpinoBulbar Muscular Atrophy (SBMA), caused by the polyQ of the androgen receptor (AR). SBMA is a motor neuronal disease and it is particularly useful since mutant AR neurotoxicity can be triggered by its ligand testosterone. We utilized AR with different polyQ length (non-

pathological Q0, Q10, Q22, or pathological Q48, Q112) expressed in immortalized motor neuronal (NSC34) cells. We also used a doxycycline-inducible PC12 cells expressing AR.Q10 or AR.Q112 (D. Merry, TJU, Philadelphia). Typically, after testosterone treatment, AR.Q46 aggregates in cytoplasm both in NSC34 and PC12 cells, while AR.Q112 aggregates in nuclei, but only when stably expressed in PC12 cells. We found that a small heat shock protein, HSPB8, prevents mutant AR.Q48 aggregation. HSPB8 acts by inducing AR.Q48 degradation via the autophagic pathway, without interfering with the proteasome. Interestingly, HSPB8 was much less efficient with the AR.Q112 (both transiently or stably expressed in PC12 or NSC34 cells). We also utilized the disaccharide trehalose and the artificial chaperone cyclodextrin (CD, a water soluble, non-toxic, cyclic oligosaccharide used as vehicle for drug delivery). Both trehalose and CD were active on AR.Q48, but less efficient on AR.Q112 aggregation and clearance. In fact, both compounds efficiently inhibited AR.Q46 aggregation rate (evaluated in ICC and filter retardation assays) and decreased protein levels (evaluated in western blot). Therefore, our data indicate that both molecular and chemical approaches can counteract aggregation and facilitate clearance of ARpolyQ with shorter pathological repeats, but showed different pro-degradative and/or anti-aggregation power on ARs with different polyQ length. Since the longest repeats described in SBMA patients are of approximately 70Qs, these aspects may be relevant in the choice of the models on which one should test compounds to be used in future therapeutical trials.

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Poster

858. Motoneuron Disease Mechanisms

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Title: Naratriptan ameliorates SBMA pathology by the repression of CGRP1-activated JNK pathway

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Abstract: SBMA (Spinal and bulbar muscular atrophy) is a motor neuron disease caused by the expansion of the CAG triplet repeats within the gene encoding the AR (androgen receptor). We performed the gene expression analysis for the spinal cord of SBMA model mice with DNA microarray, and identified the disease related gene CGRP1 (calcitonin gene-related peptide alpha). CGRP1 is a multifunctional neuropeptide and is thought to react with autocrine-paracrine mode in spinal motor neurons, but the pathological function is not well known. Therefore, we made the biochemical and pathological analysis (western blotting, ELISA, RT-PCR, cell viability assay, cell cytotoxic assay, immunohistochemistry etc.) of CGRP1 for SBMA model cells (SH-SY5Y stably expressing mutant AR) and mice (mutant AR transgenic mice), and searched for the therapeutic strategy. CGRP1 expressed intensively in the motor neuron of SBMA model mice, and was also detected in the post mortem spinal cord of the patients. In neuronal cell, the over-expression of CGRP1 reacted toxically via the activation of the c-Jun N-terminal kinase (JNK) pathway, and the genetical / pharmacological suppression of CGRP1 (siRNA / CGRP1 antagonist, CGRP8-37) or JNK inhibitor, SP-600125 rescued from the neurotoxicity. In a mouse model of SBMA, the CGRP1 expression level in the spinal cord was attenuated by the castration, and the depletion of CGRP1 ameliorated the phenotypes (survival rate, body weight, rotarod task, and grip) and deactivated the JNK pathway. We searched CGRP1 suppressor for SBMA therapy, and naratriptan, a 5HT1B/1D agonist was chosen for the long lasting reactivity and the blood brain barrier permeability among the three agonists (naratriptan, sumatriptan, and rizatriptan). Naratriptan suppressed CGRP1 expression via the induction of MKP1 (mitogen-activated protein kinase phosphatase 1), and attenuated JNK activity. The 5HT1 receptor blocker, methiothepin offset the effect of naratriptan in cellular SBMA models. Naratriptan mitigated the pathogenic AR-mediated neuronal damage in cellular and mouse SBMA models. CGRP1 is a new therapeutic target for SBMA and the pharmacological activation of the 5HT1B/1D receptor is a novel therapeutic strategy for this disease.

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Poster

858. Motoneuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Johns Hopkins Robert Packard Center for ALS Research

The Johns Hopkins Ansari Fund

Title: Development of a C9ORF72 ALS antisense therapy and a therapeutic biomarker.

Authors: C. J. DONNELLY[1], L. W. OSTROW[1], P.-W. ZHANG[1], S. VIDENSKY[1], B. N. HOOVER[1], U. BALASUBRAMANIAN[1], Y. LI[1], N. J. MARAGAKIS[1], P. TIENARI[5], B. J. TRAYNOR[6], R. SATTLER[1,2], *J. D. ROTHSTEIN[3,2,4]
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Abstract: The C9ORF72 hexanucleotide repeat expansion has been identified in ~30% of familial and ~4-10% of sporadic ALS cases and is therefore the most common genetic abnormality associated with ALS to date. Repeat expansions in non-protein coding regions are the known cause of other neuromuscular disorders (e.g., DM1/2, FXTAS) and pathogenicity is thought to arise from aberrant binding of trans acting factors to the cis repetitive elements. This is best studied in DM1 where MBNL1 is sequestered by the 'CTG' expansion in the DMPK pre-mRNA. Therefore, antisense oligonucleotide (ASO) and RNAi (siRNA) strategies designed to block binding to the cis elements may have therapeutic potential in these disorders. To test this possibility in the context of C9orf72 ALS, we designed siRNA and ASO methodologies to target the C9ORF72 transcript and repeat expansion, and have demonstrated knockdown the C9ORF72 gene in patient-derived cell lines. Furthermore, utilizing our transcriptome profiling generated from C9ORF72 ALS patient fibroblasts, iPS derived CNS cells and validated with human autopsy tissue, we show that ASO/RNAi treatment can normalize specific genes which are aberrantly expressed in cell lines that contain the 'GGGGCC' expanded repeat. These approaches will allow us to effectively develop 1) antisense mediated therapeutic approaches to ALS and 2) relevant pharmacodynamic readouts for antisense efficacy.

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Poster

858. Motoneuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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Title: RBM45 pathology in sporadic and C9ORF72-linked amyotrophic lateral sclerosis

Authors: *M. A. COLLINS[1], T. KOVALIK[2], D. RIASCOS[2], R. BOWSER[2]
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Abstract: RNA binding protein pathology is a pathological hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Using liquid chromatography tandem mass spectrometry (LC/MS-MS), we recently identified the RNA binding protein RBM45 as a putative ALS biomarker. Immunohistochemistry revealed the presence of RBM45-positive inclusions, similar in appearance to those containing TDP-43, in ALS patients. These could be detected in ALS cases with the c9ORF72 hexanucleotide repeat expansion. Recent evidence suggests that c9ORF72-linked ALS has a distinct pathological profile. This study was conducted to further describe the RBM45 pathology in the central nervous system of sporadic and c9ORF72-linked ALS cases and to assess the normal distribution, expression, and subcellular localization of the protein. ALS, C9ORF72-linked ALS, FTLD, and age-matched control patient tissues were used. Regions examined included spinal cord, hippocampus, cerebellum, and frontal cortex. RBM45 expression was evaluated by immunohistochemistry and immunoblot. Immunofluorescence was used to examine the colocalization of RBM45 with TDP-43, stress granule markers, ubiquitin, p62, and SC35. Immunoblot results demonstrated modestly increased levels of RBM45 in the CSF of ALS patients, consistent with our LC-MS/MS data. By immunohistochemistry we detected RBM45 inclusions in spinal cord motor neurons and glia of sporadic and c9ORF72-linked ALS cases. The number of surviving motor neurons and percentage of these harboring RBM45 inclusions was higher in c9ORF72-linked ALS than in sporadic ALS. Cytoplasmic RBM45 inclusions could be seen in the dentate gyrus of FTLD patients and, more rarely, sporadic ALS cases. Inclusions were not seen in control cases. RBM45 inclusions were frequently positive for TDP-43, ubiquitin, and stress granule markers. RBM45 also exhibited a speckled nuclear staining pattern that was negative for the nuclear speckle marker SC35. In this study we have further characterized RBM45 pathology in ALS and have added to existing evidence suggesting that sporadic ALS and c9ORF72-linked ALS differ pathologically. Nevertheless, the colocalization of RBM45 inclusions with TDP-43 and stress

granule markers suggests common mechanisms of inclusion formation. The speckled nuclear staining pattern observed for RBM45 distinguishes it from TDP-43. This pattern was also separate from SC35-positive nuclear speckles, implying that RBM45 is a component of a distinct subnuclear structure. Ongoing studies will determine RBM45's role in RNA metabolism, stress response, and how these processes might contribute to neurodegeneration.

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Poster

858. Motoneuron Disease Mechanisms

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Title: Decreased expression of BTBD10 in ALS motor neurons may contribute to the ALS pathogenesis

Authors: *M. NAWA, M. MATSUOKA
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Abstract: BTBD10, an Akt interactor, increases phospho-Akt levels by decreasing the protein phosphatase 2A-mediated dephosphorylation of Akt. In this study, we found that the level of BTBD10 expression is decreased in spinal cord motor neurons from the patients of sporadic ALS and transgenic mice overexpressing G93A-SOD1. We further investigated whether the reduction of BTBD10 is linked to the ALS pathogenesis. The overexpression of BTBD10 suppressed motor neuron death that was induced by a familial ALS-inked superoxide dismutase (SOD) 1 mutant, G93A-SOD1 *in vitro*. Reciprocally, the siRNA-mediated reduction of BTBD10 expression led to the death of cultured motor neuron. In *Caenorhabditis elegans*, the disruption of the *btbd-10* gene caused loss of neurons, including both motor and touch-receptor neurons, and a locomotion defect. Interestingly, the low-level expression of fused in sarcoma (FUS), an ALS-linked protein, downregulated the level of BTBD10 expression and simultaneously induced neuronal cell death. Our ongoing study is aimed at addressing the mechanism underlying the FUS-induced neuronal cell death from a standpoint of the BTBD10 involvement.

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Poster

858. Motoneuron Disease Mechanisms

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:858.19/H14

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R01NS051488 (PP)

NIH Grant RO1-NS64488 (DT)

Title: Mutated SOD1 causes mitochondrial damage in astrocytes

Authors: *N. NANICHE, W. TAN, D. TROTTI, P. PASINELLI
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Abstract: Amyotrophic lateral sclerosis (ALS) is a slowly progressing, ultimately fatal neurodegenerative disease affecting mainly motor neurons in the motor cortex, brainstem and spinal cord. ~ 20% of all inherited cases of ALS (fALS) are caused by mutations in the gene encoding for superoxide dismutase 1 (SOD1), which lead to a gain of toxic function(s) of the protein. Although motor neurons are the predominant cells that die during ALS, glial cells are also known to contribute pathologically to the progression of disease. A number of studies have shown the importance of knocking out mutSOD1 from glia towards ameliorating disease. While many of these studies looked at the toxic effects that astrocytes have on motor neurons, fewer have looked at the effect that mutSOD1 has on astrocytes themselves, in particular on astrocyte mitochondria. While a predominately cytoplasmic protein, a small portion of SOD1 has been shown to localize to the mitochondria, where when mutated the protein can cause mitochondrial damage and toxicity. In this study, we analyze the effects of mutSOD1 specifically on astrocyte mitochondria. Using wild-type (WT) or G93A-mutated SOD1 plasmid in an adeno-virus (Adv-SOD1), we infected primary non-transgenic spinal cord astrocytes with Adv-SOD1 over a time-course to look for mitochondrial morphological and bioenergetic changes. We found that over an eight-day time course, there was little to no effect of WT Adv-SOD1 on astrocyte mitochondria in terms of morphology, bioenergetics or protein level changes. However, there were dramatic changes seen in G93A Adv-SOD1 treated astrocytes. Mutated SOD1 aggregates were seen in infected astrocytes that led to aggregation of mitochondria. Shifts in mitochondrial membrane potential were seen throughout the eight-day treatment, and mitochondrial protein levels were decreased over time in these astrocytes. However, astrocytes were still alive even with these mitochondrial abnormalities after G93A Adv-SOD1 treatment. Our data suggest that mutSOD1 causes substantial mitochondrial alterations to astrocytes, without causing astrocyte death or

toxicity in the same rapid fashion as it does in motor neurons. Studies are underway to determine the cellular differences in response to mitochondrial damage between motor neurons and astrocytes.

Disclosures: N. Naniche: None. **W. Tan:** None. **D. Trotti:** None. **P. Pasinelli:** None.

Poster

858. Motoneuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Pathogenic role of astrocyte exocytosis in ALS

Authors: S. NG, N. DIAZ, *H. HIGASHIMORI, L. MOREL, P. HAYDON, Y. YANG
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Abstract: The recognition of the active roles of glial cells in the physiology of mammalian central nervous system (CNS) is significantly changing the view how neurodegeneration occurs and progresses. In ALS, many lines of evidence have clearly suggested that astrocytes are involved in pathogenesis and can significantly modulate disease phenotype. Recent in vitro studies found that SOD1G93A astrocytes can selectively induce motor neuron cell death through secreted toxic factors. This implicates a primary and direct role of astrocyte gain-of-toxicity in motor neuron death. However, the exact mechanisms/pathways that underlie astrocyte mediated toxicity in ALS remain poorly understood. Exocytosis represents one of the major pathways for the secretion of active substances from astrocytes, which is SNARE-dependent and requires membrane fusion between the vesicle membrane and plasma membrane. In this project, we investigated the effect of astrocyte exocytosis inhibition on the disease phenotypes in SOD1G93A mice in vivo. Selective inhibition of the exocytosis pathway in astrocytes has been previously achieved by expressing the cytosolic portion of the SNARE protein synaptobrevin 2 in cultured astrocytes and in doxycycline (Dox) regulated Tet on/off transgenic mice (GFAP-tTA/TetO-SNARE mice). We have bred these mice with SOD1G93A mice to generate triple SOD1G93A+SNARE+tTA+ transgenic mice. Dox was supplemented into the food during breeding and was removed once the mice were weaned (P21). Induction of SNARE was confirmed by the co-expression of the eGFP reporter in the spinal cord. Disease phenotypes, including forelimb and hindlimb grip strength, weight loss, and survival, were closely examined weekly in these mice starting from P60. A significant delay in disease onset (defined by 10% loss of forelimb grip strength) was found in SOD1G93A+SNARE+tTA+ mice (α=104, N=8)

compared to that of littermates SOD1G93A+ mice (α=94, N=13). Similarly, the late disease onset (defined by 10% weight loss) in SOD1G93A+SNARE+tTA+ mice is also significantly delayed (α=131, N=8) compared to that in SOD1G93A+ mice (α=119, N=13). However, the disease duration time (from the onset to the end-stage) between SOD1G93A+SNARE+tTA+ (α= 41, N=8) and SOD1G93A+ (α=47, N=13) mice is not significantly different. In fact, SOD1G93A+SNARE+tTA+ mice tend to have shorter disease progression than that in SOD1G93A+ mice (though not statistically significant). We are currently investigating the effects of exocytosis-inhibited SOD1G93A astrocytes on motor neuron cell survival using in vitro co-culture system.

Disclosures: S. Ng: None. **N. Diaz:** None. **H. Higashimori:** None. **L. Morel:** None. **P. Haydon:** None. **Y. Yang:** None.

Poster

858. Motoneuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant RO1-NS051419

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Robert Packard Center for ALS Research

Muscular Dystrophy Association

Title: Abnormal store-operated calcium entry and ER calcium release in mutant SOD1 astrocytes

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Abstract: Calcium release from the endoplasmic reticulum (ER) stores is the main source of calcium signaling in astrocytes. To maximize signaling effects, cytosolic calcium concentrations are tightly controlled by the interactions of multiple calcium regulating systems, including mitochondria, ER, and plasma membrane. After ER calcium release, plasma membrane calcium channels are activated to replenish the calcium that has been extruded from the cell, a mechanism defined as store-operated calcium entry (SOCE).

We investigated the mechanisms of ER release following purinergic receptor stimulation and SOCE, in cultured adult spinal cord astrocytes from mutant SOD1 transgenic mice. With ATP stimulation of the IP3 receptor, ER calcium release in mutant astrocytes was enhanced as compared to non-transgenic cells. Depleting ER calcium with the SERCA inhibitor caused higher ER calcium release from mutant astrocytes, suggesting increased ER calcium stores. The calcium clearance through the plasma membrane and mitochondria did not differ between mutant and control astrocytes. In addition, the increased ER calcium release did not correlate with changes in ER volume or decreased levels of the ER calcium-binding protein calreticulin. Mutant astrocytes had a significantly increase in the influx of calcium by SOCE, which could explain the increased ER calcium content in mutant cells. We further investigated the sensitivity to SOCE inhibitors, and found that mutant astrocytes were partially insensitive to STIM1-ORA1 inhibition.

STIM1 glutathionylation results in a constitutively active protein, which opens ORA1 calcium channels on the plasma membrane. This posttranslational modification renders STIM1 insensitive to ER calcium levels and may result in chronic increase of ER calcium filling. To test the hypothesis that glutathionylation of STIM1 results in increased calcium entry, we treated mutant SOD1 cells with the antioxidant BHA, which completely normalized the SOCE response. Vice versa, treatment with BSO, which depletes GSH levels and causes oxidative stress, resulted in increased calcium entry in control cells

These findings suggest that mutant SOD1 causes increased ER calcium levels by promoting oxidative stress and glutathionylation of STIM1 in astrocytes. This could provide a mechanistic explanation for the abnormal calcium dynamics in mutant SOD1 astrocytes. Although astrocytes are spared in mutant SOD1 associated familial amytrophic lateral sclerosis, abnormal calcium properties in the astrocytes may be involved in the non-cell autonomous toxicity to motor neurons by altering the tripartite synaptic milieu.

Disclosures: H. Kawamata: None. **G. Manfredi:** None.

Poster

858. Motoneuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: LES Turner Foundation

Blazeman Foundation

Title: Mutation in ubiquilin4 in ALS patient affects the ubiquitin proteosome system and Autophagy *in vitro*

Authors: K. AJROUD, 1[1], J. YAN, 2[1], F. FECTO, 3[1], Y. SHI, 4[1], N. SIDDIQUE, 5[1], J.-G. ZHENG, 6[1], *E. MUGNAINI[2], H.-X. DENG, 8[1], T. SIDDIQUE, 9[1]
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Abstract: Growing evidence supports the notion that impairment of the proteosome and autophagy are associated with the formation of protein aggregates and neurodegeneration including ALS. We recently reported mutations in Ubiquilin2, member of the Ubiquilin family affecting both the Ubiquitin Proteosome System (UPS) and Autophagy in vitro. We have now identified a mutation in Ubiquilin4 gene in a familial ALS patient. Ubiquilin4 is a member of the Ubiquilin family and also plays a role in the regulation of proteasomal protein degradation. The aim of the current study is to test whether the identified mutation in Ubiquilin4 affects the UPS and the Autophagy in vitro. We performed genomic DNA sequencing of familial ALS cases as well as controls and generated a cell line model (Neuro2A cells, N2A) expressing Ubiquilin4 to evaluate the expression of the Ubiquilin4 wild type and mutant and conducted colocalisation with autophagosome markers and ALS linked proteins using Confocal microscopy and Flow cytometry. We identified a mutation in the exon 3 of Ubiquilin4 gene that was absent from controls. We also found four single nucleotide polymorphisms (SNP) that were present in the controls. N2A cells transiently transfected with Ubiquilin4 tagged to mCherry showed punctuate structures in both wild type and mutant. Flow cytometry reveled a significant accumulation of LC3-GFP which corresponds to an inhibition of autophagic activity in mutant Ubiquilin4 transfected cells. Consistent with this, we observed that both wt and mutant Ubiquilin4 co-localize with LC3, p62 and subsequently Ubiquilin2. However we found a significant decrease in the Rab7-GFP accumulation in the mutant Ubiquilin4 transfected cells which may indicate impairment at the autophagosome late endosome formation and exacerbating the decrease in the autophagy activity. These data suggest that mutant Ubiquilin4-mediated autophagy impairment may be responsible for ALS pathogenesis. Such a mechanism could participate in the accumulation of the aggregation-prone proteins in ALS, and impair essential regulatory functions of autophagy and the UPS. Hence, autophagy-related proteins, like Ubiquilin4, represent an attractive target for designing rational therapeutics in ALS.

Disclosures: K. Ajroud: None. **J. yan:** None. **F. Fecto:** None. **Y. Shi:** None. **N. Siddique:** None. **J. Zheng:** None. **E. Mugnaini:** None. **H. Deng:** None. **T. Siddique:** None.

Poster

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ARS SLA

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Title: Homeostatic regulation of excitability in SOD1 mouse motoneurons

Authors: M. MANUEL[1,2], N. DELESTREE[1], S. M. ELBASIOUNY[2], C. IGLESIAS[1], C. J. HECKMAN[2], *D. ZYTNICKI[1]
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Abstract: Excitotoxicity has been proposed as a mechanism leading to the selective death of MNs in Amyotrophic Lateral Sclerosis (ALS). It is indeed known that increase excitation and hyperexcitability can cause the death of motoneurons (MNs). However, it has yet to be demonstrated that spinal MNs exhibit changes in their excitability in the days preceding their degeneration. The aim of this work is therefore to test this hypothesis.
To do so, we recorded the intrinsic properties of MNs in SOD1 mice at an age (P50) just preceding the first denervations of motor units in two regions of the spinal cord affected by the disease: the sacro-caudal cord and the lumbar cord.
At odds with the excitotoxicity hypothesis we observed that SOD1 MNs did not exhibit signs of hyperexcitability in either region of the spinal cord. In particular, we observed that the input conductance of SOD1 MNs was significantly increased compared to controls. In the lumbar cord, the input conductance of MNs was 0.31±0.13µS (N=25) in WT mice, whereas it was 0.46±0.18µS (N=25; $p<0.005$) in mutant animals. Despite this increase of conductance, the excitability of the motoneurons was preserved. Neither the onset current for repetitive firing (4.7±3.1nA; N=10 vs. 5.9±3.0nA; N=10), nor the gain of the current-frequency relationship (11±2Hz/nA; N=6 vs. 16±7Hz/nA; N=8) were statistically different between SOD1 and WT mice.
Our new results show that MN conductance continues to increase as the SOD1 animal matures, pushing some cells well above the normal limits. Yet, net excitability continues to remain normal, clearly requiring an increase in voltage sensitive currents as a compensatory mechanism. Thus MNs in ALS do not become hyperexcitable but instead undergo a notably successful

homeostatic regulation for excitability. Although initially this homeostasis helps the animal maintain normal motor output, the progressive increases in cell size and density of membrane channels could lead to their degeneration.

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Poster

858. Motoneuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CONACYT, Mexico (Project 128229)

DGAPA, UNAM (Project IN215610)

Title: Characterization of the motor neuron death processes in two models of excitotoxicity in the rat spinal cord *in vivo*.

Authors: *U. N. RAMIREZ-JARQUIN, R. TAPIA
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Abstract: Among the multiple factors involved in motor neuron (MN) degeneration in amyotrophic lateral sclerosis (ALS), glutamate-mediated excitotoxicity seems to be of relevance. Based on the overactivation of AMPA type glutamatergic receptors, we developed two models of MN degeneration in the rat spinal cord, one by AMPA perfusion during 25 min using the microdialysis technique (acute model, J. Neurochem. 89:988, 2004.), and the other by the continuous infusion of AMPA with osmotic minipumps (chronic model, J. Neuropathol. Exp. Neurol. 66:913, 2007). Both models induce MN loss and motor deficits similar to ALS, the acute one during the 12 initial hours after AMPA perfusion and the chronic one during 2-5 days after the implant of minipump. In order to know the process of MN death, we studied comparatively in the two models the time course of the morphological and biochemical changes in the tissue, by means of electronic and confocal microscopy. In the acute model, MN degeneration starts 3-4 h after the AMPA perfusion and is characterized by swelling and rupture of cytoplasmic, nuclear and mitochondrial membranes, which progress until total disruption of cell structure at about 12 h. At 24 h macrophage-like cells around remnant cell debris were observed. In this model no cleaved caspase-3 or DNA fragmentation (TUNEL) were detected at any time. In the chronic model, MN loss and motor deficit occur gradually, starting in the ipsilateral side the second day

after the beginning of AMPA infusion and progressing to the contralateral ventral horn. We identified four degenerative stages during the progress of MN degeneration, as follows: stage 1, vacuolization of the cytoplasm (days 1-2); stage 2, swelling of endoplasmic reticulum and mitochondria (days 2-4); stage 3, formation of “empty pools” (large intracellular zones without cellular components, probably resulting from the fusion of vacuoles) (days 3-5); stage 4, rupture of the cytoplasmic membrane. Macrophage-like cells also appeared from day 2. Cleaved caspase-3 and TUNEL were detected immunohistochemically in MNs, but only in stages 2-3. We conclude that necrosis is the predominant MN death process in the acute model, whereas in the chronic model degeneration starts by apoptosis but a necrotic process develops afterwards. These findings are relevant for the understanding of MN death mechanisms and consequently for the design of pharmacological strategies directed to the treatment or prevention of ALS.
Supported by CONACYT, Mexico (Project 128229) and DGAPA, UNAM (Project IN215610), U.N.R.J is recipient of a scholarship from CONACYT.

Disclosures: U.N. Ramirez-Jarquin: None. **R. Tapia:** None.

Poster

858. Motoneuron Disease Mechanisms

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: PRIN project 2006058401

Title: Group I metabotropic glutamate receptors in ALS: knocking-down mGlu1 receptors ameliorates survival and disease progression in SOD1/G93A mice.

Authors: M. MILANESE[1], A. PULITI[2], T. BONIFACINO[1], F. CONTI[3], F. GIRIBALDI[1], I. MUSANTE[2], M. MELONE[3], *G. BONANNO[1,4]
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Abstract: Amyotrophic lateral sclerosis (ALS) is a late-onset fatal neurodegenerative disease characterized by muscle wasting, weakness and spasticity, reflecting a degeneration of upper and lower motor-neurons (MNs). The cause of ALS and the mechanisms of neuronal death are still largely obscure, thus impairing the establishment of efficacious therapies. Glutamate(Glu)-mediated excitotoxicity plays a major role in the degeneration of MNs in ALS and reduced

astrocytary glutamate transport in spinal cord, which in turn increases the synaptic availability of the amino acid neurotransmitter, was suggested as a cause. On the basis of our studies, we alternatively proposed that abnormal release of Glu may well represent a possible source of excessive glutamate transmission. In this line, we recently demonstrates the existence of mGlu1 and mGlu5 Glu-releasing autoreceptors in spinal cord nerve terminals, whose activation produces higher Glu release in SOD1/G93A mice, a widely used animal model of human ALS, than in controls.
In the present work we generated mice carring half expression of mGlu1 receptors in the SOD1/G93A background, by crossing the SOD1/G93A mutant mouse with Grm1+/crv4 mouse, which lacks mGluR1 because of a spontaneous recessive mutation, thus providing a genetic tool to evaluate the role of mGlu1 receptors in ALS. Double mutants were analysed for survival and motor abilities, as well as for biochemical and histological parameters linked to the disease progression. Double mutants showed prolonged survival probability with respect to single mutant SOD1/G93A mice. Accordingly, slower disease progression and improved motor performances were observed. Histological studies showed MN preservation in spinal cord, reduced mithocondrial damage and axonal degeneration, decreased astrocyte and microglia activation in double mutant mice. Moreover, the mGluR1 deletion turned around the up-regulation of metallothioneines, glutathione S-transferase and iNOS mRNA expression present in SOD1/G93A mice. This study demonstrates that mGlu1 receptor deletion has a significant impact on survival and motor performances in mice with experimental ALS, thus providing the rationale for the selective block of these receptors as a novel pharmacological approach in the treatment of this disease.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: The UK Motor Neurone Disease Association

Title: Characterization of a unique SOD1 mouse model for Amyotrophic lateral sclerosis

Authors: R. SACCON[1], P. JOYCE[2], P. FRATTA[1], L. GREENSMITH[3], *P. M. NOLAN[2], A. ACEVEDO[2], E. FISHER[1]

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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the loss of upper and lower motor neurons (MNs). In patients, the disease leads to paralysis and is fatal within 2-5 years of onset, generally due to respiratory failure. ALS is mostly sporadic but approximately 10% of cases are familial (FALS). Mutations in the Cu/Zn superoxide dismutase type 1 (*SOD1*) gene are known to cause FALS. Several SOD1 transgenic mice modelling ALS provide evidence of a toxic gain of function mechanism. While the toxic gain of function has been proven to be causative of the disease, a reduction of SOD1 function is not sufficient to provoke loss of MNs, but it does make MNs more susceptible to damage. SOD1 dismutase activity has been measured in a variety of human ALS patients carrying different mutations in *SOD1*. In the great majority of cases, SOD1 activity is reported to be reduced to ~50%. This activity reduction is not reproduced in SOD1 transgenic mouse models, as they express mutant SOD1 in addition to the normal mouse alleles. To better represent the human disease, a new SOD1 mouse model has been generated using MRC Harwell's N-ethyl-N-nitrosourea (ENU) mutagenesis program. This new mouse strain carries a single point mutation in the mouse endogenous *Sod1* gene (D83G), a mutation previously found in a familial ALS kindred. As this new mouse model carries the D83G mutation in the mouse *Sod1* gene, mutant SOD1 will be expressed at endogenous levels, making it biochemically more relevant to the human condition. We have measured SOD1 activity in brain extracts from these mice and found that heterozygotes ($Sod1^{D83G/+}$) have a ~50% reduction whereas homozygotes ($Sod1^{D83G/D83G}$) are dismutase inactive. Data from our group shows that homozygous mice ($Sod1^{D83G/D83G}$) display weakness and motor neuron degeneration which models aspects of human ALS. In order to further characterize this model and gain some insights on the pathological mechanisms of ALS, we are presenting an immunohistochemical, biochemical and molecular analysis of these mice.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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Title: Group I metabotropic glutamate receptors in ALS: mGluR1 and mGluR5 autoreceptors induce excessive glutamate release in SOD1/G93A mice

Authors: *C. USAI[1], F. GIRIBALDI[2,3], M. MILANESE[2,3], T. BONIFACINO[2,3], S. DI PRISCO[2,3], C. TACCHETTI[4], A. PULITI[5,6], A. PITTALUGA[2,3], G. BONANNO[2,3]
[1]Natl. Rese Council, Genova, Italy; [2]Dept. of Exptl. Medicine, Pharmacol. and Toxicology Unit, [3]Ctr. of Excellence for Biomed. Res., [4]Dept. of Exptl. Medicine, Human Anat. Unit, [5]Dept. of Pediatric Sci., Univ. of Genova, Genova, Italy; [6]Mol. Genet. and Cytogenetics Unit, Gaslini Inst., Genova, Italy

Abstract: Amyotrophic lateral sclerosis (ALS) is a late-onset fatal neurodegenerative disease characterized by muscle wasting, weakness and spasticity, reflecting a degeneration of upper and lower motor-neurons (MNs). The cause of ALS and the mechanisms of neuronal death are still largely obscure, thus impairing the establishment of efficacious therapies. Glutamate-mediated excitotoxicity plays a major role in ALS and reduced astrocytic glutamate transport was suggested as a cause. Based on previous work we have proposed that abnormal release may represent another source of excessive glutamate. In this line, here we studied the modulation of glutamate release in ALS by Group I metabotropic glutamate (mGlu) receptors, that comprise mGlu1 and mGlu5 members. Synaptosomes from the lumbar spinal cord of SOD1/G93A mice, a widely used murine model for human ALS, and controls were used in release, confocal or electron microscopy and Western blot experiments. Concentrations of the mGlu1/5 receptor agonist 3,5-DHPG >0.3 μM stimulated the release of [3H]D-aspartate, used to label the releasing pools of glutamate, both in control and SOD1/G93A mice. At variance, ≤0.3 μM 3,5-DHPG increased [3H]D-aspartate release in SOD1/G93A mice only. Experiments with selective antagonists indicated the involvement of both mGlu1 and mGlu5 receptors, mGlu5 being preferentially involved in the high potency effects of 3,5-DHPG. High 3,5-DHPG concentrations increased IP3 formation in both mouse strains, whereas low 3,5-DHPG did it in SOD1/G93A

mice only. Release experiments confirmed that 3,5-DHPG elicited [3H]D-aspartate exocytosis involving intra-terminal Ca2+ release through IP3-sensitive channels. Confocal microscopy indicated the co-existence of both receptors presynaptically in the same glutamatergic nerve terminal in SOD1/G93A mice. To conclude, activation of mGlu1/5 receptors produced abnormal glutamate release in SOD1/G93A mice, suggesting that these receptors are implicated in ALS and that selective antagonists may be predicted for new therapeutic approaches.

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Poster

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Support: Martina Liebl and Murat Kaya are members of the neuro graduate school Mainz (DFG DK1044)

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Title: The neuronal calcium sensor protein VILIP1 is misslocalized and enriched in aggregates in SOD1 mouse models of Amyotrophic Lateral Sclerosis

Authors: M. LIEBL[1], M. KAYA[1], S. TENZER[2], H. SCHILD[2], K.-H. BRAUNEWELL[3], *C. W. BEHL[1], A. CLEMENT[1]
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a devastating neurological disease that leads to progressive death of motoneurons in the spinal cord and the motor cortex, with an average survival time of only 1-5 years after diagnosis. Mutations in the gene for superoxide dismutase 1 (SOD1) have been identified as the major cause of familial ALS, and mouse models based on the overexpression of mutant human SOD1 have proofed to be valuable models for the disease. Although a lot of effort has been made, the underlying pathomechanisms leading to the specific death of motoneurons still have to be elucidated. Occurrence of protein aggregates is a major hallmark in human ALS and can be observed in mutant SOD1 (SOD1-mt) transgenic mice. Whether protein aggregates play a causal role in the disease is still a matter of debate and several

additional pathomechanisms have been proposed. As one hypothesis, glutamate excitotoxicity, leading to missregulation of intracellular calcium levels and subsequent apoptosis, is discussed. Visinin-like protein 1 (VILIP1) belongs to a group of neuronal calcium sensor (NCS) proteins and contains two functionally active EF-hand motifs for the binding of Ca2+. All members of the NCS family contain a myristol residue which, dependent on a conformational change upon Ca2+-binding, interacts with cellular membranes. Cytosolic VILIP-1 has been shown to translocate Ca2+-dependently to the plasma membrane, where it has been shown to directly or indirectly affect cellular cGMP and cAMP levels and to modulate α4β2-nicotinergic acetylcholine receptors.
We found VILIP1 to be specifically enriched in an aggregate fraction of SOD1(G93A) and SOD1(G85R) overexpressing mice at disease onset and endstage, but not in SOD1(WT) overexpressing mice and non-transgenic littermates. Furthermore we could show that in SOD1-mt overexpressing mice VILIP1 localization to the plasma membrane was decreased and the protein was more uniformly distributed to the cytosol.
Considering that VILIP-1 localization is sensitively modulated by intracellular calcium levels, and VILIP-1 trapped in aggregates might not be functionally active, a reduction of available VILIP-1 protein level provides an interesting link between the role of protein aggregation and glutamate excitotoxicity in ALS.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: EY-07099

Title: CNS glutamate cysteine ligase catalytic subunit deficiency mice induce mitochondrial damage, neurodegeneration and develop als phenotype

Authors: *X. FAN[1], B. SHENG[2], R. MARIANA[3], S. SIEDLAK[2], V. MONNIER[4], P. FENG[5]
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Abstract: Amyotrophic lateral sclerosis (ALS) is devastating disease that 90% of the cases is sporadic with unknown origin. Studies carried out with postmortem tissue from sALS and fALS patients clearly show accumulation of oxidative damage to proteins, lipids, and DNA indicating a direct role of oxidative stress. Glutathione (GSH) which is considered to be the most prevalent and important intracellular non-protein thiol/sulfhydryl antioxidant in mammalian cells is reportedly decreased in spinal cord of ALS patients. We aimed to test the hypothesis that impaired GSH de novo synthesis can trigger motor neuron degeneration by creating conditional CNS knockout of glutamate cysteine ligase, catalytic subunit (Gclc), named as NEGSKO mouse. With 40-65% reduced GSH level in brain and spinal cord, NEGSKO mice developed a gait abnormality by approximately ~22 days of age with slightly reduced body size and body weight, and have a postnatal life expectancy of approximately 5 weeks. Histology at postnatal day 28 indicated, motor neurodegeneration was accompanied with increased apoptosis (TUNEL stain), activated caspase 3, cytochrome C translocation into cytoplasm, decreased cholinergic neurons with ChAT stain, muscle atrophy and denervation. EMG done in gastrocnemious muscle at postnatal day 28 showed numerous fibrillation potentials indicative of loss of muscle fiber innervation and fasciculations. The most intriguing finding is that NEGSKO mouse have increased neuronal TDP-43 fragmentation and translocation from nucleus to cytoplasm that colocalizes with elevated ubiquitination, a characteristic finding in sALS. Further mechanistic characterization indicates that mitochondrial GSH level was significantly reduced (~40% vs. wild type), and mitochondrial function was impaired, in particular state IV of mitochondrial electron transporter chain (ETC). Similar conclusions were obtained with a human neuroblastoma cells showing that TDP-43 was translocated into cytoplasm when cells Gclc was knockdown with shRNA, but not glutamate cysteine ligase, modifier subunit (Gclm). These results implicate the key role Gclc in the maintaining mitochondrial GSH homeostasis. Taken together, NEGSKO mice, with impaired GSH synthesis, closely resemble sALS, and are expected to serve as a novel mouse model for sALS research and testing of pharmacological inhibitors of neuronal apoptosis and degeneration.

Disclosures: X. Fan: None. **B. Sheng:** None. **R. Mariana:** None. **S. Siedlak:** None. **V. Monnier:** None. **P. Feng:** None.

Poster

859. Aging: Longevity and Teatment

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:859.01/H25

Topic: C.05. Aging

Title: Tin protoporphyrin XI, a competitive inhibitor of Heme oxigenase, exacerbates the cellular damage induced by an intrastriatal infusion of quinolinic acid to rats

Authors: *A. L. COLÍN GONZÁLEZ[1], M. OROZCO-IBARRA[1], M. CHANEZ-CARDENAS[1], D. SILVA-ADAYA[1], A. SANTAMARIA[1], J. PEDRAZA-CHAVERRI[2], P. D. MALDONADO[1]
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Abstract: Under physiological conditions, the tryptophan metabolite quinolinic acid (QA) is known to play a role in brain development. However, increased QA concentrations contribute to dysfunction of the CNS in certain disorders such as Huntington's disease (HD), dementia associated with HIV-AIDS, cerebral ischemia, epilepsy and multiple sclerosis. After an intrastriatal infusion of QA to rats and primates, overactivation of N-methyl-D-aspartate (NMDA) receptors yields excitotoxicity, oxidative stress and mitochondrial dysfunction, which altogether contribute to trigger a wide variety of toxic effects with biochemical, behavioral and neuropathological alterations similar to those of HD. Noteworthy, both in the brains of HD patients and QA-lesioned animals, increased expression levels of Heme oxygenase 1 (HO-1) can be found. This enzyme can exert a dual role, as it can be either protective or deleterious in the CNS. A large body of evidence indicates that HO-1 overexpression affords cellular antioxidant protection due to 1) a decrease in the concentration of its pro-oxidant substrate, 2) an increase in the antioxidant bilirubin, and 3) an increase in ferritin synthesis. On the other hand, a high expression of HO-1, and consequently, an increase in its activity, may also result in a pro-oxidizing atmosphere due to a release of Fe2+, a response that depends on the intensity of activation and the cellular microenvironment. In this work we evaluated the role of HO-1 in animals intrastriatally infused with QA (240 nmol/μL). For this purpose, the tin protoporphyrin IX (SnPP), a well-known HO inhibitor, was administered to rats (30 mmol/kg, i.p.). The ratio of cell damage, the content of carbonyl groups as an indicator of oxidative damage, and the HO activity were all quantified 3 days after the QA injection. QA induced HO-1 at 3 days after its infusion. The administration of SnPP to animals treated with QA inhibited the HO activity while increased the content of carbonyl groups and cell damage when compared to the group receiving QA alone. These data strongly suggest that HO-1 might be exerting a protective role in the toxic paradigm evoked by QA in rats.

Disclosures: A.L. Colín González: None. **M. Orozco-Ibarra:** None. **M. Chanez-Cardenas:** None. **D. Silva-Adaya:** None. **A. Santamaria:** None. **J. Pedraza-Chaverri:** None. **P.D. Maldonado:** None.

Poster

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Title: Proteasome function in the longevity *Drosophila* mutant methuselah

Authors: *J. GAO[1], C.-H. YEH[2], M. JANSEN[2], C. CHAN[2], S. DODDI[2], M. FIGUEIREDO-PEREIRA[2], T. SCHMIDT-GLENEWINKEL[2]
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Abstract: The ubiquitin-proteasome system (UPS) plays a central role in turning over 80-90% of intracellular proteins as well as in the degradation of misfolded proteins. With increasing age the UPS declines in activity in the nervous system which commonly affects steady state protein dynamics and causes enhanced protein aggregation. Most neurodegenerative disorders are associated with aggregates of ubiquitinated proteins, such as Lewy bodies in Parkinson's disease and neurofibrillary tangles in Alzheimer's disease. Here we investigate the proteasome function of the Drosophila longevity mutant, methuselah (mth) which encodes a putative G-protein coupled receptor. mth mutant flies exhibit a 35% increase in average lifespan and are resistant to numerous stressors including high temperature, starvation, and oxidants. The underlying mechanism for the increase in longevity is unknown. Our studies are the first to show that the mth mutation delays the decline in proteasome activity caused by aging. The proteasome activity and proteasome protein level of the parent control line, w1118 declines sharply during the aging process. However, the proteasome activity and proteasome level of mth are maintained at advanced age. Interestingly, proteasome activity, proteasome protein level, individual proteasome subunit level as well as ATP level were higher in w1118 than in mth at corresponding young age but differed at the oldest age. We also found that the ubiquitinated proteins and aggregates are reduced in mth mutants when compared with the w1118 strain at each age group. These results are similar to the effects of dietary restriction, which extends lifespan by reducing the rate of metabolism and mortality. Our studies will contribute to clarify the role of mth gene in the aging process and the relationship between the UPS pathway and aging.

Disclosures: J. Gao: None. **C. Yeh:** None. **M. Jansen:** None. **C. Chan:** None. **S. Doddi:** None. **M. Figueiredo-Pereira:** None. **T. Schmidt-Glenewinkel:** None.

Poster

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Topic: C.05. Aging

Support: CIHR MOP-97727

CIHR Banting and Best Studentship

Title: Sympathosensory coupling in aged mice that over express nerve growth factor

Authors: *L. J. SMITHSON[1], M. D. KAWAJA[2]
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Abstract: Since its discovery over 50 years ago, nerve growth factor (NGF) has been shown to induce directional axonal growth of postganglionic sympathetic neurons, neural crest-derived sensory neurons, and basal forebrain cholinergic neurons. In this study, we investigated the effects of age on sympathetic perineuronal plexuses formation in sensory ganglia of transgenic mice that have elevated levels of NGF in cells expressing the glial fibrillary acidic protein promoter (GFAPpr). Our group has previously shown the presence of tyrosine hydroxylase (TH)-immunopositive perineuronal plexuses around a subset of sensory somata of 2-3 month old GFAPpr-NGF mice. This sympathosensory coupling has been linked to the onset of neuropathic pain. In this current study, we demonstrated morphological changes (at both light and electron microscopy levels) of TH-immunopositive perineuronal plexuses in sensory ganglia of aged (i.e., 11-13 month and 16-18 month old) transgenic mice, compared to younger (i.e., 2-3 month old) GFAPpr-NGF mice. Double immunolabeling revealed an association between cfos- and TNFα-immunopositive reactive satellite glial cells (and some Iba1-immunopositive reactive macrophages) and TH-immunopositive plexuses in aged mice, compared to younger transgenic mice. In addition, TH-immunopositive perineuronal plexuses in aged transgenic mice appeared swollen or degenerated and were associated with dead or dying sensory neurons. No significant differences were observed in Kir4.1 expression in satellite glial cells among the different age groups of GFAPpr-NGF mice. Our results are the first to report changes in the morphology of perineuronal plexuses in aged mice. These results suggest that the presence of perineuronal plexuses of sympathetic axons around sensory somata may be slowly damaging the associated sensory neurons. That is, sympathosensory coupling in aged mice likely compromises the function of sensory neurons, which may have profound implications for neuropathic pain.

Disclosures: L.J. Smithson: None. **M.D. Kawaja:** None.

Poster

859. Aging: Longevity and Teatment

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Support: Core Research for Evolutional Science and Technology, Japan Science and Technology Agency

NOVARTIS Foundation for Gerontological Research

Title: Anti-Amyloid β drug validation using human iPS cell-derived neurons for the treatment of Alzheimer's disease.

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease that causes progressive memory and cognitive decline during middle to late adult life. The AD brain is characterized by deposition of amyloid β peptide (Aβ), which is produced from amyloid precursor protein by β- and γ-secretase-mediated sequential cleavage. Induced pluripotent stem (iPS) cells potentially provide an opportunity to generate a human cell-based model of AD that would be crucial for drug discovery as well as for investigating mechanisms of the disease.
We differentiated human iPS (hiPS) cells into neuronal cells expressing the forebrain marker, Foxg1, and the neocortical markers, Cux1, Satb2, Ctip2, and Tbr1. The iPS cell-derived neuronal cells also expressed amyloid precursor protein, β-secretase, and γ-secretase components (Presenilin 1, Nicastrin, Pen-2, and Aph-1) and were capable of secreting Aβ into the conditioned media. Aβ production was inhibited by β-secretase inhibitor, γ-secretase inhibitor (GSI), and an NSAID; however, there were different susceptibilities to all three drugs between early and late differentiation stages. At the early differentiation stage, GSI treatment caused a temporal increase at lower dose (Aβ surge) and drastic decline of Aβ production in three hiPS/human embryonic stem cell lines. These results indicate that the hiPS cell-derived neuronal cells express functional β- and γ-secretases involved in Aβ production; however, anti-Aβ drug screening using these hiPS cell-derived neuronal cells requires sufficient neuronal differentiation.

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Poster

859. Aging: Longevity and Teatment

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Topic: C.19. Drug Discovery and Development

Title: Blood transcriptomic biomarkers of AD patients treated with EHT 0202

Authors: *L. DESIRE, J. CARRIÈRE, P. BEURDELEY, M. PANDO
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Abstract: Monitoring patients genomic expression in clinical trials for Alzheimer's Disease (AD) can assist trial design and treatment response analysis. Here, we report on the identification in AD patients of blood-based transcriptomic signatures associated with treatment response of EHT 0202, a new compound with potential disease-modifying and symptomatic properties.
EHT 0202 recently completed a 3-month, placebo-controlled, Phase IIA study aimed at determining the clinical safety, tolerability and exploratory efficacy of EHT 0202 (40 and 80mg bid) as adjunctive therapy to one cholinesterase inhibitor in mild to moderate AD patients. During the study, blood samples were collected from each randomized patient at different time points, notably before treatment initiation and at study completion. Using sixty AD patients having either responded or declined during the study period with regards to ADAS-cog total score, we report on the identification of blood-based transcriptomic signatures associated with treatment response of EHT 0202, using Exonhit's SpliceArray^{TM}. This new generation of microarray extends transcriptomic profiling to the monitoring of alternative splicing.
Two transcriptomic signatures were identified: a predictive (prior to treatment), and efficacy profile (post-treatment) from peripheral blood specimens that correlated with treatment response and efficacy of EHT 0202, respectively. In the patients responding to EHT 0202, the pre-treatment (baseline) signature showed activation of pathways related to AD, CNS disorders, diabetes, inflammation, and autoimmunity, while the post-treatment signature indicated reduced activation of these pathways with induced metabolic and transcription stimulation.
These signatures should be replicated in a larger patient population. Nevertheless, this pilot study demonstrates the utility of blood transcriptomic signatures used as biomarkers for predicting patient response or monitoring efficacy, through the administration of a therapeutic drug in a complex disease such as AD. For EHT 0202 or other AD drugs, such biomarkers may help to improve strategies to better: identify appropriate patient populations for treatment, understand the drug mechanism of efficacy, and/or clarify the inherent subjectivity in most clinical endpoints used in this disease.

Disclosures: **L. Desire:** Employment; Exonhit. **J. Carrière:** Employment; Exonhit. **P. Beurdeley:** Employment; Exonhit. **M. Pando:** Employment; Exonhit.

Poster

859. Aging: Longevity and Teatment

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Support: NIAAA Intramural Research Program AA11034

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Title: Dopamine D2 plays an important role in the lifespan increases related to an Enriched Environment

Authors: *J. O'ROURKE[1], J. HAMILTON[1], M. SUBRIZE[1,2], J. G. PAGIAZITIS[1], J. BARNETT[1], A. R. NAPOLI[2,3], D. GRANDY[4], G.-J. WANG[1], N. D. VOLKOW[1,5], P. THANOS[1,2,5]
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Abstract: Dopamine (DA) is involved in a variety of brain functions including reward, locomotion, emotion, cognition, and memory (Jaber et al. 1996). Age-related decreases in DA D_2 receptors (*Drd_2)* have previously been associated with decreases in motor function (Umegaki et al. 2003). Exposure to an enriched environment (EE) has been widely implicated in an increase of lifespan, physical performance, and cognitive function (Warren et al. 1982). This study examined the role and interaction of *Drd_2* with an EE on lifespan and locomotor activity. ***Methods***: Male and female wild-type (*Drd_2* +/+), heterozygous (*Drd_2* +/-), and knockout (*Drd_2* -/-) mice were divided into two housing groups, an enriched environment (EE) and a deprived environment (DE). EE mice were group housed (4-6 mice) with access to a running wheel, an igloo, and an exploratory tunnel while DE mice were deprived of these objects and were single housed in a standard cage. Beginning at 20 weeks of age, all mice were measured bi-weekly for body weight and spontaneous locomotor activity. When mice approached their endpoint, they were euthanized and brain tissue was collected for future analysis. ***Results***: While final results

will be presented at the meeting, the majority of the results at this point show the following: Lifespan: Overall, *Drd$_2$* +/+ EE mice lived about 20% (p<0.001) longer than their DE counterparts (32% in males; p<0.01, 13% in females; p<0.05); whereas the lifespan in *Drd$_2$* +/- EE and *Drd$_2$* +/- DE mice was not found to be statistically significant (p>0.05). Finally, the *Drd$_2$* -/- mice showed no significant differences in lifespan between EE and DE mice (p>0.05). Bodyweight: Overall, when comparing within the DE housed mice, *Drd$_2$* +/- and *Drd$_2$* -/- mice weighed more than *Drd$_2$* +/+ mice by 10% and 9% respectively (p<0.05 for both). Comparison within the EE housed mice showed no significant differences (p>0.05).
Locomotor Activity: *Drd$_2$* +/+ and *Drd$_2$* +/- EE mice showed greater spontaneous locomotor activity compared to *Drd$_2$* -/- EE mice (33% and 28%, respectively; p<0.001 for both). Although *Drd$_2$* -/- DE mice showed 29% more locomotor activity compared to their EE counterparts (p<0.001), this was primarily influenced by the female mice (48%; p<0.001), since this trend was not seen in male mice, which was statistically not significant (p>0.05). In conclusion, these results show that *Drd$_2$* plays an important role in interaction with an enriched environment to produce significant changes in lifespan, body weight and locomotor activity.

Disclosures: J. O'Rourke: None. **J. Hamilton:** None. **M. Subrize:** None. **J.G. Pagiazitis:** None. **J. Barnett:** None. **A.R. Napoli:** None. **D. Grandy:** None. **G. Wang:** None. **N.D. Volkow:** None. **P. Thanos:** None.

Poster

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Topic: C.05. Aging

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Title: Identification of molecular mediators in the dysregulation of aged microglia

Authors: *S. C. HOPP[1], H. M. BROTHERS[2], I. BARDOU[2], R. M. KAERCHER[2], G. WENK[2]
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Abstract: Neuroinflammation is a normal process that occurs in the brain in response to disease and injury. One of the primary effector cells of neuroinflammation are microglia, the resident macrophages of the CNS. Microglia activation can exert either neuroprotective or neurodegenerative effects in the CNS. As such, there are two general phenotypes of activated microglia. The first, classical activation (M1), is associated with pro-inflammatory markers and destruction of threats such as bacteria. The second, alternative activation (M2), is associated with

anti-inflammatory markers and the repair of surrounding tissue. Chronic classical activation of microglia is generally maladaptive, leading to neuronal death and decreases in neurogenesis. Interestingly, activated microglia are observed in aged rats as well as old humans before the onset of Alzheimer's disease in areas where degeneration usually occur, implicating that their dysregulation plays a role in disease pathogenesis. We hypothesize that a cyclic relationship between glutamate, pro-inflammatory cytokines, and Alzheimer's biomarkers exists. Here, we compare the inflammatory response of the aged rat brain to acute application of IL-1β, a pro-inflammatory cytokine that has been linked to Alzheimer's disease. Following acute injection of IL-1β, we examined the time course of biomarkers. Using multiplexed magnetic bead immunoassays, we measured 15 rat cytokines associated with M1 and M2 microglia phenotypes. We also analyzed mRNA expression of these and other inflammatory biomarkers using qPCR to confirm changes in cytokine expression. We found age- and treatment-dependent changes in the levels of cytokines expressed at the various time points. Our findings contribute to the overall understanding of the key molecular mediators in the dysregulation of neuroinflammation in aging, which is ultimately important in the development of treatments for age-related neurodegenerative diseases.

Disclosures: S.C. Hopp: None. **H.M. Brothers:** None. **I. Bardou:** None. **R.M. Kaercher:** None. **G. Wenk:** None.

Poster

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OSEO

Alsace region

Mulhouse Alsace Agglomeration

SODIV

Title: Development of a striatum modeling and simulation platform to identify treatments for Huntington's disease

Authors: *F. J. PERNOT[1], R. GREGET[1], S. BISCHOFF[1], J.-M. C. BOUTEILLER[1,2], M. BAUDRY[1,3]
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Abstract: Huntington's disease (HD) is caused by an autosomal dominant mutation of the huntingtin gene. One of the major difficulties in identifying efficacious therapeutic agents to treat this disease is its complexity, as highlighted by the broad spectrum of multiple and often opposite pathological syndromes. In order to improve efficacy and limit the side effects of therapeutic agents new and better targets (or combinations of targets) need to be identified. As a first step, Rhenovia is developing a computational model of striatal GABAergic medium spiny neurons (MSN), which incorporates glutamatergic terminals originating from cortico-striatal inputs, and dopaminergic terminals from the nigro-striatal pathway. These synapses exhibit a large number of molecular targets that collectively regulate the activity of MSNs and thereby participate in the control of motor activity. The model was first validated by reproducing the features of glutamatergic and dopaminergic transmission under various stimulation conditions. In particular, the model reproduced the pattern of dopamine release under various stimulation frequencies and in the presence of dopamine transporter blockers. In addition, the model was used to determine the effects of various compounds affecting glutamatergic and dopaminergic targets on second messenger pathways in MSN dendritic spines. The model was also used to determine the role of presynaptic and postsynaptic dopaminergic receptors on the control of dopamine release and cAMP dynamics under a variety of experimental conditions. A major benefit of our computational framework has the potential to: (i) integrate the large knowledge base of HD biology to create pathological models; (ii) make data-based predictions about the success of drug development programs; (iii) predict potential clinical success of different drug targets; (iv) predict new drug potential side effects and (v) determine mechanism-based combination principles.
This work was supported in part by the French Agency for Innovation (OSEO), the "Fonds Unique Interministériel", the French Ministry of Defense, the Region Alsace, Mulhouse Alsace Agglomeration, Alsace Business Angels and SODIV.

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Poster

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Topic: C.05. Aging

Support: NIH Grant 5P01-AG05119

NIH Grant P30-AG028383

Title: Elevated 4-hydroxyhexenal in Alzheimer's disease (AD) progression

Authors: *M. BRADLEY[1], S. XIONG-FISTER[2], M. A. LOVELL[1]
[1]Chem., [2]Univ. of Kentucky, Lexington, KY

Abstract: Oxidative damage to nucleic acids, proteins, and lipids is not only a feature of normal aging but is associated with many neurodegenerative diseases, including Alzheimer's disease (AD). Lipids are particularly vulnerable to oxidative damage by reactive oxygen species (ROS) resulting in compromised integrity of cellular membranes and the generation of a variety of diffusible aldehydic by-products. Toxicity of α,β-unsaturated aldehydes including 4-hydoxynonenal and acrolein has been established in culture models and levels of both are elevated in the progression of AD. More recently 4-hydroxyhexenal (HHE) a third α,ß-unsaturated aldehydic by-product of peroxidation of docasahexaenoic acid (DHA) peroxidation has been identified. The current study aims to quantify levels of extractable and protein-bound HHE during the progression of AD in both vulnerable (hippocampus/parahippocampal gyrus (HPG) and less vulnerable brain regions (superior and middle temporal gyrus (SMTG) and cerebellum (CER). Levels of extractable HHE were quantified using gas chromatography mass spectrometry with negative chemical ionization (GC/MS/NCI) and protein-bound levels of HHE were determined by dot blot immunochemistry with an HHE specific antibody. Toxicity and glucose uptake were assessed in response to HHE treatment in primary cortical neurons. Levels of both extractable and protein-bound HHE were significantly elevated early in disease progression in the HPG and showed a significant positive correlation with levels of PBS soluble Aβ1-42 and FA soluble Aβ1-40. Treatment of primary cortical neurons with HHE lead to time- and concentration- dependent decreases in neuron survival. Treatment of primary cortical neurons with sub-toxic concentrations of HHE led to significant impairment in glucose uptake. These data suggest HHE is generated early during the pathogenesis of AD with potentially detrimental cellular effects further supporting the hypothesis that oxidative stress is an early event in the pathogenesis of AD.

Disclosures: M. Bradley: None. **S. Xiong-Fister:** None. **M.A. Lovell:** None.

Poster

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Topic: C.05. Aging

Title: Tyr682 in the APP intracellular domain regulates cholinergic function and cognitive performance

Authors: *C. MATRONE[1], S. LUVISETTO[1,2], L. LA ROSA[1], R. TAMAYEV[2], L. D'ADAMIO[2]
[1]Inst. of Mol. Biol. and Neurobio., Natl. Council of Res., Rome, Italy; [2]A Einstein Med. Sch., New York, NY

Abstract: Processing of Aβ-precursor protein (APP) plays an important role in Alzheimer's Disease pathogenesis. The APP intracellular domain contains residues important in regulating APP function and processing, in particular the 682YENPTY687 motif. To dissect the functions of this sequence in vivo, we created an APP knock-in allele mutating Tyr682 (Y682) to Gly (APPYG/YG mice). This mutation alters processing of APP and TrkA signaling, and leads to postnatal lethality and neuromuscular synapse defects when expressed on an APP-like protein 2 knock-out background. The dramatic alterations present in mice bearing this mutation prompted us to characterize further the APPYG/YG mice. Here, we show that APPYG/YG mice develop aging-dependent decline in cognitive and neuromuscular functions. This phenotype correlates with a progressive reduction in cholinergic tone and TrkA levels, suggesting a mechanistic connection between cholinergic tone depression, TrkA signaling deficits, cognitive and neuromuscular performance decline of APPYG/YG mice. The profound deficits caused by the Y682 mutation underscore the biological importance of APP and indicate that APPYG/YG are a valuable mouse model to study APP functions in physiological and pathological processes.

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Poster

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Topic: C.05. Aging

Support: P01-AG 22550

Title: Short-term caloric restriction initiated at different ages had minimal effect on activation of Nrf-2 pathway

Authors: *N. THANGTHAENG, N. SUMIEN, M. J. FORSTER
Pharmacol. and Neurosci., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Caloric restriction (CR) has been reported to confer health benefits, prolonged lifespan and attenuates age-related brain dysfunction. These outcomes have been hypothesized to be the results of alteration of redox homeostasis and reduction in oxidative damage. The purpose of this study was to determine if short-term CR affects antioxidant and detoxification response via the activation of the NF-E2-related factor 2 (Nrf2) pathway in different brain regions. Three age groups of C57BL/6J males, young (3 mos), middle-aged (12 mos), and old (19 mos) were assigned to either ad libitum (AL) or 30% CR. After four-months, nuclear translocation of Nrf-2 (n=4), and activities of Nrf-2 down-stream target genes (n=5-8) such as glutamate-cysteine ligase (GCL), and glutathione S-transferase (GST), and glutathione levels (GSH; n=6-9) were measured within the cerebral cortex (CX), cerebellum (CB), hippocampus (HP), and striatum (ST). Furthermore, aldehyde reactive probe (ARP) was used to assess oxidative damage proteins in each region (n=5-8). With age, Nrf-2 translocation was enhanced only in CB and HP, GST and GCL activities were increased, and GSH concentrations showed a small decrease. The short regimen of CR had little effect on Nrf-2 activation and its downstream effectors, except in CB and HP where CR attenuated age-related induction of Nrf-2. However, CR did not seem to change GSH concentrations in any region. The current results suggest that while CR may be able to activate Nrf-2 and promote an antioxidant and detoxification response in a region-specific manner; this effect did not have any impact on the level of reduced glutathione.

Disclosures: N. Thangthaeng: None. **N. Sumien:** None. **M.J. Forster:** None.

Poster

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Program#/Poster#:859.12/I7

Topic: C.05. Aging

Support: DFG Grant FOR-926

Title: Circuit specific functions of cannabinoid CB1 receptor in aging

Authors: A. LEGLER[1], R. LERNER[1], A. BILKEI-GORZÓ[2], B. LUTZ[1], *K. MONORY[1]
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Abstract: Aging of the brain holds the risk of neurodegenerative diseases and cognitive decline. As, thanks to the modern medical standards, the human population is getting older and older, this is an increasingly important theme for neuroscientists and society as a whole.
The endocannabinoid system (ECS) has emerged as a multifunctional neuromodulatory system, implicated in a plethora of physiological and pathophysiological processes. Among others it plays a role in learning, memory, neuroprotection and metabolism. By analyzing rodents in various memory tests, it has been shown that the ECS is required in acquisition and/or extinction of memories. The most prominent receptor that mediates the effects of endocannabinoids in the brain is the cannabinoid type 1 (CB1) receptor. This G protein coupled receptor is most abundant in the brain, but is also found in peripheral organs. Lack of the CB1 receptor leads to enhanced neuroinflammation in the hippocampus during aging.
We aim at understanding the dynamics of the activity status of the ECS in the adult mouse brain during the progression of normal aging and to dissect which neuronal circuits containing CB1 receptors are receptive to these age-dependent effects. By testing hippocampus-dependent behavior in conditional mutant mice lacking the CB1 receptor in cortical glutamatergic or GABAergic neurons, we will unravel the role of the ECS in age-dependent cognitive decline. A gene expression study of the ECS components via quantitative PCR shall elucidate age-related variations of the system. A better understanding of how the ECS in involved in aging could provide new therapeutical options for age related diseases.

Disclosures: A. Legler: None. **R. Lerner:** None. **A. Bilkei-Gorzó:** None. **B. Lutz:** None. **K. Monory:** None.

Poster

859. Aging: Longevity and Teatment

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:859.13/I8

Topic: C.19. Drug Discovery and Development

Support: grant

Title: Tau oligomer protease, a therapeutic target for Alzheimer’s disease

Authors: J. G. MOE, P. LOPEZ, D. ROMERO, *E. J. DAVIDOWITZ
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Abstract: Tau is a microtubule binding protein that normally functions in axons to maintain microtubule structure but is hyperphosphorylated in AD facilitating its dissociation from microtubules and accumulation in the somatodendritic compartment where it can aggregate (Iqbal et al. 2010). Neurofibrillary tangles, primarily composed of tau protein are a pathological hallmark in Alzheimer's disease (AD). However, the oligomeric forms of tau appear to be most closely associated with neuronal loss and memory impairment in mouse models of tauopathy (Berger et al. 2007; Yoshiyama et al. 2007). Tau oligomers were also found to accumulate in human AD specimens (Maeda et al. 2006; Patterson et al. 2011; Lasagna-Reeves et al. 2012). Importantly, extracellular tau oligomers have been shown to cause memory impairment and toxicity in mice inhibiting formation of long-term potentiation in hippocampal slices and formation of associative fear memory (Fá et al. 2010) and to induce neurodegeneration by affecting mitochondrial and synaptic function (Lasagna-Reeves et al. 2012). However, the particular structure and mechanism by which tau oligomers exert their deleterious effects has not been determined. We have found that upon the formation of oligomeric structures tau acquires the additional function of a protease leading to self-cleavage and the ability to cut other proteins. Here, we describe work characterizing this activity, the development of tools for studying its role in disease, and approaches for targeting tau protease for the development of small molecule drugs and antibody therapeutics.

Disclosures: J.G. Moe: Employment; Oligomerix, Inc. **P. Lopez:** Employment; Oligomerix, Inc. **D. Romero:** Employment; Oligomerix, Inc. **E.J. Davidowitz:** Employment; Oligomerix, Inc..

Poster

859. Aging: Longevity and Teatment

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:859.14/I9

Topic: C.05. Aging

Support: NIH grant R01 AG027910-01A1

Saunders Family Fund

Title: Cortical and hippocampal expression of insulin-degrading enzyme and neprilysin in normal aging: Implications for amyloid-beta clearance

Authors: *M. C. MILLER[1], A. A. MESSIER[2], D. P. OSGOOD[2], V. HOVANESIAN[4], J. T. MACHAN[5], C. CHIU[2], E. G. STOPA[3], C. E. JOHANSON[2], G. D. SILVERBERG[2]
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University/ Rhode Island Hopsital, Providence, RI; [4]Core Image Analysis Lab., [5]Biostatistics, Rhode Island Hosp., Providence, RI

Abstract: Background: Age is the single most important risk factor in the accumulation of amyloid-beta (Aβ) peptide in both normal aging and non-familial Alzheimer's disease (AD). Impaired clearance of Aβ from the brain interstitial fluid has been postulated to be an important component in the accumulation of brain Aβ in both processes. It has been recognized that enzymatic degradation of Aβ occurs in situ by way of enzymes such as neprilysin and insulin-degrading enzyme (IDE). However, previous studies seem to indicate that this local degradation of Aβ accounts for only a minimal amount of clearance at physiological concentration in both aging and AD. In this study, we measured neprilysin, IDE, and Aβ peptide levels in the frontoparietal cortex and hippocampus across normal aging in Fischer 344/ Brown-Norway hybrid rats.
Methods: Rats at selected ages from 3 to 36 months were analyzed by: (A) immunohistochemistry (IHC) and (B) Western blotting for cortical and hippocampal neprilysin and IDE protein expression. Software-assisted and manual neuronal cell counting was performed on the IHC-stained sections, and optical density readings were obtained from the immunoblots at all age points. In addition, cortical Aβ40 and Aβ42 concentrations were measured by ELISA.
Results: The concentrations of cortical Aβ40 and Aβ42 increased significantly with age ($p<0.001$), with the sharpest rise occurring from 9 to 12 months. In contrast, IDE expression did not significantly change across any of the age groups tested by both IHC and immunoblot. Neprilysin expression did not significantly change by immunblot, but a significant decrease in total number of immunopositive hippocampal neurons (after 9 months of age) and cortical neurons (after 12 months of age) was observed. However, it is important to note that these changes manifest after Aβ has already begun to increase (initially happening between 6 and 9 months of age).
Conclusions and future directions: Our results suggest that in aging, in situ degradation does not play a major role in Aβ clearance, but rather, the majority of Aβ clearance is by transport across the brain-fluid barriers and by the bulk flow of cerebrospinal fluid (CSF). These data are in keeping with our previous finding of a strong relationship between Aβ accumulation and the expression of Aβ transporter genes at the blood-brain and blood-CSF barriers.
Conflict of interest declaration: No conflict of interest to declare.

Disclosures: M.C. Miller: None. **A.A. Messier:** None. **D.P. Osgood:** None. **V. Hovanesian:** None. **J.T. Machan:** None. **C. Chiu:** None. **E.G. Stopa:** None. **C.E. Johanson:** None. **G.D. Silverberg:** None.

Poster

859. Aging: Longevity and Teatment

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Topic: C.19. Drug Discovery and Development

Support: NIH Grant DK054441

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American Diabetes Association (1-08-RA-139)

Seahorse Biosciences (to A.N.M.)

Title: TZDs may offer neuroprotection through a mitochondrial target (mTOT)

Authors: A. DIVAKARUNI[1], S. WILEY[1], A. ANDREYEV[1], G. ROGERS[2], J. COLCA[3], *A. N. MURPHY[1]
[1]UCSD, LA JOLLA, CA; [2]Seahorse Biosci., Billerica, MA; [3]Metabolic Solutions Develop. Co., Kalamazoo, MI

Abstract: Thiazolidinediones (TZDs), such as pioglitazone, promote neuroprotection in a wide variety of preclinical models of neurodegeneration including Parkinson's and Alzheimer's Disease and stroke, and are in human trials for these indications. As PPAR-γ agonists, however, these TZDs cause marked side effects that can be clinically prohibitive (including plasma volume expansion, increased adiposity, congestive heart failure, and cardiovascular risk). A target of insulin sensitizers located in the mitochondrial inner membrane, mTOT, was recently identified and may contribute to the neuroprotective and insulin sensitizing pharmacology of TZDs.

We find that TZDs previously approved for treating type 2 diabetes (pioglitazone, rosiglitazone, and troglitazone) selectively inhibit pyruvate oxidation to varying degrees in both permeabilized rat cortical neurons ans also human and rodent myocytes. This action is also shared by a prototype PPARg-sparing TZD, MSDC-0160, which was designed to have a more selective interaction with the mitochondrial target and is now in clinical trials for both type 2 diabetes and Alzheimer's disease.

Clinically relevant concentrations of the drug (1uM<Kd<7uM) selectively inhibit respiration on pyruvate, but have no effect on either alternative Complex I-linked substrates, such as glutamate and palmitoylcarnitine, or the complex II-linked substrate succinate.

Our data suggest mTOT is a heretofore uncharacterized regulator of mammalian pyruvate oxidation. Moreover, the inhibition of pyruvate oxidation via TZD binding to mTOT may be a crucial mediator of the effects of TZDs.

Disclosures: A. Divakaruni: None. **S. Wiley:** None. **A. Andreyev:** None. **G. Rogers:** Employment; Seahorse Biosciences. **J. Colca:** Ownership Interest; Metabolic Solutions Development Company. **A.N. Murphy:** None.

Poster

860. Aging: Pathophysiology and Treatment

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Topic: C.05. Aging

Support: NIH grants P01AG11370

R01AG38747

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R01NS056218

Ellison Medical Foundation

American Heart Association

Title: Early-life deficiency of growth hormone and IGF-1 affects outcomes of endothelin-induced cerebral ischemia

Authors: H. YAN[1], M. MITSCHELEN[1], K.-M. FUNG[2], J. FARLEY[1], Z. UNGVARI[1], A. CSISZAR[1], J. P. WARRINGTON[1], *W. E. SONNTAG[3]
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Abstract: Numerous experiments and clinical evidence have shown that age-related IGF-1 deficiency negatively affects the health span in the aged population as well as the morbidity and mortality of cerebrovascular disease. However, recent studies suggest that peripubertal deficiency of growth hormone (GH) and IGF-1 increases lifespan. Nevertheless, the cerebrovascular effects of early-life GH/IGF-1 deficiency remain controversial. To investigate the effects of IGF-1 deficiency on the outcome of cerebral ischemia, dwarf rats with GH/IGF-1 deficiency and their normal heterozygous littermates (HZ) as well as dwarf rats injected with GH were subjected to endothelin-induced stroke. Endothelin-1, a potent vessel constrictor, was unilaterally injected proximal to the middle cerebral artery. Neurological deficits and sensorimotor function were monitored on post-surgical days 1 and 3. After behavioral tests on

day 3, animals were euthanized and the brains were cryosectioned. Sections were evaluated for infarct volume, neurogenesis, oxidative stress, inflammation, and gliosis. Animals in the three groups did not differ in levels of endothelin receptors. Compared to HZ and GH-replaced groups, dwarf animals demonstrated significantly smaller infarct volume, a reduction in Nestin-positive immature neurons in the peri-infarct region and reduced levels of 4-HNE (a marker of lipid oxidation), MMP9 (an inflammatory marker), and activated microglia (assessed by *Iba-1*$^{+}$ staining) migrating into the infarct core. Our results indicate that in response to ischemic challenge animals with early-life IGF-1 deficiency accumulate less oxidative stress and signs of inflammation. Our results provide evidence that early-life GH and IGF-1 deficiency has unique effects on infarct volume and recovery in response to endothelin induced cerebral ischemia.

Disclosures: H. Yan: None. **M. Mitschelen:** None. **K. Fung:** None. **J. Farley:** None. **Z. Ungvari:** None. **A. Csiszar:** None. **J.P. Warrington:** None. **W.E. Sonntag:** None.

Poster

860. Aging: Pathophysiology and Treatment

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:860.02/I12

Topic: C.19. Drug Discovery and Development

Title: Exogenous nicotinamide application prevents neurite degeneration by the mechanism independent from nicotinamide adenine dinucleotide salvage pathway *in vitro* DRG neurons

Authors: *S. TOKUNAGA[1,2], T. ARAKI[1,2]
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Abstract: Axonal degeneration occurs in various neurological disorders, including stroke, Parkinson's disease and peripheral neuropathies. Studies in animal models of neurological disorders indicate that axonal degeneration precedes the appearance of disease symptoms, suggesting that delaying the degeneration process can prevent the progression of disorders. A previous study demonstrated that exogenous application of high conc nicotinamide (Nam) or nicotinamide adenine dinucleotide (NAD) (> 5 mM) at the time of neurite transection can delay neurite degeneration concurrently with increased intracellular NAD level. Although Nam is utilized as a NAD precursor in salvage pathway in mammalian cells, Nam is also a potent natural modulator of several proteins, including cytokines and NAD-dependent protein deacetylases. To determine whether Nam-mediated neurite protection depends on NAD salvage pathway, we inhibited nicotinamide phosphoribosyltransferase (Nampt), which synthesizes the substrate for NAD biosynthetic enzyme from Nam in DRG neuron cultures and assessed the impact on the

protective effect of Nam exogenous application. We found that the inhibition of Nampt did not affect the protective effect by Nam exogenous application. In addition, we found that the decline of mitochondrial membrane potential after neurite transection was suppressed promptly after exogenous Nam application. These results suggest that Nam may exert neurite-protective effect through mitochondrial protection independently from NAD salvage pathway

Disclosures: S. Tokunaga: None. **T. Araki:** None.

Poster

860. Aging: Pathophysiology and Treatment

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:860.03/J1

Topic: C.19. Drug Discovery and Development

Support: Quincy Bioscience

Title: Apoaequorin protects neurons from ischemia and alters cytokine mRNA levels in rat hippocampus.

Authors: *E. L. HOCHSTETTER[1], J. A. DETERT[1], J. D. LESCHER[1,2], J. R. MOYER, Jr.[1,2]
[1]Psychology, [2]Biol. Sci., Univ. of Wisconsin Milwaukee, Milwaukee, WI

Abstract: During an ischemic attack, elevated levels of intracellular calcium can produce deleterious effects, up to and including cell death. Calcium binding proteins (CaBPs) are one mechanism by which cells sequester excess calcium. Aequorin is a CaBP isolated from the jellyfish Aequoria Victoria. We previously demonstrated that an intrahippocampal injection of apoaequorin (AQ, the CaBP component of aequorin) significantly protects hippocampal neurons from ischemic cell death. The neuroprotective effects of AQ were first observed at 24 hrs and endured for up to 48 hours. Interestingly, western blot analysis suggested that AQ protein levels dramatically decrease over this time period. Although the neuroprotective effects of AQ were strongest at 48 hrs, the relatively low level of AQ protein present at that time suggests that other factors may play a role in the ability of AQ to protect neurons from ischemic cell death. One possibility is that AQ administration leads to a change of levels of anti-inflammatory cytokines. Interleukin-10 (IL-10), an anti-inflammatory cytokine, has previously demonstated the ability to protect neurons from hypoxia in vitro. To test the hypothesis that cytokine expression may play a role in the neuroprotective effects of AQ, cytokine mRNA levels were measured at different time points following a single, intra-hippocampal administration of AQ. Twelve male F344 rats were bilaterally cannulated in the dorsal hippocampus. Following recovery, 4% AQ was injected unilaterally (vehicle in the other side, counterbalanced) and after 1, 24, or 48 hrs, the brains were

removed and the hippocampi dissected, placed on dry ice, and homogenized. RNA was isolated using Qiagen's RNeasy mini kit. Reverse transcription was performed using Qiagen's RT2 HT First Strand Kit-96 and qPCR was used to quantify IL-10 and β-actin. IL-10 was significantly increased in the AQ treated hemispheres at 1 hour ($t(14) = 5.30$, $p < .05$), but not 24 or 48 hours ($p > .05$) post injection. AQ treatment did not affect β-actin mRNA levels. Further PCR array analyses with Qiagen's RT2 Profiler Arrays for cytokines and chemokines were performed in an effort to evaluate whether other cytokines and chemokines are activated at these time points. Results suggest that multiple anti-inflammatory cytokine and chemokine mRNA levels change in a time-dependent manner following AQ administration. Ongoing studies are investigating IL-10 and IL-10Rα protein levels to determine how their time courses compare with those of the mRNA. Collectively, these results suggest that AQ is neuroprotective and that this neuroprotection may at least partly involve a neuroimmunomodulatory mechanism.

Disclosures: E.L. Hochstetter: Other; Quincy Bioscience. **J.A. Detert:** Other; Quincy Bioscience. **J.D. Lescher:** Other; Quincy Bioscience. **J.R. Moyer:** Other; Quincy Bioscience.

Poster

860. Aging: Pathophysiology and Treatment

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:860.04/J2

Topic: C.05. Aging

Support: GACR Grant 309/09/1597

Title: Diffusion parameters in the brain and behavioral changes in hereditary hypertriglyceridemic rats

Authors: I. VORISEK[1], *L. VARGOVA[2], M. ORT[1], E. SYKOVA[2,1]
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Abstract: We studied the diffusion parameters in the brain and cognitive changes during aging in hereditary hypertriglyceridemic (HHTg) rats, an experimental non-obese model of insulin resistance. Cognitive decline in old age has been linked not only to neuronal loss and changes in the efficacy of synaptic signal transmission, but also to changes in extracellular diffusion, which affects extrasynaptic transmission. Changes in diffusion parameters during aging result mainly from structural rebuilding of astrocytic processes and the quantitative and qualitative changes of extracellular matrix (ECM) molecules.
The cognitive abilities of 4- and 21-month-old rats were assessed using the active allotethic place

avoidance (AAPA) test, in which an animal has to learn to avoid the prohibited sector in a rotating circular arena. Then, the apparent diffusion coefficient of water (ADC_W) was measured in the HHTg rats and Wistar control animals using diffusion-weighted magnetic resonance and a stimulated echo sequence. HHTg rats exhibited significantly increased glycemia, triglyceridemia and impaired glucose tolerance at the ages of 4 and 21 months. The AAPA test was performed 1.5 and 24 hours after training.

In the short-term memory test, aged HHTg rats performed better than age-matched controls (time of avoidance was 216 ± 20 s vs. 87 ± 15 s, n = 5). In the long-term memory test, the aged HHTg rats' performance was not significantly different from that of age-matched controls (104± 12 s vs. 113 ± 15 s, n = 5). There were no significant differences between young-adult HHTg and control rats: 233± 11 s vs. 238 ± 10 s (n = 6). ADC_W was measured in the cerebral cortex (CX) and hippocampal CA1area. ADC_W in young adult HHTg rats (CX: 615 ± 9 $\mu m^2 s^{-1}$, CA1: 754 ± 15 $\mu m^2 s^{-1}$, n=6) was not significantly different from that in age-matched controls (CX: 622 ± 13 $\mu m^2 s^{-1}$, CA1: 767 ± 18 $\mu m^2 s^{-1}$, n=6). In 21-month-old control rats, significantly lower values were observed: 578 ± 10 $\mu m^2 s^{-1}$ (CX), 718 ± 17 $\mu m^2 s^{-1}$ (CA1), n = 5. In contrast, aged HHTg rats did not exhibit a significant decrease in ADC_W: 606 ± 12 $\mu m^2 s^{-1}$ (CX), 750 ± 19 $\mu m^2 s^{-1}$ (CA1), n = 5.

We conclude, that the formation of long-term memories in aged HHTg rats is affected by aging to the same extent as in control rats. However, HHTg rats did not show a decline in short-term memory during aging, which could be related to preserved extracellular space diffusion. This might be due to changes of slowly metabolized ECM molecules (e.g. advanced glycation and different water content), which affect the extracellular volume and diffusion barriers and in turn extrasynaptic as well as synaptic transmission and cognitive functions.

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Disclosures: I. Vorisek: Research Grant; GACR 309/09/1597. **L. Vargova:** Research Grant; GACR 309/09/1597. **M. Ort:** None. **E. Sykova:** None.

Poster

860. Aging: Pathophysiology and Treatment

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:860.05/J3

Topic: C.05. Aging

Support: CIHR-TPRM

Title: Promoting endogenous repair the aged brain following stroke

Authors: *N. SACHEWSKY[1], D. PICCIN[2], T. ZARIN[2], J. HUNT[2], C. MORSHEAD[2]
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Abstract: Stroke is the third leading cause of death and disability in both Canada and the US. Seventy-five percent of strokes occur in individuals over the age of 65 and there are no current medical therapies for alleviating the chronic functional impairments that result. Regenerative strategies to repair the ischemically injured brain have not been fully realized or adapted to reflect the aged cohort of stroke patients. We have previously demonstrated that endogenous neural stem and progenitor cells (together termed precursor cells, NPCs) residing in the periventricular region of the adult forebrain can be activated with growth factors or the small molecule Cyclosporin A (CsA) in a permanent occlusion model of stroke, ultimately resulting in newly generated tissue and functional recovery in young adult animals. We have shown that CsA acts directly on NPCs to promote their survival by selectively inhibiting mitochondrial pore formation, thereby enhancing their survival. Reports suggest that the size of the NPC pool is depleted with age hence the efficacy of these endogenous stimulation strategies in the aged brain is not clear. We first examined whether NPCs derived from the young and aged brains were inherently different or whether the NPC niche could account for the apparent differences between young and aged brain. We transplanted young and aged NPCs into both young and aged brains and found that the decreased NPC migration and olfactory bulb neurogenesis seen in aged brain was completely independent of the transplanted cells indicating that the NPC niche dictated the behaviour of the transplanted cells. *In vitro* co-culture experiments confirmed these findings whereby aged NPCs plated to an overwhelming young environment resulted in a complete rescue of the total numbers of neural stem cells as shown by a rescue of the numbers of neurospheres back to the numbers observed from the young adult brain. Given that the total numbers of stem cells is unchanged with age, we reasoned that we could activate the endogenous cells using our endogenous stimulation paradigms. We demonstrated that CsA *in vitro* lead to a similar increase in the survival of NPCs from the aged brain as reported from the young adult brain. Moreover, in an endothelin-1 (ET-1) model of stroke, which closely parallels ischemia in humans by mimicking the loss of blood flow and the subsequent reperfusion of the lesion, CsA administration was able to activate aged NPCs *in vivo*, resulting in tissue regeneration and functional recovery in aged mice. These findings suggest that endogenous neural precursor activation holds great promise for cell-based strategies to treat stroke in a clinically relevant model and age cohort.

Disclosures: N. Sachewsky: None. **D. Piccin:** None. **T. Zarin:** None. **J. Hunt:** None. **C. Morshead:** None.

Poster

860. Aging: Pathophysiology and Treatment

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Program#/Poster#:860.06/J4

Topic: C.19. Drug Discovery and Development

Support: NIH Grant 1R15NSO70268

Title: Evaluation of apoptosis-related gene expression in a cell culture model of the blood brain barrier following poly(butylcyanoacrylate) nanoparticle exposure.

Authors: H. DASENBROCK[1], *R. HEMMER[1], R. SPAULDING[1], E. ESHAM[1], B. SABEL[2], P. HENRICH-NOACK[2], P. SCHULTHEIS[1], H. BULLEN[1], K. HAIK[1]
[1]Northern Kentucky Univ., Highland Heights, KY; [2]Otto-von-Guericke Univ., Magdeburg, Germany

Abstract: Nanoparticles (NPs) are studied in a variety of biological systems, and the use of NPs as a novel approach to treat disease has been described in several experimental models, including cancer, eye diseases, cerebral palsy, diabetes and coronary artery disease. The focus of this project is to examine the toxicity of NPs used to deliver drugs to the brain. Coating NPs with surfactants, such as Tween 80, has been shown to facilitate the crossing of various biological barriers including the blood brain barrier (BBB). Poly(butylcyanoacrylate) (PBCA) NPs are biodegradable and can be loaded with anti-tumor drugs, such as doxorubicin and gemcitabine and coated with surfactants including polysorbates (Tween 80) or poloxamers. These drug-conjugated PBCA NPs have been successfully used to treat glioblastoma in animals, providing indirect evidence that the PBCA NP can cross the BBB. However, there are concerns that the Tween 80 surfactant coating may be toxic at therapeutic doses. A detailed characterization of PBCA-Tween 80 NP toxicity is important for the design and use of these NPs in drug delivery to the brain. In the present study, a cell co-culture model of the BBB consisting of primary rat brain astrocytes and endothelial cells was used to investigate gene expression patterns in co-cultures following exposure to (1) PBCA NPs coated with Tween 80; (2) PBCA NPs loaded with doxorubicin and coated with Tween 80; and (3) untreated controls. After six days in culture, co-cultures were treated with PBCA-Tween 80 or PBCA-Tween 80-doxorubicin NPs at the following concentrations: 7.8 μg/ml, 31.25 μg/ml and 125 μg/ml. RNA was collected 8 hours post-treatment and analyzed for expression of 84 apoptosis-related genes using Apoptosis RT^2 Profiler PCR Arrays. The results indicate that co-cultures exposed to PBCA-Tween 80-doxorubicin NPs at the two lower concentrations exhibit a downregulation of a number of apoptosis-related genes relative to controls. Specifically, co-cultures treated at 7.8 μg/ml PBCA-Tween 80-doxorubicin NPs had a significant downregulation of *Cflar, Ripk, Cradd, Aven, Mapk8ip1, Nfkb1* and *Bcl2*. Co-cultures treated with 31.25 μg/ml PBCA-Tween 80-doxorubicin NPs had a significant downregulation of *Ripk, Tradd, Traf4, Cradd, Aven* and *Sphk2,* whereas very few changes were observed in the expression of apoptosis-related genes in co-cultures exposed to PBCA-Tween 80 NPs. These results indicate that PBCA-

Tween 80 NPs induce a much lower level of toxicity than those loaded with doxorubicin in this co-culture system and that use of Tween 80 as a NP coating for drug delivery systems should be further evaluated.

Disclosures: H. Dasenbrock: None. **R. Hemmer:** None. **R. Spaulding:** None. **E. Esham:** None. **B. Sabel:** None. **P. Henrich-Noack:** None. **P. Schultheis:** None. **H. Bullen:** None. **K. Haik:** None.

Poster

860. Aging: Pathophysiology and Treatment

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Program#/Poster#:860.07/J5

Topic: C.05. Aging

Support: NIH Grant

Title: Could mechanical obstruction by slowly degenerating nerve debris cause a delay in axon regeneration in aged animals?

Authors: *H. KANG, J. W. LICHTMAN
Mol. and Cell. Biol., Harvard Univ., CAMBRIDGE, MA

Abstract: In the aging population, delay in functional recovery following motor impairment is a major health concern because it restricts mobility and independence. However the underlying causes of this delay are not known. Ideas such as an intrinsic decline in a peripheral neuron's ability to regenerate, or decreases in the supply of trophic or tropic factors from supporting cells, are often suggested as underlying causes, but there have only been a few direct analyses of age-related axon regeneration.
Our aim has been to directly observe regenerating axons as they grow and ultimately reoccupy denervated neuromuscular synaptic sites in order to learn what changes in this process are age-related. We have accomplished this by *in vivo* imaging of transgenic mice that express fluorescent proteins in their axons using a custom video-rate 2-photon microscope in order to obtain high spatiotemporal resolution time-lapse images of growing axons in living animals. The results show that axon growth in aged animals is more saltatory than in younger animals. The growth rate is impaired by frequent halting associated with axonal bulging suggesting that the intracellular transport rate is faster than axon extension leading to temporary increases in volume. In addition axons show more extensive branching within the fasciculated nerve bundle. Immuno-staining for myelin debris indicated that more uncleared axonal debris was present in the Schwann cells within old damaged nerves than in younger ones suggesting that delayed

debris clearance by glia or other cells may be hindering the advance of growing axon tips by mechanically blocking the passage in the closed Schwann cell tubes. Direct visualization of aged axons shows them attempting to navigate around the debris-containing cells. Our data imply that facilitating clearance of axon debris might be a good target for treatment of nerve injury in the aged.

Disclosures: H. Kang: None. **J.W. Lichtman:** None.

Poster

860. Aging: Pathophysiology and Treatment

Location: Hall F-J

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Program#/Poster#:860.08/J6

Topic: C.05. Aging

Support: 103527

Title: Protective effect of curcumin on behavioral and morphological alterations induced in a model of striatal degeneration in rats

Authors: *R. A. SANTANA MARTINEZ[1], J. PEDRAZA-CHAVERRÍ[2], A. SANTAMARIA[1], P. D. MALDONADO[1]
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Abstract: Quinolinic acid (QUIN), an endogenous competitive agonist of NMDAr, is considered an excitotoxin, and its intrastriatal administration to rats has been used to reproduce some biochemical, behavioral and morphological alterations similar to those observed in some neurodegenerative disorders. QUIN induces selective neuronal death in the striatum due to overactivation of NMDAr (excitotoxicity), which has been related to acute oxidative stress. Curcumin (CUR) is a major polyphenol derived from the rhizome *Curcuma longa* and its biological properties as direct (free radical scavenger) and indirect (Nrf2 inducer) antioxidant are well known. Additionally, CUR is highly lipophilic and is able to reach the brain. In this context, we studied the protective effects of CUR in a neurodegenerative model produced by QUIN in rats.
Animals intrastriatally infused with QUIN (240 nmol/µl) received CUR (400 mg/kg, i.g.) daily during 6 consecutive days, and on day seven, animals were euthanized to evaluate histological changes by hematoxylin & eosin staining. The behavioral study was assessed by the limb-use asymmetry test at day -1 (one day before QUIN microinjection), as well as on days 2 and 6 after QUIN injection. In this test, we estimated the number of contacts the animals on the walls using

the ipsilesional (affected limb), the contralesional (unaffected limb), and both limbs simultaneously. CUR treatment significantly decreased the QUIN-induced striatal morphological alterations by reducing the number of lesioned cells. CUR administration also partially recovered the ability of QUIN-lesioned animals to use both limbs, while decreased the single use of the ipsilesional limb (20 % below the QUIN condition). Our results suggest that CUR treatment may constitute an effective therapeutic tool to ameliorate some features of neurodegenerative events in which the optimal maintenance of the redox status is crucial for neuronal survival. Further studies are needed to elucidate the precise mechanism of CUR actions and to explain the protective effect observed in the toxic paradigm induced by QUIN.

Disclosures: R.A. Santana Martinez: None. **J. Pedraza-Chaverrí:** None. **A. Santamaria:** None. **P.D. Maldonado:** None.

Poster

860. Aging: Pathophysiology and Treatment

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:860.09/J7

Topic: C.19. Drug Discovery and Development

Support: NIH Grant DK054441

NIH Grant DK081298

ADA Grant 1-08-RA-139

Title: Systematic review of thiazolidinediones (TZDs) in pre-clinical studies reveals beneficial effects on cognition

Authors: *S. P. MURPHY[1], C. L. GIBSON[2], A. N. MURPHY[3]
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Abstract: Used in the treatment of Type 2 diabetes, thiazolidinediones (TZDs) are insulin sensitizers that normalize blood glucose and lipid levels, improve metabolic flexibility, and have anti-inflammatory activity. A member of this drug class, pioglitazone, is in clinical trials for multiple neurological indications, including Alzheimer's and Parkinson's Diseases, and stroke, while similar trials of rosiglitazone have been halted due to concerns over increased risk of adverse cardiovascular events. Using meta-analysis, we have assessed the potential benefits of pioglitazone and rosiglitazone on cognitive function in preclinical studies across a variety of

models, including those for Alzheimer's and Parkinson's Diseases, aging, traumatic brain injury, cerebral ischemia, diabetes, epilepsy, and chemical-induced amnesia and vascular dementia. Analysis of the 24 studies we identified has revealed an overall neuroprotective effect of TZDs, with significant improvement of cognitive function by both pioglitazone and rosiglitazone. Evaluation of study quality raised some concern that higher quality studies reported less benefit. Interestingly, efficacy was similar whether TZDs were administered either acutely or chronically, and there appeared to be less of an effect in 'control' animals. Overall, the animal studies thus far suggest a beneficial effect of TZDs on cognitive function, and we await the results of human trials in order to evaluate how well these preclinical models predict efficacy.

Disclosures: S.P. Murphy: None. **C.L. Gibson:** None. **A.N. Murphy:** None.

Poster

860. Aging: Pathophysiology and Treatment

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Program#/Poster#:860.10/J8

Topic: C.19. Drug Discovery and Development

Support: DoD

Title: Derivation of scalable, NURR1 expressing dopaminergic progenitor cells from human embryonic stem cells

Authors: *J. M. CHILTON[1], K. L. GALLAND[2], J. A. MCCABE[3], S. L. STICE[3]
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Abstract: Parkinson's disease (PD) is one of the most common neurodegenerative diseases affecting approximately 1-2% of the population over 65 years of age. The lack of an appropriate cell type that can be expanded for high throughput drug screening has hindered progress in PD drug discovery, as well as investigating disease mechanisms underlying PD. Our goal was to generate human dopaminergic progenitor cells, derived from human embryonic stem cells (hESCs), which are homogeneous, expandable and demonstrate a propensity for dopaminergic differentiation. We isolated neural progenitor-like cells that exhibited greater than 95% immunoreactivity for nuclear receptor related 1 protein (NURR1 or NR4A2), a transcription factor essential for the generation of midbrain dopaminergic neurons, and we refer to these cells as DopaPro™ cells. DopaPro™ cells displayed normal karyotype, formed adherent monolayers and were proliferative over multiple passages, allowing for high throughput screening (HTS) scale production and dopaminergic differentiation. To determine if these cells could generate

dopaminergic neurons, DopaPro™ cells were differentiated in the presence of leukemia inhibitory factor (LIF), brain derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), transforming growth factor beta 3 (TGF-β3), forskolin and ascorbic acid (AA). By 3 weeks, approximately 10% of differentiated DopaPro™ cells showed immunoreactivity for tyrosine hydroxylase (TH). In addition, since the DopaPro™ cells are putative midbrain progenitor cells, we asked whether these cells required regionalization signals such as Sonic Hedgehog (SHH) or fibroblast growth factor 8 (FGF8), which are normally used in directed differentiation of hESCs to dopaminergic neurons. The DopaPro™ cells did not require, nor was their differentiation to TH expressing populations significantly enhanced, when cultures were exposed to SHH and FGF8 for 2 weeks prior to culture in LIF, BDNF, GDNF, TGF-β3, forskolin and AA. Both control and SHH/FGF8 treated populations contained approximately 10% dopaminergic neurons. Here, hESC-derived progenitor cells poised for differentiation into TH expressing populations and potentially dopaminergic cell derivation were generated, thus reducing time and need for midbrain induction factors. Our results show our DopaPro™ cells are a unique, renewable and scalable cell source for PD research.

Disclosures: **J.M. Chilton:** Employment; ArunA Biomedical, Inc.. Other Research Support; DoD. **K.L. Galland:** Employment; ArunA Biomedical, Inc.. Other Research Support; DoD. **J.A. McCabe:** Employment; University of Georgia. **S.L. Stice:** Employment; ArunA Biomedical, Inc.. Other Research Support; DoD.

Poster

860. Aging: Pathophysiology and Treatment

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:860.11/J9

Topic: C.19. Drug Discovery and Development

Support: CONACYT 82482

CONACYT 103907

CONACYT scholarship 372072

UNAM PAPIIT IN-211709-22

Title: Evaluation of biomaterials for the release of chemotropic proteins into the brain.

Authors: A. RIOS RAMIREZ[1], *M. GIORDANO[2], E. TAMARIZ[3]
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Querétaro., Mexico; [2]Cognitive and Behavioral Neurobio., Univ. Nacional Autónoma De México, Querétaro, Qro., Mexico; [3]Biomedicine, Univ. Veracruzana, Xalapa, Veracruz, Mexico

Abstract: Protein release into the central nervous system is restricted by the presence of the blood brain barrier. One potential strategy is the release of substances of interest into the central nervous system by the use biomaterials that should be biocompatible, and biodegradable. Also, the products of biodegradation should not be neurotoxic. In particular, biomaterials could be used for site-specific delivery of proteins with chemotropic properties, to form concentration gradients and to stimulate and direct axonal growth of implanted cells, for example in models of Parkinson's disease, promoting proper reinnervation and restoration of the nigro-striatal pathway. In this study two biomaterials were evaluated, methylcellulose hydrogels and microspheres of a copolymer of lactic acid-glycolic acid (PLGA) wherein was encapsulated recombinant semaphorin 3A (sema 3A-Fc). This protein has an attractive effect on dopaminergic ventral midbrain neurons (VM) during the formation of the nigro-striatal pathway and repellent in dorsal root ganglion neurons (DRG) during embryonic development. In vitro tests showed that the encapsulation and release of proteins in PLGA microspheres is of low efficiency and no concentration gradient is formed, as compared with methylcellulose hydrogels (MCH). Evaluation of the biological effect of released Sema 3A-Fc in primary cultures of VM and DRG showed that the chemotropic protein is efficiently released from MCH and retains its functionality. We observed increased growth of VM dopaminergic axons compared with control; in contrast, in primary cultures of DRG retraction of axons near the Sema3A-Fc source was detected. These results indicate that MHC may be used for continual release of proteins into the brain. Furthermore behavioral tests in rats unilaterally implanted with MCH in the striatum at 1 week, 15 days and 1 month post-transplantation showed no alterations in locomotor activity and other behavioral tests. The results so far indicate that MCH could be used to release chemotropic proteins into the brain in a controlled way, without inducing neurological or behavioral alterations. It must be noted that it is also important to verify that this vehicle does not cause an inflammatory reaction after implantation into the brain.

Disclosures: A. Rios Ramirez: None. **M. Giordano:** None. **E. Tamariz:** None.

Poster

860. Aging: Pathophysiology and Treatment

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:860.12/J10

Topic: C.05. Aging

Title: CaMKIV reduction may contribute to age-related adult neurogenesis reduction

Authors: *S. MIYAKE[1], N. WALTON[1], A. NAGAKURA[2], Y. SHITAKA[2], A. MIYAKE[2], K. NI[2], N. MATSUOKA[2], C. HEUSNER[1], J. KOGAN[1], Q. CHEN[1], R. SHIN[1], A. GROSS[1], K. TAJINDA[2], K. TAMURA[1], M. MATSUMOTO[1]
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Abstract: Age-related memory impairment is a common occurrence in many mammalian species. Previous work suggests that the hippocampus, a brain region strongly implicated in the formation and retrieval of memories, is often impaired with age. Interestingly, a marked, age-associated decline in hippocampal adult neurogenesis has been described in rodents and primates, including humans. These declines in neurogenesis have been hypothesized to be related to hippocampal function, however the mechanism(s) for this decline have not yet been fully elucidated.
Here, we show that a significant reduction in expression of pCaMKIV(T196) and total-CaMKIV occurs in the hippocampus of aged rats compared to younger controls. Reduction of total CaMKIV is evident at 19 months and 25 months of age. A significant reduction in pCaMKIV is only observed at 25 months of age. To determine if CaMKIV expression is related to changes in adult neurogenesis, we examined CaMKIV expression in the dentate gyrus (DG) of CaMKIIa hKO mice, which exhibit increased hippocampal adult neurogenesis. CaMKIV mRNA in the DG is significantly increased in CaMKIIa hKO mice compared with age-matched WT mice, suggesting a role for CaMKIV in hippocampal adult neurogenesis. Additionally, CaMKIV mRNA is strongly upregulated following the terminal differentiation adult hippocampal neural stem cells.
These data suggest that CaMKIV may be positively correlated with neurogenesis and CaMKIV reduction may be one cause of the observed decline in age-related adult neurogenesis.

Disclosures: S. Miyake: Employment; CNS Astellas Research Institute of America LLC, Skokie, IL. **N. Walton:** None. **A. Nagakura:** None. **Y. Shitaka:** None. **A. Miyake:** None. **K. Ni:** None. **N. Matsuoka:** None. **C. Heusner:** None. **J. Kogan:** None. **Q. Chen:** None. **R. Shin:** None. **A. Gross:** None. **K. Tajinda:** None. **K. Tamura:** None. **M. Matsumoto:** None.

Poster

860. Aging: Pathophysiology and Treatment

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Program#/Poster#:860.13/J11

Topic: C.05. Aging

Support: NIA P01 AG16765

NIA AG 006647

NIA AG 010606

NIA R01 AG10606

NIA R37 AG06647

NIA AG 016765

Title: The effects of aging on dendritic spine density and morphology in macaque dorsolateral prefrontal and primary visual cortices

Authors: *M. E. BAILEY, D. T. OHM, D. DUMITRIU, J. H. MORRISON
Mount Sinai Sch. of Med., New York, NY

Abstract: Previous results from our lab indicate that aging decreases both spine density and the proportion of small, thin spines in layer III neurons of the dorsolateral prefrontal cortex of rhesus monkeys.
In the present study, we used intracellular injection of Lucifer Yellow dye and 3D confocal microscopy to compare the effects of aging on dendritic spine density and morphology in the dorsolateral prefrontal cortex and primary visual cortex. A cohort of behaviorally-characterized rhesus monkeys ranging in age from 9 to 33 years was used for this analysis. Dendritic spine density, head diameter, and head volume were measured in order to evaluate the effect of aging on spine architecture.
Our results confirm that pyramidal neuronal spine density decreases with age in the prefrontal cortex, but not in primary visual cortex. Additionally, thinner spines make up a larger proportion of the total population in young animals in both cortical areas, though this effect is more pronounced in the prefrontal cortex. Further analysis will examine the relationship between performance on the delayed response and delayed nonmatching to sample tasks and spine density and morphology in both regions.

Disclosures: M.E. Bailey: None. **D.T. Ohm:** None. **D. Dumitriu:** None. **J.H. Morrison:** None.

Poster

860. Aging: Pathophysiology and Treatment

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Topic: C.05. Aging

Support: NIH-NIDCD DC000210-25S1

NIH-NIA AG028054-05

Neurobiology Training Grant

NIH-NIDCD NRSA F32 DC010098-01A1

Title: Age-dependent effects on neuroblasts in the rostral migratory stream

Authors: *A. S. MOBLEY[1], A. BRYANT[1], M. B. RICHARD[3,4], J. H. BRANN[5], S. J. FIRESTEIN[5], C. A. GREER[1,2]
[1]Neurosurg., Yale Univ., NEW HAVEN, CT; [2]Neurobio., Yale Univ., New haven, CT; [3]Lyon Neurosci. Res. Ctr., INSERM, Lyon, France; [4]Univ. of Lyon, Lyon, France; [5]Biol. Sci., Columbia Univ., New York, NY

Abstract: Throughout life the subventricular zone (SVZ) is a source of new olfactory bulb (OB) interneurons. Neuroblasts born in the SVZ migrate to the OB tangentially in chains through an astrocyte restricted route, the rostral migratory stream (RMS)[1]. In mice the RMS is ~5 mm long with a pervasive vasculature which may aid migration; neuroblasts take 10-14 days to reach the OB from the SVZ[2]. During migration neuroblasts retain the ability to divide. Upon arrival in the OB neuroblasts migrate radially to the granule and glomerular layers and differentiate into granule and subpopulations of periglomerular (PG) cells that integrate into synaptic circuits. SVZ neurogenesis decreases with age, and is suggested as a factor in age-related olfactory deficits[3]. How the decrease in SVZ neurogenesis may translate into neuroblast migration, proliferation within the RMS, and the differentiation of interneuron subpopulations is less well understood. To address these issues we injected 6, 12, 18 and 23 month old mice with BrdU and sacrificed them at different intervals. We divided the RMS analyses into three regions, the vertical arm (VA) that extends from the SVZ and descends beneath the corpus callosum, the elbow, composed of the rostral curve towards the OB at the base of the VA, and the horizontal arm (HA), the final rostral extension into the OB. PG cell subpopulations were quantified within defined regions of the OB. Our data extends the finding that neuroblasts continue to proliferate in the RMS. Proliferation in the VA decreased during aging, similar to the SVZ, while proliferation in the elbow and HA remained stable. Further, we found that aging affects RMS size in a regional manner due to decreased numbers of neuroblasts, with no effect on the vascular or glial surround. The rate of neuroblast migration remained stable with age; 14 days after BrdU injection there were no significant differences in BrdU+ cells in the RMS across ages. While the number of newborn PG cells decreases with age, the population numbers and their distribution remain stable, consistent with our previous data[4]. These data suggest that despite a decrease in neurogenesis and changes in RMS organization cellular populations in the OB remain largely stable during aging, with implications for olfactory-related behavior.
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1. Lois, C., Garcia-Verdugo, J.M., and Alvarez-Buylla, A. (1996) Science *271*, 978-981.

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3. Enwere, E., Shingo, T., Gregg, C., Fujikawa, H., Ohta, S., and Weiss, S. (2004) J Neurosci *24*, 8354-8365.
4. Richard, M.B., Taylor, S.R., Greer, C.A. (2010) PNAS *107*, 15613-15618.

Disclosures: A.S. Mobley: None. **A. Bryant:** None. **M.B. Richard:** None. **J.H. Brann:** None. **S.J. Firestein:** None. **C.A. Greer:** None.

Poster

860. Aging: Pathophysiology and Treatment

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:860.15/J13

Topic: C.19. Drug Discovery and Development

Support: Dean's Academic Enrichment Fund, University of Colorado - Anshutz Medical Campus

Title: Neural stem cells and hydrogels: Enhancing neural tissue engineering potential

Authors: *E. R. AURAND[1], J. WAGNER[2], C. LANNING[2], K. B. BJUGSTAD[3]
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Abstract: Neural tissue engineering could greatly benefit from the use of hydrogels; however, the hydrogels must be customizable and, ideally, mimic the various properties of brain tissue in addition to being therapeutically functional. Currently, hydrogels used for neural tissue engineering are not thoroughly characterized for their chemical, physical, and mechanical properties. The goal of this research was to create a well-characterized hydrogel using commercially available materials suitable for the implantation of neural stem cells. Hydrogels were created from a range of ratios of hyaluronic acid (HA) to poly(ethylene glycol) (PEG). These hydrogels were assessed for the physical properties of polymerization and degradation, the mechanical property of compressive modulus, and the cytocompatibility with encapsulated neural stem cells. The polymerization and degradation rates of the HA:PEG hydrogels were a function of the amount of HA incorporated into the hydrogel. With increasing amounts of HA, the rate of polymerization increased (>120 min to <2 min); conversely, with increasing HA content, there was a decrease in the rate of degradation (<12 hr to >10 d). The compressive modulus was also a function of the HA content. Compressive modulus (hydrogel stiffness) increased with increasing HA content and ranged from 0.1 to 32kPa (rodent brain tissue ranges

from 2-10kPa). In addition to physical and mechanical characterization, neural stem cells derived from fetal rats (fNSC) were encapsulated within a three-dimensional (3D) HA:PEG hydrogel environment. Twenty-four hours post-encapsulation, cell survival was measured and compared to cells plated on standard tissue culture plates (TCP; controls). Results demonstrated that 3D cultures typically had good survival/proliferation, with an average survival rate of 122.51±2.69% compared to only 34.62±3.47% in TCP controls. fNSC survival was found to be dependent on the relative proportion of HA or PEG. At low concentrations of PEG, fNSC survival was high, regardless of HA content. At high concentrations of PEG, fNSC survival was dependent on the amount of HA, with greater survival at higher HA concentrations. These results indicate that there can be a wide range of HA:PEG-based hydrogels which are compatible with neural stem cells. Furthermore, the physical and mechanical properties can be readily altered by changing the proportion of HA without substantially altering neural stem cell survival. This bodes well for neural tissue engineering strategies, allowing for the possibility to create customized hydrogels for specific applications.

Disclosures: E.R. Aurand: None. **J. Wagner:** None. **C. Lanning:** None. **K.B. Bjugstad:** None.

Poster

861. Animal Models and Mechanisms III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:861.01/J14

Topic: C.06. Developmental Disorders

Title: Molecular pathogenesis of a central diabetes insipidus rat strain

Authors: *T. ITO, N. TAKEUCHI, K. YOSHIDA, K. YUKAWA
Physiol., Meijo Univ., Nagoya / Aichi, Japan

Abstract: As previously reported, our animal facility maintains a Wistar rat strain showing polydipsia and polyuria. The rat strain offspring gain the pathologic phenotype in an autosomal recessive manner. Significantly lower urine osmotic pressure and plasma arginine vasopressin (AVP) level of the rat strain indicated diabetes insipidus as the primary cause of the symptom. Sequence analysis of the preprovasopressin (preproAVP) mRNA did not reveal any mutations in the nucleotide sequences. Real-time PCR analysis of vasopressin mRNA did not detect any significant differences in the vasopressin mRNA level in supraoptic and paraventricular nuclei of the hypothalamus between the diabetes insipidus strain and the normal Wistar rat. Subdermal injection of AVP analog (dDAVP, 0.4μg/Kg) to the strain could maintain both high urine osmolality and decreased urine volume as longs as dDAVP remained at the effective levels,

which demonstrated normal water reabsorption system in the kidney. To examine if lower plasma AVP level of the strain results from either the decrease of AVP axonal transport in hypothalamic neurons or disturbed AVP secretion from axon terminals into blood, we quantified the expression level of AVP in the hypothalamus and pituitary from both the diabetes insipidus strain and the normal Wistar rat by immunohistochemistry and western blotting. Immunohistochemical studies showed higher AVP level in hypothalamic nuclei and pituitary of the diabetes insipidus strain as compared with the normal Wistar rat, suggesting a defect of AVP secretion from the posterior lobe of puituitary in the central diabetes insipidus rat strain.

Disclosures: T. Ito: None. **N. Takeuchi:** None. **K. Yoshida:** None. **K. Yukawa:** None.

Poster

861. Animal Models and Mechanisms III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:861.02/J15

Topic: C.06. Developmental Disorders

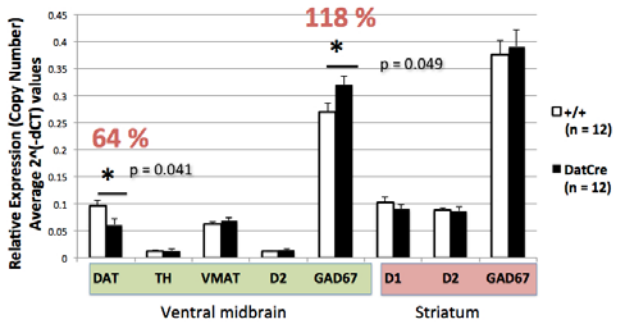
Support: PHS Award P50 MH086404

Title: Subtle Impact of the reduction in dopamine transporter (DAT) gene expression in DAT-Cre mice

Authors: S. MINGOTE[1], M. O. CHOHAN[2], N. CHUHMA[1], C. GELLMAN[1], B. INBAR[2], S. RAYPORT[1], *H. M. MOORE[2]
[1]Psychiatry and Mol. Therapeut., Columbia Univ. and New York State Psychiatric Inst., New York, NY; [2]Psychiatry and Integrative Neurosci., Columbia Univ. and New York State Psychiatric Inst., NEW YORK, NY

Abstract: DAT-Cre knock in mice, with a heterozygous insertion of the Cre allele in the dopamine transporter (DAT) locus (Zhuang et al., 2005) have been widely used for conditional targeting strategies in DA neurons. We have found that DAT expression in DAT-Cre mice, backcrossed onto a congenic C57/BL6 background at P60, is 64% of wt DAT levels (see Figure), as compared to DAT hemizygous mice that have 36% of wt DAT levels (Giros et al., 1996). There were no significant changes in tyrosine hydroxylase, VMAT, dopamine D1 or D2 receptor expression in either the ventral midbrain or the striatum of DAT-Cre mice; GAD expression was slightly increased in the ventral midbrain. There was no significant change in DA content in the nucleus accumbens or dorsolateral striatum, but there was a significant reduction in prefrontal cortex. Decreases in DA content were accompanied by increases in the ratio of homovanillic acid to DA, consistent with a reduction in intracellular DA but increased levels and metabolism of

extracellular DA. In ventral midbrain slices from DAT-Cre mice, there were no significant differences in the intrinsic properties of DA neurons, or in their firing patterns. The firing characteristics of DA neurons in vivo, assessed by extracellular single-unit recordings of electrophysiologically identified DA neurons under chloral hydrate anesthesia, were unchanged in our preliminary analyses. In contrast to DAT-Cre mice, DAT-IRES-Cre mice (Bäckman et al., 2006) show no significant impact on DAT gene expression, or in the other DA system genes. Thus, the DAT-Cre allele, but not the DAT-IRES-Cre allele, significantly compromises DAT expression and produces subtle changes that should be considered in the interpretation of the apparent effects of conditional deletion of target genes in DA neurons in DAT-Cre mice.



Disclosures: S. Mingote: None. **M.O. Chohan:** None. **N. Chuhma:** None. **C. Gellman:** None. **B. Inbar:** None. **S. Rayport:** None. **H.M. Moore:** None.

Poster

861. Animal Models and Mechanisms III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:861.03/J16

Topic: C.06. Developmental Disorders

Support: Klarman Family Foundation Grants Program in Eating Disorders Research (to NCBM)

Title: Activity-based anorexia reduced cell proliferation in the ventral hippocampus of adolescent female rats: Do changes remain following recovery and relapse?

Authors: *N. S. BURGHARDT[1], K. LAURINO[2], R. HEN[1], N. BARBARICH-MARSTELLER[2]
[1]Dept Neurosci, Psych & Pharmacol, Columbia Univ., NEW YORK, NY; [2]Dept. of Psychiatry, Col. of Physicians and Surgeons, Columbia Univ., New York, NY

Abstract: Anorexia nervosa is a psychiatric disorder that is characterized by severe self-starvation and life-threatening weight loss. The predominately female disorder typically develops during adolescence and is often associated with compulsive exercise and anxiety. Aspects of anorexia nervosa can be modeled in animals using a translational approach known as activity-based anorexia (ABA), which combines limited access to food with unlimited access to a running wheel. Under these conditions, rodents run continuously, even throughout the daily hour of food access, thereby promoting self-starvation. This failure to adapt food intake to increasing energy demands leads to significant weight loss and possible death. ABA in rats induces changes in GABA receptor subunits and decreases gliogenesis in the hippocampus, a structure known to play an important role in regulating anxiety. Here, we used adolescent female rats to investigate the effects of ABA on the generation of new neurons in the dentate gyrus of the hippocampus. Neurogenesis was evaluated following three time points: 4 days of ABA (ABA), 7 days of recovery from ABA (Recovery), and 4 days of a second exposure to ABA after recovery (Relapse). At each time point, four groups of rats were tested: control (24 h/day food access), food-restricted control (1 hr/day food access), exercise (24 h/day food and wheel access), and ABA (1 h/day food access, 24 h/day wheel access). Rats were injected once with 5-bromo-2'-deoxyuridine (BrdU) (200 mg/kg, i.p.) and perfused two hours later so that the effects of ABA, recovery, and relapse on cell proliferation could be evaluated. BrdU-positive (+) cells were quantified throughout the rostro-caudal extent of the granule cell layer of the dentate gyrus. As expected, the ABA group dramatically increased running wheel activity, leading to a decrease in body weight with each day of food restriction. Four days of ABA led to a significant decrease in BrdU+ cells (40%) in the ventral but not dorsal hippocampus. ABA was also associated with significantly fewer BrdU+ cells in regions outside of the granule cell layer, confirming that ABA decreases gliogenesis. Ongoing analyses will determine the extent to which the decrease in BrdU+ cells in the ventral dentate gyrus represents reduced neurogenesis rather than reduced gliogenesis. We will also evaluate the independent effects of exercise and food restriction on cell proliferation in the control groups and explore whether alterations in cell proliferation remain following recovery and relapse. Our results indicate that the ventral hippocampus is sensitive to stress induced by ABA.

Disclosures: N.S. Burghardt: None. **K. Laurino:** None. **R. Hen:** None. **N. Barbarich-Marsteller:** None.

Poster

861. Animal Models and Mechanisms III

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Program#/Poster#:861.04/J17

Topic: C.06. Developmental Disorders

Support: NIH Grant HD021350

Title: Global 30% maternal nutrient reduction (MNR) from 0.16 to 0.9 of gestation (G) decreases leptin receptor (Ob-R) protein expression in the fetal baboon hypothalamic arcuate (ARC) and paraventricular nuclei (PVN)

Authors: *C. LI, P. W. NATHANIELSZ, T. J. MCDONALD
OB/GYN, Univ. Texas Hlth. Sci. Ctr, San Antonio, San Antonio, TX

Abstract: INTRODUCTION: In human pregnancy the adipose hormone leptin is increased in diabetic pregnancy and pre-eclampsia and reduced in maternal and fetal plasma in intrauterine growth restriction (IUGR; Placenta 23:103). Leptin has many central nervous system functions including signaling to inhibit appetite. The ARC and PVN are recognized as critical appetite regulatory nuclei producing neuropeptides that control feeding behavior and energy balance. We previously showed that MNR: 1) increases protein expression of fetal baboon ARC and PVN orexigenic peptides, 2) decreases anorexigenic peptides and 3) decreases placental leptin and Ob-R by 0.9G. In the present study, we hypothesized that MNR regulates Ob-R protein expression in both the ARC and PVN at 0.9G. **METHODS:** Pregnant baboons were fed a *ad lib* controls (CTR; n=7) or 70% CTR global diet (MNR; n= 6) from 0.16 to 0.9G and fetuses ecovered at CSsection with general anesthesia. Ob-R protein expression was determined by immunohistochemistry with Ob-R antibody (Santa Cruz, sc-1833) and quantified by image analysis (ImageJ, NIH) for density (arbitrary density units and fraction (area immunostained/-area of field x 100%). Analysis, Student's t-test, set at 0.05; data = mean ± SUM. RESULTS:

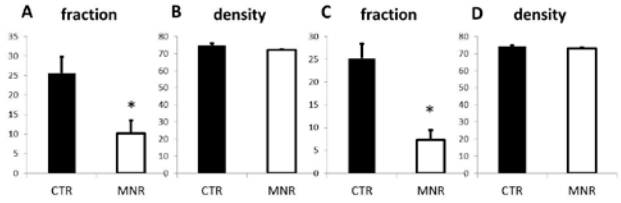


Figure 1. Fetal baboon hypothalamic leptin receptor immunoreactivity in arcuate (A & B) and paraventricular nuclei (C & D) CTR n=7, MNR n=6. Data =mean ± SEM. *$p < 0.05$.

CONCLUSIONS: The observed decrease in OB-R in the appetitive centers would decrease the efficiency of leptin negative feedback on appetite. If this change persists post-natally it could contribute the predisposition to postnatal obesity and accompanying pathologies associated with IUGR.

Disclosures: C. Li: None. **P.W. Nathanielsz:** None. **T.J. McDonald:** None.

Poster

861. Animal Models and Mechanisms III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:861.05/J18

Topic: C.06. Developmental Disorders

Support: COECYTJAL Grant PS-2009-500

Title: Dopaminergic alterations related with impairment of work memory in a perinatal asphyxia model.

Authors: *S. J. LOPEZ-PEREZ, A. MORALES-VILLAGRÁN
Mol. and Cell. Biol., Univ. of Guadalajara, Zapopan, Jalisco, Mexico

Abstract: Perinatal asphyxia is a process that produce neurotoxic milieu in the brain, by an initial glutamate accumulation in the extracellular space. This represents a potential risk of permanent brain damage, with high probability to develop
important neurological sequelae like learning and memory disabilities. Both these capacities are integrated in the frontal cortex (FC) and are related directly with dopaminergic neurotransmission in the striatum (ST). The aim of this work
was to analyze the response of dopaminergic D1 and D2 receptors and dopaminergic transporter in striatum and frontal cortex of juvenile Wistar rats (30 days old) subjected to 45 min of asphyxia in early postnatal period (11 days old). We also evaluate the work memory, related with the frontal circuits, in the asphyxiated animals, through a standard object recognition test. 11 days old pup rats were subjected to an asphyxia protocol in a sealed chamber, fill with a mixture of 8% O_2 and 92% N_2 for a period of 45 min. At 30 postnatal days, asphyxia and controls animals were selected to one of the next experimental procedures: 1) Animals were sacrificed to obtain the brains and dissected the FC and ST. Tissues were homogenate and centrifuged and final supernatants were assayed to DA and DOPAC content by HPLC. 2) Animals were sacrificed to obtain the brains and dissected the FC and ST. Tissues were homogenate and
centrifuged and final supernatants were assay to total protein concentration. Aliquots containing 80 µg of total protein were tested to D1/D2 receptors and dopaminergic transporter (DAT) in each region, by Western blot. 3) Finally, a third group of animals, formed by asphyxiated and control animals, were used to carry out a standard object recognition test. Results from total DA and DOPAC quantification shows a generalized decrease in the DA content in FC and ST, while DOPAC show no changes in FC and low level in ST. Regarding to D1, D2 and DAT protein expression, it was observed an increase in the expression of D2 receptor and DAT, with no changes in expression of D1 receptor. The object recognition test allowed us to observe that asphyxiated animals fail in the recognition of an object that previously has been explored by

them. These results suggest alterations in the dopaminergic neurotransmission in ST and FC, which could be generating a neurochemical substrate to failures in the work memory task.

Disclosures: S.J. Lopez-Perez: None. **A. Morales-Villagrán:** None.

Poster

861. Animal Models and Mechanisms III

Location: Hall F-J

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Program#/Poster#:861.06/J19

Topic: C.06. Developmental Disorders

Title: The effects of fourth ventricle intraventricular hemorrhage on the development of the postnatal mouse cerebellum

Authors: J. YOO, G. MAK, *D. GOLDOWITZ
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Abstract: Intraventricular hemorrhage (IVH) is the most common neurological problem in premature infants and often leads to neurological sequelae, such as cerebral palsy, post-hemorrhagic hydrocephalus, and cognitive dysfunction. With the increase in survival of preterm infants and the improvement in neuroimaging technology, the detection of IVH in the fourth ventricle of preterm infants is becoming increasingly common. The cerebellum, which is involved in motor control, motor learning and cognition, is positioned immediately above the fourth ventricle, but how fourth ventricle IVH affects the development and function of cerebellum remains largely unknown. Therefore, our study focuses on developing a mouse model of fourth ventricle IVH to determine the anatomical and behavioural phenotypes resulting from the hemorrhage and to understand the mechanisms of injury in the neonatal cerebellum. To induce a hemorrhage in the germinal matrix of the cerebellum, we injected collagenase, which breaks down surrounding blood vessel walls, into the fourth ventricle of postnatal (P) day 2 mice. Controls were injected with saline. We then analyzed cell proliferation, apoptosis, and inflammation in the developing cerebellum. Also, we performed various histological assessments to detect any changes in size of cerebellum and the number of cerebellar cell types, including granule cell, Purkinje cell, interneuron, and oligodendrocyte. Behavioral analyses were also conducted on P60 mice to assess locomotor and cognitive abilities. In our model of fourth ventricle IVH, we found a delay in cerebellar development, reduction in granule cell number, and persistent neurobehavioral abnormalities similar to premature infants with IVH. Therefore, our study provides a novel pre-clinical model of IVH that can be used to understand the

pathophysiology of the disease, as well as for the development and evaluation of preventive therapies and post-hemorrhagic treatments.

Disclosures: J. Yoo: None. **G. Mak:** None. **D. Goldowitz:** None.

Poster

861. Animal Models and Mechanisms III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:861.07/J20

Topic: C.06. Developmental Disorders

Title: Perinatal asphyxia and fetal asphyctic preconditioning: Global inflammatory impact on brain cortex, cerebellum and liver

Authors: *E. VLASSAKS[1,2], J. S. H. VLES[3], T. BRUDEK[4], E. STRACKX[1,2], H. W. STEINBUSCH[1], B. W. KRAMER[2], B. PAKKENBERG[4], D. A. W. GAVILANES[2]
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Abstract: Background:
Fetal and perinatal asphyxia are the major cause of neonatal mortality and morbidity worldwide. During periods of moderate and severe asphyxia (hypoxia-ischemia), fetuses develop multi-organ failure with involvement of many vital organs such as heart, kidneys, liver and brain.
Objectives:
1. To investigate inflammatory changes after fetal and perinatal asphyxia in brain cortex, cerebellum and liver.
2. To investigate if the combination of both insults (preconditioning) would have any hepatic or neuroprotective effect.
Methods:
In pregnant rats, fetal asphyxia was induced at E17 by clamping the uterine circulation for 30 min. Perinatal asphyxia was induced at E21/22 by submersing the uterine horns in a saline bath (37ºC) for 19 min. At different time points after both insults, we assessed inflammatory changes by means of quantitative RT-PCR and immunohistochemistry in brain cortex, cerebellum (results are pending) and liver.
Results:
Fetal asphyxia induced down regulation of the acute brain cytokine response with decreased IL-1β, TNF-α and IL-10 mRNA levels. However, IL-6 mRNA levels increased 96 h after fetal

asphyxia compared to controls. After birth, we found increased IL-10 and IL-6 mRNA expression after severe perinatal asphyxia, though this increase was not present in newborns that were previously exposed to fetal asphyctic preconditioning. Opposite effects were found in the liver, where we examined increased levels in IL-1β, IL-6 and IL-10 mRNA after fetal asphyxia. Postnatal IL-6 mRNA expression increased after perinatal asphyxia and was comparable with baseline levels in neonates that were previously exposed to fetal asphyctic preconditioning.
Conclusion:
In the brain cortex, fetal asphyctic preconditioning leads to down regulation of the acute inflammatory response, while the systemic response in the liver was reversed. This modulation of the cytokine response probably results in a protective phenotype postnatally. Therefore, elucidating the inflammatory pathways involved in asphyxia and preconditioning may have significant implications for preventing post-asphyctic multi-organ failure.

Disclosures: E. Vlassaks: None. **J.S.H. Vles:** None. **T. Brudek:** None. **E. Strackx:** None. **H.W. Steinbusch:** None. **B.W. Kramer:** None. **B. Pakkenberg:** None. **D.A.W. Gavilanes:** None.

Poster

861. Animal Models and Mechanisms III

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Program#/Poster#:861.08/K1

Topic: C.06. Developmental Disorders

Support: NIH Grant P01HD57853

Title: A behavioral assessment of sex differences in a mouse model of severe neuronal migration anomalies

Authors: *D. T. TRUONG[1], C. E. SZALKOWSKI[1], G. D. ROSEN[2], R. H. FITCH[1]
[1]Behavioral Neurosci., Univ. of Connecticut, Storrs, CT; [2]Neurol., Harvard Med. Sch., Boston, MA

Abstract: Neuronal migration anomalies have been linked to a wide range of neurobehavioral and cognitive disorders including epilepsy, autism, and developmental language and reading disabilities. Moreover, researchers have suggested that underlying dysfunction in rapid auditory processing (RAP) may contribute to the etiology of developmental language/learning impairments. As such, rodent models have been used as an invaluable tool to study the link between neuronal migration anomalies and auditory processing ability. These studies have found RAP deficits in rodents with spontaneous, genetically manipulated, and injury-induced cortical

anomalies. Such results suggest a link between neuronal migration disruption and RAP, and further provide one possible underlying biological and behavioral etiology for language related dysfunction in clinical populations. Interestingly, males are more commonly diagnosed with neurodevelopmental disorders associated with language learning impairments, and research using rodent models of disrupted neuronal migration has also revealed sex differences in RAP, with affected males typically performing worse than affected females.
The current study was designed to examine possible sex differences in behavioral measures (RAP, Morris spatial water maze, non-spatial water maze) using a recently discovered model of spontaneously occurring severe neuronal migration disruption (bilateral subcortical nodular heterotopia and callosal agenesis) in a BXD29 recombinant inbred strain of mice (BXD29-Tlr4^{lps-2J}/J). Current results replicate our previous behavioral findings, with affected male mice showing a specific impairment in rapid auditory processing in comparison to the isogenic BXD29/Ty mice (no cortical anomaly). Interestingly, comparable deficits were also seen in affected females (indicating a lack of sex differeneces). Overall, these data suggest that the severity of the neuronal migration anomaly in BXD29-Tlr4^{lps-2J}/J mice impairs auditory processing in both sexes. The current findings provide insight to the neurodevelopmental anomalies that may mediate underlying emergent impairments of language-related processing in humans by providing a model wherein both sexes appear to be affected equally (consistent with clinical evidence that despite much smaller numbers, females are also affected with language/learning disorders).

Disclosures: D.T. Truong: None. **C.E. Szalkowski:** None. **G.D. Rosen:** None. **R.H. Fitch:** None.

Poster

861. Animal Models and Mechanisms III

Location: Hall F-J

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Program#/Poster#:861.09/K2

Topic: C.06. Developmental Disorders

Support: NIH Grant MH065702 to AS

Title: Neural mechanisms underlying selective loss of social learning in neurofibromatosis type 1 gene (Nf1) deficient mice and its restoration by co-deletion of p21-activated kinase 1 (Pak1) gene.

Authors: *A. I. MOLOSH[1,2], J. P. SPENCE[1,3], L. M. FEDERICI[4,3], S. JANASIK[1], Z. M. SEGU[11,12], C. GOSWAMI[5], W. ZHU[6,2], R. KHANNA[6,2,3], P. L. JOHNSON[4,7,1], L. LI[8,5], Y. S.

MECHREF[11,12], D. W. CLAPP[9,10], A. SHEKHAR[1,2,3,6,7]
[1]Psychiatry, [2]Stark Neurosciences Res. Inst., [3]Program in Med. Neurosciences, [4]Anat. and Cell Biol., [5]Ctr. for Computat. Biol. and Bioinformatics, [6]Pharmacology&Toxicology, [7]Clin. and Translational Sci. Inst., [8]Med. and Mol. Genet., [9]Pediatrics, [10]Microbiology&Immunology, IU Sch. of Med., Indianapolis, IN; [11]Chem., [12]METACyt Biochem. Analysis Ctr., Indiana Univ., Bloomington, IN

Abstract: Neurofibromatosis type 1 (NF1) is an autosomal dominant disease with mutation in one copy of the NF1 gene (NF1+/-) that affects roughly 1 in 3500 individuals, and frequently presents with learning disabilities, attention deficit hyperactivity disorder, and social deficits similar to autism spectrum disorders (ASDs). The NF1 gene encodes neurofibromin, a cytoplasmic GAP-like protein that is expressed throughout the central nervous system. Previously, we demonstrated that Nf1-deficient (Nf1+/-) mice show social learning deficits, express greater activation of MAPK pathway following growth factor stimulation, which were rescued by deleting Pak1 gene activity (Nf1+/-/Pak1-/- mice). We also showed that projection neurons in basolateral amygdala (BLA) region in Nf1+/- mice exhibit increased frequency (but normal amplitude) of miniature inhibitory post-synaptic currents (mIPSCs). Here, we further characterized the mechanisms of social learning deficits in Nf1+/- mice. We confirmed that compared to WT, the Nf1+/- mice demonstrate selective deficit only in social learning but not in elevated-plus maze avoidance learning, olfactory habituation, novel object recognition or passive avoidance tests. Using the whole-cell patch clamp technique, we also determined that deletion of Nf1 gene decreases long-term potentiation (LTP) in the amygdala. Interestingly, we also observed a significant increase in amplitude of miniature excitatory post-synaptic currents (mEPSCs) in Nf1+/-/Pak1-/- and Pak1-/- mice. Utilizing mass spectrometry, we measured expression levels of 338 proteins in amygdala region in WT, Nf1+/-, Nf1+/-/Pak1-/- and Pak1-/- mice. Our plan was to specifically identify proteins that were changed in Nf1+/-, compared to WT, and those that were restored in Nf1+/-/Pak1-/- mice but were not similarly altered by deletion of Pak1 gene alone. Using INGENUITY pathway analysis software, we identified reduction in several key proteins (ADAM22, CAP1 and HSP70) associated with glutamate neurotransmission and synaptic plasticity in the amygdala. We further confirmed loss of ADAM22 in the amygdala region with immunohistochemical analysis. In conclusion, our findings demonstrate that Nf1+/- mice express a selective deficit in social learning that is seen in Nf1+/- mice and rescued in Nf1+/-/Pak1-/- mice appears to involve disruption of GABA-mediated inhibition and glutamate excitation of the projection neurons of the amygdala, perhaps resulting from altered expression of important synaptic proteins such as ADAM22 in the amygdala.

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Poster

861. Animal Models and Mechanisms III

Location: Hall F-J

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Program#/Poster#:861.10/K3

Topic: C.06. Developmental Disorders

Support: NIMH grant MH080069

NICHD grant P30 HD03110

Title: Neuronal activation following social challenge in adolescent Grin1 knockdown mice

Authors: N. V. RIDDICK[1], V. D. NIKOLOVA[1], K. L. AGSTER[1], L. K. BAKER[1], B. H. KOLLER[2], G. E. DUNCAN[1], *S. S. MOY[1]
[1]Psychiatry, [2]Genet., Univ. NC Sch. Med., CHAPEL HILL, NC

Abstract: Genetically-altered mice with reductions in the NR1 subunit of the *N*-methyl-D-aspartate (NMDA) receptor, encoded by *Grin1*, have been proposed as a model for aberrant glutamatergic neurotransmission and behavioral phenotypes in schizophrenia and autism. *Grin1* knockdown mice recapitulate impaired social behavior, cognitive deficits, and other features of severe neuropsychiatric disorders, and also show deficient neuronal activation, measured by induction of Fos protein, following a social challenge. However, most abnormal phenotypes have been observed in adult mice. We have recently reported that, in contrast to a marked lack of sociability in adulthood, *Grin1* knockdown mice have significant hypersociability during adolescence. The present study examined Fos induction in several brain regions of adolescent (postnatal day 30-35) male and female *Grin1* and wild type mice following a modified resident/intruder challenge. For this procedure, an adult C57BL/6J mouse ("resident") was individually housed for 24 hrs prior to the test. An adolescent *Grin1* knockdown or wild type mouse ("intruder") was introduced to a sex-matched resident and behavior was video-recorded. After 15 min of social interaction, the resident mouse was placed under a wire cup, and the pair remained together for 2 hours, when the intruder was anesthetized and perfused. Non-social challenge controls were introduced to an empty cage and an empty wire cup. Results indicate that, in contrast to the marked deficits in Fos induction observed in adulthood, adolescent *Grin1* knockdown mice have significantly increased levels of Fos in cingulate cortex, infralimbic cortex, and lateral septum, following either a social challenge or exposure to a novel environment. These results suggest that the hypersociability phenotype in adolescent *Grin1* knockdown mice is associated with developmental increases in neuronal activation. Findings will be compared to profiles of Fos expression in young adult *Grin1* and wild type mice (postnatal day 60-65), to investigate the window for emergence of the deficient neuronal activation observed in older *Grin1* knockdown mice, relevant to age-dependent social phenotypes.

Disclosures: N.V. Riddick: None. **V.D. Nikolova:** None. **K.L. Agster:** None. **L.K. Baker:** None. **B.H. Koller:** None. **G.E. Duncan:** None. **S.S. Moy:** None.

Poster

861. Animal Models and Mechanisms III

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Program#/Poster#:861.11/K4

Topic: C.06. Developmental Disorders

Support: CIHR

SHRF

NARSAD Young Investigator Award

Title: Maternal diabetes as a risk factor for psychiatric disorders in the offspring: A new animal model of schizophrenia?

Authors: *A. CHANDNA, C. A. THAI, Q. GREBA, N. KUHLMANN, C. A. BRYCE, V. A. CAMPANUCCI, J. G. HOWLAND
Physiol., Univ. of Saskatchewan, Saskatoon, SK, Canada

Abstract: Inflammatory responses to infection during pregnancy increase the risk of neurodevelopmental psychiatric disorders such as schizophrenia in the offspring. However, whether maternal diabetes leads to a similar inflammatory state with long-term consequences for the offspring is not known. A major source of inflammatory signaling observed in diabetes is activation of the receptor for advanced glycation end products (RAGE). Interestingly, several studies have shown increased RAGE activation in psychiatric disorders. Thus, our hypothesis is that maternal diabetes creates a proinflammatory state, triggered largely by RAGE signaling, that alters normal neural development of the offspring. We tested this hypothesis in rats using the streptozotocin (STZ; 50 mg/kg; i.p.) model of maternal diabetes during the last week of pregnancy. Following STZ treatment, we observed a significant increase in protein expression of RAGE and the pro-inflammatory nuclear factor NF-κB in the forebrain of the offspring (postnatal day 0). Electrophysiological characterization of hippocampal cultures grown from the offspring of STZ-treated dams also showed a striking increase in excitability. Using whole-cell current clamp techniques, neurons from the offspring of diabetic rats showed a robust increase in the number of action potentials generated in response to a series of 500 ms depolarizing current steps. Further analysis of the action potentials generated also showed that hippocampal neurons from the offspring of diabetic rats generated action potentials of larger amplitudes and faster rise

and decay times. When tested in a battery of cognitive tasks in early adulthood, the offspring of STZ-treated dams had significantly lower prepulse inhibition than control offspring (22.9% vs. 42.7%) without changes in anxiety or recognition memory. In an operant-based task, STZ offspring did not differ from controls on a visual discrimination task but took significantly longer to shift to a new strategy to solve the task (i.e., set-shift; controls: 52.3 trials, STZ: 75.9 trials). These results suggest that maternal diabetes alters normal hippocampal development of the offspring, effects that may be mediated by RAGE signaling. Given the types of cognition affected in early adulthood, this preparation may be useful as a rodent model for some aspects of schizophrenia.

Disclosures: A. Chandna: None. **C.A. Thai:** None. **Q. Greba:** None. **N. Kuhlmann:** None. **C.A. Bryce:** None. **V.A. Campanucci:** None. **J.G. Howland:** None.

Poster

861. Animal Models and Mechanisms III

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Topic: C.06. Developmental Disorders

Support: NARSAD Independent Investigator Award from the Brain and Behavior Research Foundation

LSUHSC Research Enhancement Fund Grant

NIH Grant P20RR16816

Title: Neurodevelopmental role for VGLUT2 in pyramidal neuron plasticity and spatial learning

Authors: H. HE[1], A. H. MAHNKE[2], S. DOYLE[1], N. FAN[1], C.-C. WANG[2], B. J. HALL[2], Y.-P. TANG[1], F. M. INGLIS[2], C. CHEN[1], *J. D. ERICKSON[1]
[1]Neurosci., LSUHSC, NEW ORLEANS, LA; [2]Dept. of Cell and Mol. Biol., Tulane Univ., New Orleans, LA

Abstract: The level and integrity of glutamate transmission during postnatal development plays an important role in the regulation of pyramidal neuron structure and plasticity, and in cognition. The synaptic release of glutamate is mediated predominantly by VGLUT1 and VGLUT2 in cortico-limbic circuits. An important developmental window for the maturation of VGLUT1-encoded transmission in pyramidal neurons occurs during the 2nd and 3rd postnatal weeks in rodents. We hypothesize that VGLUT2 may play an important role in this process as it is

expressed in a considerable number of VGLUT1-containing developing synapses throughout the forebrain, and then either disappears, is inducible, or is retained in subsets of excitatory neurons into adulthood. To assess a potential neurodevelopmental role for VGLUT2 in pyramidal neuron plasticity, and in cognition, we have generated recombinant VGLUT2 knockout mice and inactivated VGLUT2 in cortico-limbic circuits throughout development. We used a Morris water maze to evaluate spatial learning and memory. Novelty-induced exploratory activity was measured in an open-field chamber. Hippocampal CA1 field potentials were evoked by constant current stimuli to CA3 Schaffer collateral commissural projections while recording field potentials in the *stratum radiatum* of CA1. We measured LTD, LTP, paired-pulse facilitation and input-output function. Region-specific changes in VGLUT1 and VGLUT2 expression was performed by Western and immunohistochemical analysis. We show that VGLUT2-deficiency in Emx1-$Cre^{+/+}$VGLUT2$^{fx/fx}$ mice results in reduced evoked glutamate transmission, release probability, and LTD at CA3-CA1 synapses during a formative development period (P11-14). In adults, CA1 dendritic arbor and spine number are reduced together with LTP and the levels of synaptic markers spinophilin and VGLUT1. VGLUT2-deficient mice exhibit increased novelty-induced exploratory activity, yet impaired spatial learning and memory; endophenotypes similar to NMDA receptor knockdown mice. Remarkably, the impairment in spatial learning can be partially restored by selectively increasing NMDA-receptor mediated glutamatergic transmission in adult mice *in vivo*. Our results support the notion that LTD induced by LFS in young CA3-CA1 connections may involve plasticity in VGLUT2-encoded vesicle release. Furthermore, our data suggest that VGLUT2 transmission in cortico-limbic circuits during development is critical for the maturation of normal structure and plasticity of CA1 pyramidal neurons and that such glutamatergic deficiency leads to cognitive malfunction as observed in NMDA receptor implicated psychiatric disorders.

Disclosures: H. He: None. **A.H. Mahnke:** None. **S. Doyle:** None. **N. Fan:** None. **C. Wang:** None. **B.J. Hall:** None. **Y. Tang:** None. **F.M. Inglis:** None. **C. Chen:** None. **J.D. Erickson:** None.

Poster

861. Animal Models and Mechanisms III

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:861.13/K6

Topic: C.06. Developmental Disorders

Support: NIH HD057488

NIH HD057488-S1

NIH HD50201

Title: Outcomes of intrauterine asphyxia in the full term rat

Authors: *J. J. MORGAN[1], C. D. TULBERT[2], J. OLSON[3], G. A. KLEVEN[4], A. E. RONCA[2]
[1]Obstetrics & Gynecology, Wake Forest Univ., WINSTON SALEM, NC; [2]Obstetrics & Gynecology, [3]Ctr. for Biomolecular Imaging, Wake Forest Sch. of Med., Winston-Salem, NC; [4]Psychology, Wright State Univ., Dayton, OH

Abstract: Intrauterine asphyxia, observed in 1-6 out of every 1,000 live full term births and nearly 60% of preterm births, is a component of many obstetric complications, including infection, preeclampsia, maternal diabetes, and intrauterine growth restriction. Numerous population-based studies show risk for developing schizophrenia is more than doubled following intrauterine asphyxia. The mechanistic link between intrauterine asphyxia and schizophrenia has yet to be elucidated. Intrauterine asphyxia causes an acute lack of oxygen in the blood, resulting in metabolic acidosis, hypercapnia, and hypoxia. Preclinical models have identified postnatal sequelae involving oxidative stress and inflammation, as well as alterations in the dopamine (DA) system that parallel abnormalities found in the schizophrenic brain. In particular, preclinical studies have identified an increased sensitivity to DA agonists, consistent with observed increases in DA activity in prodromal schizophrenic populations, suggesting a key DA system disturbance observed in the brain at the onset of schizophrenia can be induced by asphyxia at birth. How DA supersensitivity arises from birth asphyxia is unknown, but it is developmentally regulated, meaning that DA sensitivity may be preventable with early identification and intervention. However, there is no way to identify an individual that will develop DA supersensitivity prior to symptom onset.
The aim of this experiment was to identify changes in the neurochemistry of forebrain dopamine targets using *in vivo* magnetic resonance spectroscopy at ages prior to the emergence of DA supersensitivity. We observed developmental changes in the dorsal striatum (Str) and medial prefrontal cortex. In Str, the ratio of glutamine (Gln) to glutamate (Glu) measure on postnatal day (P) 7 was significantly increased in birth asphyxiated rats (APX) compared to non-asphyxiated (NON) littermates ($p=.030$), normalizing thereafter. On P35, APX show reduced Glu+Gln in the Str ($p=.018$) and increased Glu in the mPFC, relative to NON ($p=.028$). Str GABA was increased in APX at P7 ($p=.023$) and then decreased at P60 ($p=.007$) relative to NON. Further experiments are identifying *in vitro* dopamine metabolite and receptor levels in scanned brains.

Disclosures: J.J. Morgan: None. **C.D. Tulbert:** None. **J. Olson:** None. **G.A. Kleven:** None. **A.E. Ronca:** None.

Poster

861. Animal Models and Mechanisms III

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Program#/Poster#:861.14/K7

Topic: C.06. Developmental Disorders

Support: NSERC

Title: The effects of "housing-stress" manipulations and sex differences on the wheel-induced feeding suppression in adult rats

Authors: *A. MASTROIANNI, R. EIKELBOOM
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Abstract: Excessive exercise has been implicated in the etiology of Anorexia Nervosa (AN) as they are highly co-morbid (Davis, 1997). The wheel-induced feeding suppression (WIFS) rodent model suggests a paradoxical relationship between exercise and feeding, which may parallel with aspects of AN. The WIFS model has found that adult male rats introduced to a running wheel will reduce feeding for 8-12 days compared to non-wheel controls. Wheel rats experience a drop in body weight, which is maintained as long as there is wheel access (Afonso & Eikelboom, 2003). The WIFS occurs despite increasing energy expenditure through high levels of running. In addition to exercise, stress has been shown to reduce feeding (Vallès et al., 2000). The stress levels of rats may be mitigated through pair or group housing, as avoiding isolation better mimics their natural colonies (Sharp et al., 2002). A pilot study has found that the WIFS was 33% shorter in pair housed females and 50% shorter in pair housed males, compared to earlier studies using individually housed male rats. This suggested that pair housing may have offered protection against the WIFS. METHODS: Since AN is more prevalent in females, female adult rats were housed individually (n=32) or in pairs (n=32; 16 pairs). Within each condition, half of the rats were given access for a running wheel for 32 days, while the remaining animals were housed in cages with locked wheels. Wheel revolutions, body weight and food intake were measured daily. It was predicted that individual housing, presumably more stressful, would result in a larger and longer lasting WIFS compared to pair housed wheel rats. RESULTS: Pair housed rats showed fewer wheel turns than individual rats; anecdotally, the female pair rats ran in the wheel together, potentially a gender-specific trait. Paired and individually housed wheel rats dropped in weight compared to non-wheel controls. A brief, yet pronounced, WIFS occurred in all (paired and individually housed) female wheel rats; much shorter in duration than typically seen in males. The feeding results suggest that pair housing failed to significantly alleviate the WIFS in female wheel animals. A male replication will allow for exploration of gender differences in WIFS and housing-stress conditions.

Disclosures: A. Mastroianni: None. **R. Eikelboom:** None.

Poster

861. Animal Models and Mechanisms III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:861.15/K8

Topic: C.06. Developmental Disorders

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Promep103.5/07/2753 (RT)

Title: Social play behavior in rats prenatally treated with valproic acid

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Abstract: Valproic acid (VPA) is an anticonvulsant drug used mainly to treat epilepsy and bipolar disorder. Previous studies demonstrated the teratogenic effect of VPA shown in some cases as an increased risk of autism spectrum disorder (ASD) in children of pregnant women who received VPA. This situation is used as a model for autism studies in rats. The model reproduces the main features of ASD such as anomalies in communication and altered social interaction. Play behavior is an important precursor of social behaviors in humans and other mammals. The study of play behavior allows determining whether there is any change in their performance and development. Thus, we measured play behavior in Wistar rats. We used several age groups: P22, P24, P26, P28, and P30. Both, a Sham (n = 8) and a treatment group (n = 8) were organized. For the treatment group a single intraperitoneal injection of 600mg/kg was applied to the pregnant female on E12.The behavioral test was performed every other day for 5 sessions.
To analyze play behavior we isolated pups for 24 hours before the test in a single box. Then we placed a pair of rats in an acrylic box of 20x40x60cm. The parameters analyzed were: latency to first contact, pinning frequency, frequency of sniffing, tail bit rate, frequency of complete defensive rotation and partial defensive rotation. One way repeated measure ANOVA was used for statistical analysis. There were no statistical differences between treatment and control groups in the parameters of: tail bit rate, pinning frequency, frequency of sniffing, frequency of

partial defensive rotation and complete defensive rotation. However there is an increase in the latency to first contact in the treatment group that may indicate less intention of social contact in comparison with the control group.

Disclosures: P. Saft: None. **B. Brug:** None. **M. Pérez-Pouchoulén:** None. **C. Morgado-Valle:** None. **L.I. García:** None. **M.E. Hernández:** None. **R. Toledo-Cárdenas:** None. **J. Manzo:** None.

Poster

861. Animal Models and Mechanisms III

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Topic: C.06. Developmental Disorders

Support: PHS R01 MH069826

Title: Maternal ethanol consumption transiently increases Cajal-Retzius cells during corticogenesis

Authors: *A. G. SKORPUT, H. H. YEH
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Abstract: Cajal-Retzius (CR) cells are among the earliest generated neurons in the embryonic cerebral cortex. They synthesize and secrete a number of factors that regulate radial and tangential migration of immature cortical neurons. Cajal-Retzius cells are depolarized by GABA, which has also been shown to influence neuronal migration. Abnormal migration of cortical neurons leads to cortical malformations and cognitive deficits, including Fetal Alcohol Spectrum Disorder (FASD). Here we hypothesize that ethanol exerts its effects on cortical migration in part by altering CR cell disposition. We report that, in the neonatal offspring, moderate maternal ethanol consumption increases the number of CR cells and diminishes their responsiveness to GABA.
Time pregnant Ebf2-GFP dams (GENSAT) were fed a liquid diet with or without 2% ethanol beginning on E9.5-E10.5, corresponding to the start of CR cell birth. The maternal blood-alcohol level (BAL) averaged 24$\pm$ 6 mg/dL, and this was comparable to the BAL in the embryos through birth (P0). Beyond P0, BAL became undetectable in the offspring, despite continued maternal ethanol consumption. GFP^+ CR cells in the marginal zone of the prefrontal cortex (PFC) and somatosensory cortex (SSC) were counted in 30μm fixed-frozen coronal sections. In both cortical regions, ethanol-exposed neonates (P0, P2) exhibited ~33% more CR cells relative to controls. This increase in CR cell number was transient, insofar as it was no longer apparent by

P4. Interestingly, at P6, counts of CR cells revealed a decrease in the PFC, but not in the SSC, of the ethanol-exposed group compared to controls, suggesting a cortical region-selective effect. Patch clamp analysis of CR cells revealed a reduced apparent potency of GABA in ethanol-exposed vs. control pups at P3 that was absent at P8.
Our results to date indicated that gestational exposure to ethanol increases the number of CR cells and alters their pharmacological properties in the neonatal cortex. These alterations in CR cell fate/function may be an important early contributor to the neurodevelopmental abnormalities in FASD. Ongoing studies are extending our initial observations to examine aspects of migration and circuit formation associated with CR cells in the developing cortex.

Disclosures: A.G. Skorput: None. **H.H. Yeh:** None.

Poster

861. Animal Models and Mechanisms III

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Support: PEDECIBA-Biología, Universidad de la República

Agencia Nacional de Investigación e Innovación

Title: Possible contribution of astrocyte dysfunction to myelination failure in Glutaric Acidemia Type I

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Abstract: Glutaric Acidemia Type I (GA-I) is a devastating neurometabolic disease caused by accumulation of high concentrations of glutaric acid (GA) derivatives in tissue and body fluids. White matter abnormalities together with striatal and cortical lesions are common pathologic findings in GA-I. The pathomechanisms involved are mostly unknown, including those leading to myelin disturbances. Our group has previously showed that, instead of neurons, astrocytes are the main cellular target of GA in a rat model of GA-I. We hypothesized that astrocyte dysfunction triggered by GA could participate in the white matter injury reported in GA-I. We first analyzed the effect of GA on cultured preoligodendrocytes (preOL) and differentiated oligodendrocytes (OL) obtained from postnatal day 1 rat cortices. 5 mM of GA, a concentration that enhanced astrocytes proliferation and oxidative stress in vitro, did not affect preOL survival,

functional status or proliferation. At that concentration, GA neither affect OL number or maturation, suggesting that OL lineage cells are not directly vulnerable to GA. However, conditioned media from astrocytes previously treated with GA, affected OL survival together with significant inhibition of process growth in surviving cells, 24h after treatment. Further, we studied OL lineage population and myelin status at 1, 12, 21 and 45 days after a single intracerebroventricular injection of GA in neonatal rats. The dose of GA employed mimicked GA-I encephalopathic crisis and survival rates. At 1 day post injection (DPI), animals that received GA did not present signs of striatal OL death, but a delayed decrease of striatal myelinated areas at 12, 21 and 45 DPI. In addition, OLs from GA-treated pups showed delayed cytotoxic signs such as swelling of ER and Golgi apparatus, decreased levels of myelin associated proteins and anomalous axonal myelin wrapping that persisted days after GA administration. Thus, our data allow to discard a cytotoxic direct effect of GA on preOL/OL cells, even at concentrations found during encephalopathic crisis. Instead, it suggests that astrocytes activated by GA may participate in GA-I myelination failure.

Disclosures: E.E. Isasi: None. **L. Barbeito:** None. **S. Olivera-Bravo:** None.

Poster

861. Animal Models and Mechanisms III

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Topic: C.06. Developmental Disorders

Support: NSERC of Canada to RG and BK

CIHR to BK

CIFAR to BK

NIH to DF

Title: Experience and the developing prefrontal cortex

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Abstract: The prefrontal cortex receives input from all other cortical regions and functions to plan and direct motor, cognitive, affective, and social behavior across time. It has a prolonged development, which allows the acquisition of complex cognitive abilities through experience, but makes it susceptible to factors that can lead to abnormal functioning often manifested in neuropsychiatric disorders. When the developing prefrontal cortex is exposed to different environmental events the prefrontal cortex is set on different trajectories that predispose the entire cerebrum to changes later in life. Here we review and contrast the effects of pre-conceptual, prenatal, infant, juvenile and adult experiences on development of dendritic morphology (dendritic length and spine density) in the medial and orbital prefrontal regions (mPFC, OFC). Experiences include tactile stimulation, complex housing, various classes of psychoactive drugs, parental-infant interactions, stress, and play. All of these factors alter prefrontal pyramidal cell morphology, regardless of age at experience, although the changes are age and experience-dependent, and in some cases sexually-dimorphic. Some examples will illustrate. Prenatal stress (E12-16) increases spine density in mPFC and OFC when measured at weaning but decreases spine density when measured in adulthood. Olanzapine (an atypical antipsychotic) decreases spine density in mPFC and OFC when administered to infants but increases spine density when administered to juveniles. When play is manipulated in juveniles, the spine density is reduced in both mPFC and OFC in animals with extensive play relative to no play whereas dendritic length is decreased in mPFC and increased in OFC.
Not only do these early experiences alter neuronal morphology directly but they also change the way later experiences influence prefrontal morphology. For example, pre- or postnatal tactile stimulation as well as pre- and postnatal complex housing dramatically attenuate the effects of exposure to amphetamine in adulthood. Conversely, juvenile exposure to methylphenidate or adult exposure to amphetamine block the later effects of complex housing.
In sum, the prefrontal cortex can be changed dramatically by events beginning prenatally and continuing throughout the life span. These changes have effects on metaplasticity later in life. The plasticity and prolonged development of the prefrontal cortex provides an opportunity for continuing modification of the level of cognitive function but creates a potential susceptibility to the formation of abnormal circuitry leading to compromised behavioral functions.

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Poster

861. Animal Models and Mechanisms III

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Program#/Poster#:861.19/K12

Topic: C.06. Developmental Disorders

Title: Long term effects of intermittent versus continuous methylphenidate administration in adolescent wistar kyoto rats

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Abstract: Attention-deficit/Hyperactivity Disorder (ADHD) is the most commonly diagnosed childhood disorder. Methylphenidate (MPH) remains the most common pharmacological treatment for ADHD. Epidemiological studies indicate a relationship between ADHD and drug abuse. The current studies assessed the impact of chronic adolescent delivery of either high (3.2) or low (1.6) doses of MPH delivered either via intermittent, twice daily injections or continuously via osmotic minipumps. Wistar-Kyoto (WKY) represent a control strain for spontaneously hypertensive rats, the most commonly used rat model of ADHD, and have been suggested to model certain features of depression and anxiety. WKY were characterized by severe hypoactivity, severely decreased acquisition of the T-maze task, and robust behavioral anxiety. Intermittent MPH increased locomotor activity, but had no effect on T-maze performance or behavioral anxiety.
By contrast, continuous MPH treatment appears to enhance acquisition of the T-maze task and powerfully reduced behavioral anxiety. Continuing studies aim to relate these behavioral changes to alterations in dopamine transporter expression in prefrontal cortex and nucleus accumbens.

Disclosures: G. Miller: None. **E.C. Peck:** None. **C. Cosme:** None. **J.D. Rice:** None. **T. Koeltzow:** None.

Poster

861. Animal Models and Mechanisms III

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PMD 03/10

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Faculty of Medicine fellowship

Title: Adenosine uptake by equilibrative nucleoside transporters is decreased in astrocytes from mice fetus exposed to gestational diabetes

Authors: *P. A. ARROYO ZÚÑIGA[1], C. SALOMON[1], E. GUZMAN-GUTIERREZ[1], F. WESTERMEIER[1], M. RICCA[3], P. CONGET[3], A. LEIVA[1], F. PARDO[1], L. SOBREVIA[2]
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Abstract: Gestational diabetes (GD) is a syndrome associated with high risk of developing cognitive alterations in the fetus. Physiological extracellular level of the endogenous nucleoside adenosine is determinant in maintaining cognitive functions and brain vascular reactivity. Adenosine transport via equilibrative nucleoside transporters (ENTs) in astrocytes is one of the mechanisms maintaining physiological extracellular levels of this nucleoside in the brain. However, adenosine plasma levels are increased in human fetus from GD, a phenomenon associated with low ENT1 isoform expression and transport activity. We here assayed whether adenosine transport and mouse ENT1 (mENT1) and mENT2 expression were altered by GD in astrocytes from fetal brains. **Methods**. Pregnant mice with normal (*Lepr*+/+) or reduced (*Lepr*^{db}/+) expression of leptin receptor were subjected to a glucose tolerance test (GTT). Animals were then separated into non-GD (*Lepr*+/+, 11 ± 0.1 µM/min area under the GTT curve) or GD (*Lepr*^{db}/+, 18 ± 0.15 µM/min area under the GTT curve) study groups. Fetal astrocytes were cultured under standard conditions (DMEM, 10% fetal bovine serum, 5% CO_2) and used for adenosine transport (0-500 µM adenosine, 2 µCi/ml [^{3}H]adenosine, 20 sec, 37ºC). mENT1 and mENT2 protein abundance was assayed in total protein extracts (80 µg) by Western blots. **Results**. Total adenosine uptake (mENT1+mENT2) was reduced (40%) in GD compared with non-GD cells. GD reduced the V_{max} (0.09 ± 0.04 and 0.04 ± 0.006 pmol/µg protein/sec, mENT1 and mENT2, respectively) without altering the apparent K_m (24 ± 11 and 67 ± 15 µM for mENT1 and mENT2, respectively) compared with non-GD cells (V_{max} 2.01 ± 0.04 and 1.1 ± 0.02 pmol/µg protein/sec, K_m 30 ± 11 and 60 ± 15 µM for mENT1 and mENT2, respectively). The mENT1 and mENT2 protein abundance was lower (61 and 35%, respectively) in GD compared with non-GD groups. **Conclusion.** GD associates with reduced mENT1 and mENT2-mediated adenosine transport likely due to reduced mENT1 and mENT2 protein abundance.

Disclosures: P.A. Arroyo Zúñiga: None. **C. Salomon:** None. **E. Guzman-Gutierrez:** None. **F. Westermeier:** None. **M. Ricca:** None. **P. Conget:** None. **A. Leiva:** None. **F. Pardo:** None. **L. Sobrevia:** None.

Poster

861. Animal Models and Mechanisms III

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Program#/Poster#:861.21/K14

Topic: C.06. Developmental Disorders

Title: Alterations in social behavior and mTOR signaling in the valproic acid-induced model of autism spectrum disorder

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Abstract: Valproic acid (VPA) has traditionally been used as an anticonvulsant in the treatment of epilepsy and migraine. Recent epidemiological evidence, however, suggests that maternal administration of VPA during pregnancy increases the likelihood of autism spectrum disorder (ASD) in the offspring. ASD is a highly prevalent, pervasive developmental disorder characterized by abnormal social interactions, communication deficits and ritualistic, repetitive behaviors. While the underlying basis of ASD remains obscure, speculation has increased around the mammalian target of rapamycin (mTOR) signaling pathway. Mutations affecting mTOR signaling result in disorders such as tuberous sclerosis complex (TSC). Since autistic behaviors are seen in many TSC patients, it follows then that disruptions in the mTOR pathway might underlie the pathophysiology of certain forms of ASD. Here, we demonstrate that prenatal exposure to VPA results in altered social behaviors described in ASD patients. To generate a model of ASD, 500mg/kg VPA (dissolved in saline; 250ug/mL) was injected IP in pregnant Wistar rats on E12.5 [PMID: 15238991]; controls received only saline. Dams were weaned on postnatal day (P)21 and the litters were sacrificed at P35. Non-play (allogrooming and sniffing) and play (complete, partial, and horizontal rotation) behaviors were quantified as measures of social interaction in rats prenatally exposed to VPA or saline. In the VPA-treatment group (n=9), rats showed significantly decreased non-play and play behaviors compared to their saline-injected counterparts (n=12) during 10-minute observation periods (24% and 100% decrease, respectively; $P<0.05$ one-way ANOVA). During this period, VPA-induced ASD rats also spent less time allogrooming (80% decrease; $P<0.05$ one-way ANOVA). To examine whether the behavioral changes noted by reflect alterations in mTOR signaling, we measured biomarkers using Western blots prepared from lateral temporal neocortices, specifically upstream pAkt and downstream pS6 and p4EBP1. In VPA-induced ASD (n=3), pAkt, pS6, and p4EBP1 levels were significantly decreased compared to saline-injected controls (n=4; 35.7%, 33.4% and 52.8% decreases, respectively; $P<0.05$ one-way ANOVA). Our data, contrary to expectations based on the existing literature, indicate that prenatal VPA exposure triggers a down-regulation of mTOR signaling. While these findings may be specific to the prenatal VPA model of ASD, our results

provide additional evidence that alterations in mTOR signaling may play a role in the pathogenesis of ASD.

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Poster

861. Animal Models and Mechanisms III

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Topic: C.06. Developmental Disorders

Support: DFG, Schw 559/8-3

Title: Ultrasonic communication in rats: Post-weaning but not post-adolescence social isolation leads to social impairments - Phenotypic rescue by re-socialization

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Abstract: Rats are social animals in which rough-and-tumble play during adolescence has an important role for social development. Separation from conspecifics during this phase is known to impair social behavior. Post-weaning social isolation in rats is a widely used animal model to induce behavioral phenotypes and changes in neural development relevant to psychiatric disorders like schizophrenia (Fone & Porkess, 2008).
Ultrasonic vocalizations (USVs) are an important component of the rat's social behavioral repertoire. Rats emit distinct types of USVs, which serve as situation-dependent affective signals with important communicative functions. Low-frequency 22-kHz USVs occur in aversive situations such as fear-conditioning and lead to freezing behavior in the recipient, indicating an alarming function. In contrast, high-frequency 50-kHz USVs are produced in appetitive situations such as rough-and-tumble play and induce social approach behavior, supporting the notion that they serve as social contact calls (Wöhr & Schwarting, 2007).
Here, we tested whether social isolation impairs social approach behavior in response to playback of pro-social 50-kHz USVs and whether isolation-induced deficits depend on the time period of isolation during development. Male rats were housed in one of the following conditions: group housing, short-term isolation, i.e. 24 hours, or long-term isolation, i.e. 28 days. Rats were isolated either as weanlings with three weeks of age or as post-adolescent young adults with seven weeks of age, after going through rough-and-tumble play. As recent findings demonstrate that social deficits can be improved by peer intervention (Yang et al., 2011), we also

tested for phenotypic rescue in this paradigm by exposing a subgroup of rats to one additional week of peer-rearing. Subsequently, all rats were tested for social approach behavior in response to 50-kHz USVs. As acoustic control stimuli served 22-kHz USVs and background noise. While group-housed and short-term isolated rats displayed approach behavior in response to pro-social 50-kHz USVs, post-weaning long-term social isolation lead to a pronounced deficit, since rats displayed avoidance rather than approach behavior when exposed to 50-kHz USVs. Such deficits were not observed after post-adolescence long-term social isolation, indicating a critical period for social development during puberty. Importantly, approach deficits to 50-kHz USVs induced by post-weaning long-term isolation were reversed by peer-mediated re-socialization. This phenotypic rescue highlights the importance of social experience for affiliative behavior.

Disclosures: D. Seffer: None. **H. Rippberger:** None. **R.K.W. Schwarting:** None. **M. Wöhr:** None.

Poster

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Topic: C.06. Developmental Disorders

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Title: Effects of early life stress on ionic currents in the CNS neurons and neuro-behaviors and its attenuation by natural sounds

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Abstract: Early life stress (ELS) has been implicated in psychiatric diseases, such as depression and schizophrenia, in adulthood. In this study, we investigated the influence of ELS on neurotransmitter responsiveness of single brain neurons and on various neuro-behaviors in Wistar rats. ELS was applied by maternal deprivation (MD) and white noise (WN). [Methods] Neonatal Wister rats were isolated from their mother and siblings, or exposed to WN at 85 dB for 4 hours a day during postnatal day (PND) 2 to 9. During WN exposure, pops were separated from their mother but reared with siblings. GIRK2 mRNA level in the hippocampus of pops was measured at PND10 and ionic currents in acutely dissociated neurons were measured during PND10 to 15 using perforated whole-cell patch-clamp technique. Behavioral tests were done at 1 month after birth. All experiments were conducted in strict accordance with the Guidelines of the Japanese Pharmacological Society and Kumamoto University for the Care and Use of Laboratory

Animals. [Results] MD augmented 5-HT-incused G-protein coupled inwardly rectifying K^+ (GIRK) channel currents in ventral hippocampal CA1pyramidal neurons (1.1±0.1 → 3.6±0.6 pA/pF) and decreased both dopamine-induced GIRK channel currents (2.1 ±0.6 → 0.9±0.4 pA/pF) and glutamate-induced cationic currents (16.3 ± 2.2 → 10.4 ± 1.3 pA/pF) in dopaminergic ventral tegmental area neurons. GIRK2 mRNA level in the hippocampus was reduced but that in ventral CA1 area was not affected by MD stress. Behaviors in the open-field, plus-maze and hole-board tests were not affected by MD stress. Interestingly, when pops were exposed to the music of little birds and the sound of a stream together with MD stress, the augmentation of 5-HT-induced GIRK channel currents was relieved (1.5±0.4 pA/pF). WN also augmented 5-HT-induced currents in ventral CA1 pyramidal neurons (2.0±0.4 pA/pF) but the influence was weaker than that of MD stress. In the same group of neurons, GABA-induced Cl^- currents were decreased from 54.8±10.8 to 36.2± 3.7 pA/pF and the ratio of neurons responding to baclofen was largely increased from 44% to 76% by WN stress. Noradrenaline-induced GIRK channel currents in locus ceruleus neurons were not affected by WN stress. The numbers of head dips and crossing from the wall-side area to the center area and the time spent in the center area in 5 min were increased from 8.1± 2.4 to 10.9±4.2 times, 3.7±2.6 to 6.5±1.6 times, and 2.8±1.6 to 6.4±3.6 %, respectively in hole-board test. These suggest that 1) ELS may alter the responsiveness of developing brain neurons to neurotransmitter, 2) effects of ELS may depend on the kind of stress and 3) soothing natural sound may attenuate the effect of ELS.

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Poster

862. Ischemia: Perinatal

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Topic: C.08. Ischemia

Support: NIH Grant A09032

Title: The effects of P7 HI injury on visual attention in a rodent model, and therapeutic benefits of caffeine treatment

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Abstract: Hypoxic Ischemic (HI) brain injury is common among children born prematurely (< 35 weeks) or at very low birth weight (VLBW; < 1500 grams). In these populations,

injuries can follow reperfusion failure, intracranial bleeding, increased ventricular pressure, or drops in blood pressure/flow. HI injuries can also occur in term-born infants with birth complications where blood and/or oxygen are reduced. Long-term cognitive/behavioral impairments following HI injury are evident in both in preterm and term-injured populations, and include cerebral palsy, epilepsy, and cognitive delays (e.g., deficits in attention, language, and/or learning). Children diagnosed with PVL (a non-hemorrhagic HI injury) also show impairments on visual memory, recognition, and spatial tasks. Finally, children born prematurely show deficits in visual attention (as measured behaviorally and by evoked potential) related to alterations in dorsal stream activity. With regard to protective treatments, caffeine is a non-selective adenosine antagonist that has been used to promote respiratory drive in premature infants. Previous studies have demonstrated neuroprotective effects of caffeine in these babies, and also in rodent models of early HI. The current study employed a postnatal day (P)7 HI rodent model to assess the therapeutic potential of caffeine treatment in reducing subsequent impairments on a visual attention task. Wistar rats received HI surgery on P7 (right common carotid artery cauterized with 2 hrs 8% oxygen) while shams received a skin incision and open air for 2 hrs. HI and sham animals were injected with either 10 mg/kg of caffeine or saline after surgery. In adulthood, animals underwent visual attention (5-choice serial reaction time; CCRT) testing for a period of two months. Data show that P7 HI injured rodents exhibit significant deficits in visual attention, and also reveal a therapeutic benefit of caffeine treatment in HI injured animals as measured by attention indices.

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Poster

862. Ischemia: Perinatal

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Topic: C.08. Ischemia

Support: P20 RR16457-11

Title: Improved longitudinal learning performance following treatment with inter-alpha inhibitor proteins in neonatal hypoxic-ischemic rats

Authors: *S. W. THRELKELD[1], E. DUGAS[1], C. A. HILL[3,4], C. GAUDET[2], L. MABARDI[2], Y.-P. LIM[5], B. S. STONESTREET[3,6]
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Abstract: Neonatal hypoxia-ischemia (HI), commonly associated with preterm birth and very-low birth weight, is accompanied by a prolonged neuro-inflammatory response. This response has been linked to injury progression and severity, which may mediate long-term neurobehavioral outcome. Inter-alpha-Inhibitor proteins (IAIPs) have been shown to significantly reduce inflammation in a model of neonatal sepsis and brain infarct size in an adult rodent stroke model. The current study sought to investigate the effects of IAIPs on long-term neurobehavioral outcome in a rat model of neonatal hypoxia-ischemia. At birth, animals were randomly assigned into one of three groups (Sham n= 12, HI n=13, HI+IAIPs n=9). On postnatal day (P) seven, 30mg/kg IAIPs or saline was administered prior to hypoxia and again 24 hours after HI. Spatial and non-spatial learning was assessed during juvenile (P38-49) and adult periods (P84-95) using the Morris water maze (MWM) and a non-spatial water maze. Juvenile results, for the MWM task revealed a significant effect of injury, with HI and HI+IAIP subjects performing worse than shams. However, results for the juvenile non-spatial task showed that HI animals took longer to locate the hidden platform as compared to sham and HI+IAIP subjects. In adulthood, HI+IAIP treated subjects no longer showed a deficit relative to shams on the spatial water maze task, while HI subjects continued to show impairments as compared to shams. Finally, while no deficit was seen in the HI+IAIP group as compared to shams on the adult non-spatial task, HI animals showed persistent learning impairments. The current results reveal that treatment with IAIPs after neonatal HI improves multimodal learning performance across juvenile and adult maturational periods. These data suggest that IAIP may serve as a novel anti-informatory treatment to reduce neonatal brain injury and improve long-term behavioral outcome.

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Poster

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Topic: C.08. Ischemia

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Title: Effects of neonatal hypoxic ischemic brain injury on rapid auditory processing and spatial working memory

Authors: *A. L. SMITH, M. L. ALEXANDER, J. J. CHROBAK, R. H. FITCH
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Abstract: Conditions of reduced oxygen and/or blood flow in the developing brain (hypoxia/ischemia or HI) are associated with tissue damage, as well as cognitive and/or motor deficits of varying degree. In the premature infant, HI may occur through intra-cranial bleeding or deficiencies in circulatory regulation, while in the term infant HI often results from prolonged labor, placental dysfunction, or other birth events. Damage to the hippocampus, cortex, corpus callosum as well as enlargement of the ventricles are frequently seen, although patterns of damage vary depending on the timing of initial injury. These injuries can be modeled in rats, with damage experimentally induced in the postnatal day 7 pup (P7) thought to roughly equate to that seen following term HI in infants. Research has shown that male rats with HI on P7 display deficits in rapid auditory processing, as well as spatial learning.
The current study sought to replicate previous findings that male rats with an induced HI injury show deficits on a rapid auditory processing (RAP) task. Based on previous data, we hypothesized that male P7 HI rats would be significantly impaired when compared to shams. Following auditory testing (gap detection), a subset of subjects (Group A) were tested on a delayed match-to-position radial water maze paradigm. This task allowed a more detailed characterization of the memory impairments associated with postnatal HI injury. Group B subjects received additional (more complex) auditory testing. Using two different behavioral tasks on the same group of animals, this further allowed us to assess whether impairments in RAP were associated with, or independent from, impairments in spatial working memory. Findings replicated RAP deficits in HI males, with HI subjects significantly impaired on gap detection (indicating deficits in the ability to detect rapidly changing cues). Subjects in Group B also evidenced HI deficits on more complex acoustic tasks. Within Group A, HI males performed significantly worse than sham animals in regards to working memory errors made. However, early RAP performance was not predictive of working memory deficits. These results suggest that HI effects are evident on “independent” neural systems, and degree/patterns of damage may differ within subjects.

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Poster

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Title: Adenosine 2A receptors contribute to striatal injury after neonatal hypoxia-ischemia

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Abstract: Striatal medium spiny neurons are highly vulnerable to hypoxia-ischemia (H-I) in term newborns. Approximately half of these striatal neurons express adenosine 2A (A2A) receptors. Because A2A receptors are coupled to adenylate cyclase and PKA activation, we tested the hypothesis that an A2A receptor antagonist would attenuate phosphorylation selectively at PKA-sensitive sites on dopamine- and cAMP-regulated phosphoprotein-32 (DARPP32), NMDA receptor NR1, and Na,K-ATPase and provide protection of neurons in putamen after H-I. Anesthetized newborn piglets were subjected to 40 min of ventilation with 10% O_2 and 7 min of airway occlusion. At 5 min after reoxygenation, piglets received vehicle or 1 of 3 doses of the A2A receptor antagonist SCH58261 (0.01, 0.1, or 1 mg/kg, iv) followed by a continuous infusion for 6 h (0.0033, 0.033, or 0.33 mg/kg/h, iv, respectively). At 4 days of recovery, the density of viable neurons in putamen in the H-I group treated with vehicle was 19±9% (±SD; n=7) of the sham value (n=8). Neuronal density was significantly increased to 33±8%, 43±11%, and 40±9% in the SCH58261 groups treated with the low (n=7), medium (n=8), and high doses (n=7), respectively. Treatment with medium dose of SCH58261 attenuated the H-I-induced phosphorylation of the PKA-sensitive sites Thr34 DARPP-32, Ser897 NR1, and Ser943 Na,K-ATPase, but had no effect on phosphorylation of the PKC-sensitive sites Ser896 NR1 or Ser23 Na,K-ATPase at 3 h of recovery. The drug also blunted H-I-induced increases in the oxidative stress markers, nitrotyrosine and carbonyls. Because A2A receptors can also contribute to cerebral vasodilation, cortical perfusion was monitored with laser-Doppler flowmentry. However, post-treatment with SCH58261 did not alter the recovery of laser-Doppler flow, which returned to baseline levels within 1 h. We conclude that A2A receptors contribute to loss of a subpopulation of neurons in putamen after reoxygenation from H-I. The mechanism of toxicity may be related to DARPP-32-dependent phosphorylation of PKA-sensitive sites on NR1 and on Na,K-ATPase, thereby augmenting excitotoxic-induced oxidative stress after reoxygenation.

Disclosures: R.C. Koehler: None. **B. Wang:** None. **L.J. Martin:** None. **Z. Yang:** None.

Poster

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NIH NS 074559

Title: A critical role of vascular endothelial growth factor (VEGF) in germinal matrix-intraventricular hemorrhage

Authors: *C.-Y. KUAN, D. YANG, J. BAUMANN, Y.-Y. SUN, H.-C. LIANG, S. POTTER
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Abstract: Germinal matrix-intraventricular hemorrhage (GMH-IVH), the most important neuorological complication of preterm birth, affects approximately 12,000 infants each year in the USA and causes either neonatal death or permanent diabilities depending on the severity of hemorrhage. While past research has elucidated GMH-IVH as a result of both vascular fragility and disturbance of cererbal blood flow, the signaling mechanism of tenuous vascular integrity in permature brains is unclear. Here we test the hypothesis that hyper-stimulation of vascular endothelial growth factor (VEGF) signaling in the unique envionment of germinal matrix may activate neuovascular proteases and trigger IVH.

To test this hypothesis, we used a tetracycline-regulated transgenic system to express murine VEGF in the telencephalic germinal matrix by crossing either GFAP-tTA (Tet-Off) or Nestin-rtTA (Tet-On) drivers to the Tet-respondent TetO-VEGF-A164 mice. This transgenic strategy led to over-growth of endothelium incompletely covered by pericytes, similar to the immature vascular rete detected in human fetal brains. Gene-profiling and biochemical analysis indicated that early effector of VEGF include transcriptional factors ETS1 and HIF2α, the VEGF/PDGFβ/TGFβ signaling pathways, MMP-9, and cathepsin. By E18, bitransgenic embryos developed ventricular dilation and IVH from avascular periventricular areas resulted from Caspase-3 activation and regression of blood vessels. In contrast, antenatal treatment with steroid (betamethasone) significantly decreased the incidence of hemorhage, similar to its effects in human perterm infants.

Together, these results not only introduce a transgenic mouse model that recapitulates the full spectrum of disease presentation from GMH to IVH to ventriculomegaly, but also suggests a pathogenic cascade from VEGF to neurovascular proteases that interacts with important risk factors, such as perinatal hypoxia (via HIF2a) and infection (through MMP activation). This new experimental model offers opprotunities to develop biomarkers and therapeutic strategies in perinatal GMH-IVH.

Disclosures: C. Kuan: None. **D. Yang:** None. **J. Baumann:** None. **Y. Sun:** None. **H. Liang:** None. **S. Potter:** None.

Poster

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NIH NS074559

Title: FTY720 mitigates infection-sensitized hypoxic-ischemic brain injury in newborns by blocking T-cell recruitment

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Abstract: Intrauterine infection (chorioamnionitis) and the ensuing fetal inflammation response sensitizes brain vulnerability to secondary hypoxic-ischemic (HI) insults in newborns. While a critical role of microglia in infection-sensitized neonatal HI brain injury is well established, the functions of blood-borne immune cells, especially T lymphocytes and Th17 cells, are yet to be determined. It is important to understand the roles of T-cells in neonatal HI brain injury for at least two reasons. First, preterm infants with elevation of CD45RO+ active T-cells in the cord blood tend to develop white-matter injury (Lancet 358: 1699, 2001). Second, FTY720, a FDA-approved drug for treating multiple sclerosis, inhibits the sphingosine 1-phosphate (S1P) receptor signaling to block T-cell infiltration into the CNS. Because FTY720-treated subjects maintain adequate resistance to bacteria infection, this oral medication may attenuate neuroinflammation without increasing the risk of severe sepsis, if T-cells contribute to infection-sensitized HI injury in neonates significantly.
To test the roles of T cells in neonatal infection/HI, we first determined that IL-23 receptor (a Th17 cell marker) was elevated and S1PR1 was reduced in the blood mononuclear cells of both preterm infants with chorioamnionitis and 7-day-old rat pups at 4 hour following LPS/HI injury (0.3 mg/kg LPS, IP injected 4 h before the start of the Rice-Vannucci model of unilateral cerebral HI). This result suggests the possibility of infection/HI-induced S1P signaling activation and mobilization of Th17 cells. Next, we conducted comparative study between administering

FTY720 (1.5 μg/g, IP) or saline at 1h after LPS/HI for mechanistic and efficacy investigation. These experiments showed that FTY720 markedly inhibited NF-kB signaling and microglia activation after LPS/HI injury and significantly reduced the resultant brain tissue loss and white-matter damage. Moreover, FTY720 blocks T-cell, including Th17 cell, infiltration into the brain with evidences ranging from immunostaining, quantitative RT-PCR analysis of Th17 cell markers, and fluorescence-activated cell sorting (FACS). Surprisingly, we found that FTY720 has no direct inhibitory on microglia in both in-vivo and in-vitro experiments.
Together, these results have two implications. First, the blood-borne T-cells, or Th17 cells alone, have a pivotal role in "priming" microglia in response to infection-sensitized neonatal HI. Second, using agents like FTY720 to block T-cell entry into the brain may be an effective strategy of brain protection against infection/HI injury of newborns.

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Poster

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Title: Gliotransmitter release from reactive hippocampal astrocytes results from enhanced Na+/H+ exchange following *in vitro* ischemia

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Abstract: Hypoxia ischemia is one of the common causes of brain injury in neonates. After HI, the intracellular pH (pHi) drops resulting in acidosis. Na+/H+ exchanger isoform 1 (NHE1) is abundantly present in the rodent hippocampus and is one of the primary proteins involved in maintenance of pHi under physiological and pathological conditions. We recently reported that inhibition of NHE1 resulted in less hippocampal pyramidal neurodegeneration and improved long-term learning and memory in the mouse model of neonatal HI. NHE1 expression in hippocampal reactive astrocytes was significantly up-regulated after HI. We hypothesize that the intracellular acidosis after HI in hippocampal reactive astrocytes results in sustained activation of

NHE1 and leads to subsequent neuronal damage. In the current study, using an in-vitro ischemia model (oxygen/glucose deprivation and reoxygenation, OGD/REOX), we investigated whether inhibition of NHE1 reduces intracellular Na+/H+ dysregulation and the gliotransmitter release from reactive hippocampal astrocytes. Hippocampal astrocytes cultured from postnatal day 4 mouse brains underwent 2 h exposure to OGD and 1-24 h REOX. The pH-sensitive dye BCECF and Sodium Green were used to determine changes of pHi and intracellular Na+. Glutamate and cytokine release into the media were determined using the amplex® red glutamic acid/glutamate oxidase assay kit and ELISA, respectively. Statistical analysis was performed using either T-test or Mann-Whitney Rank Sum test and ANOVA with Bonferroni post-test, for two and multiple comparisons respectively. 2 h OGD led to a significant increase in NHE1 expression and 1.6-fold elevation in NHE1 activity, which was sustained for 1-5 h REOX. Inhibition of NHE1 activity with its potent inhibitor HOE642 reduced sustained H+ efflux and Na+ overload in astrocytes after OGD/REOX. Blocking NHE1 activity also decreased the release of gliotransmitters (glutamate and the pro-inflammatory cytokines such as IL-1, IL-6 and TNFα) from hippocampal astrocytes at 1-5 h REOX. Administration of either HOE642 or the excitatory amino acid transporter inhibitor TBOA attenuated glutamate release. These data suggest that over-stimulation of NHE1 activity disrupt both H+ and Na+ homeostasis in hippocampal astrocytes following in-vitro ischemia, which not only reduces Na+-dependent glutamate uptake but also increases cytokine release. This work was supported by UW Department of Pediatrics R & D Grant (Cengiz P), Clinical and Translational Science Award Program thru NCATS 9U54TR000021 (Cengiz P & Ferrazzano P), NIH grants RO1NS38118 and RO1NS48216 (Sun D), Waisman IDDRC P30HD003352.

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Poster

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Title: Porcine model for designing cell-based regenerative strategies in postnatal brain injury

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Abstract: The subventricular zone (SVZ) maintains the largest source of neural stem/progenitor cells in the postnatal and adult brain. Defining the regenerative potential of SVZ neural stem/progenitor cells is crucially important to design cell-based regenerative strategies in postnatal brain injury. However, recent evidence demonstrates that the cellular and anatomical features of the human SVZ are dissimilar from the well-studied rodent SVZ. In spite of these crucial differences, both technical and ethical difficulties prevent the investigation of cellular/molecular and functional mechanisms in the human SVZ. Here we show that the juvenile porcine SVZ (1-15 weeks of age) shares the same anatomical and cellular structure of the human SVZ. The adult human lateral-SVZ (L-SVZ) comprises four distinct cellular layers. In particular, the hypo-cellular gap and astrocyte ribbon are unique features of the human L-SVZ. The juvenile porcine L-SVZ also comprised four layers, including the $GFAP^+$ astrocyte ribbon. In the second layer of the porcine L-SVZ, we observed the presence of Dcx^+ neuroblasts. It has been recently shown that the presence of neuroblasts in this layer is a specific feature of human neonates/infants. In human neonates/infants, the astrocyte ribbon is absent in the anterior L-SVZ. This was also observed in the porcine SVZ. Furthermore, porcine SVZ $GFAP^+$ processes expressed the immature neuroepithelial marker Vimentin, as demonstrated in human neonates. Another significant difference between human and rodent SVZ is the existence of a neurogenic niche in the outer SVZ (O-SVZ). We identified the O-SVZ in the posterior porcine SVZ, based on total cell density, the presence of $Ki67^+$ cells, and of $Sox2^+Pax6^+$ progenitor cells. Furthermore, it is well established that the O-SVZ contains outer radial glia (O-RG) cells. $Pax6^+Sox2^+Vimentin^+$ O-RG was also found in the porcine O-SVZ. In the O-SVZ, $Vimentin^+GFAP^+$ processes of O-RG cells not only extend radially, but also line the lateral ventricle. This mesh-like distribution of $GFAP^+$ fibers was reported only in the human fetus. This indicates that the porcine model allows us to investigate development of the O-SVZ, an important cell source in the evolution of the gyrencephalic neocortex. Altogether, our studies in the porcine SVZ will provide novel insights on cellular/molecular and developmental mechanisms of the human SVZ under both normal physiological and pathological conditions, and will help in designing novel cell-based regenerative strategies in postnatal brain injury.

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Poster

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Title: Chronic valproic acid and phenobarbital administration impacts neurogenesis after neonatal stroke.

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Abstract: Background: Stroke in the immature brain usually presents with seizures 12-24 hours after the insult which are treated with anticonvulsants. Post-stroke neurogenesis in the SGZ of the immature brain is decreased and may contribute to the long-term cognitive impairments seen. Histone deacetylase (HDAC) inhibitors prevent HDAC from blocking DNA transcription and replication. Valproic acid (VPA) is a HDAC inhibitor, but also enhances GABA neurotransmission and blocks sodium channels. Phenobarbital keeps chloride channels open longer, thereby enhancing GABA. In higher concentrations, phenobarbital can block sodium channels and block glutamate.
Objective: To determine the impact of chronic HDAC inhibition or phenobarbital on post-stroke neurogenesis in the immature brain.
Methods: P12 CD1 mice received right common carotid artery ligation and acute seizures were scored. Mice were assigned to receive either VPA (200mg/kg) or phenobarbital (25mg/kg) or vehicle twice daily from P16 to P28. 5-bromo-2'-deoxyuridine (BrdU; 50mg/kg) was injected five times between P24 and P26 prior to sacrifice on P42. Percent of brain atrophy was quantified and immunohistochemistry was performed for analysis of neurogenesis (Brdu/NeuN colabeling). In a subset of mice, serum was collected at P26 and analyzed for concentrations of VPA or phenobarbital.
Results: Among injured VPA-treated mice, there was a 208% increase in BrdU/NeuN-positive cells in the ipsilateral dentate gyrus (DG) when compared to counts in saline treated mice ($p= 0.024$); there was a 55.4% increase in contralateral DG ($p= 0.023$). In uninjured VPA-treated mice, there was a 49.4 and 61.5 % increase in cell counts in contralateral and ipsilateral DG versus vehicle treated ($p= 0.007$ and 0.004 respectively).
Our prior serum studies showed that half-lives for VPA and phenobarbital in neonatal mice after chronic dosing are much lower than previously reported. Peak drug levels of VPA in serum one

hour after dose were: 251.7 ± 13.3 mcg/ml with a maximum half-life of 1.24 ± 0.26 hours. The average peak phenobarbital level was 20.5 mcg/ml with an average half-life of 3.5 hours. Half-life of phenobarbital was significantly longer in injured vs. unligated mice (4.4 ± 0.4 and 3.2 ± 0.2 hours respectively, p= 0.025).
Conclusion: VPA causes increased neurogenesis; the neurogenesis in injured hippocampi was more marked. This is likely related to HDAC inhibition given that previous studies with Trichostatin A produced similar results. We are currently investigating chronic phenobarbital effects upon post-stroke neurogenesis. Chronic administration of valproate and phenobarbital results in very short half-lives.

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Poster

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Topic: C.08. Ischemia

Support: Grant-in-Aid for Young Scientists (B)23791603

Title: The effect of hypothermia on the laminar disruption and neural injury in neonatal mice following ischemic injury

Authors: *H. KIDA[1], S. NOMURA[2], M. SHINOYAMA[2], M. IDEGUCHI[2], Y. OWADA[3], M. SUZUKI[2]
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Abstract: Hypothermia has been proposed for reducing neuronal damages in the brain induced by hypoxia-ischemia (HI). However, little is known about the effect of hypothermia on immature brains of neonatal mice with HI. In this study, we conducted histological examination in the cortex during 4 first postnatal weeks and neurobehavioral tests.
To induce cerebral ischemia in postnatal 3 days ICR mice, we performed right common carotid artery ligation followed by hypoxia (6%O2, 37°C) for 30 minutes. Immediately after HI, mice were divided into a hypothermia group (32°C) or a normothermia group (37°C) by placed in a chamber in a water bass at the stable temperature for 24h. Mice were sacrificed at P4, P7 and 5 weeks. We focused on 1) the laminar structure in the cortex using Cux1/Ctip2 immunohistochemistry, 2) the change of the number of neurons, and 3) the comparison in

expression for myelin basic protein (MBP) between ischemic and contralateral normal hemisphere.
Histological analysis revealed that the laminar distribution was observed within 7 days after normothermia treatment. At 4 weeks of age, there appeared to be a district loss of volume with the structural changes in the deep layers. The thickness of layers V/VI was 1.9×106 μm2 in the ischemic hemisphere, while that in the normal hemisphere was 1.2×106 μm2. In addition to the focal necrosis in the deep layers, there was significant decrease in the intensity of immuoreactivity for MBP by 27% in the ischemic hemisphere. The density of neurons was not different between both hemispheres (Ipsi, 1070cells/μm2; Contra 1076cells/μm2). Hypothermia immediately after HI improved these developmental disturbances to form the normal laminar structures. Hypothermia also could improve neurological outcomes in the beam balance test and rotarod test at 4 weeks of age.
Because 3-d-old in rodents coincides with gestation between 23-32 weeks of gestational age in very preterm infant human in which infants have a risk for cerebral palsy (CP), these results reveal pathophysiology of CP and may contribute to hypothermal therapies of CP patients.

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Poster

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Topic: C.08. Ischemia

Title: Phosphorylation of NR2B Tyr1472 contributes to brain injury following neonatal hypoxia-ischemia in mice

Authors: *R. KNOX[1], D. YANG[1], A. BACH[1], S. LOWENSTEIN[1], M. LAM[1], T. NAKAZAWA[2], T. YAMAMOTO[2], D. FERRIERO[1], X. JIANG[1]
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Abstract: Neonatal brain hypoxia-ischemia (HI) is an important contributor to morbidity and mortality in infants and children. However, there are few treatments. We have recently demonstrated that Src Family Kinase (SFK) activity and NMDA receptor (NMDAR) tyrosine phosphorylation are increased following neonatal HI in mice. Particularly, phosphorylation of the NR2B subunit at Tyr1472 is upregulated rapidly after HI (peaking at 15min after HI). However, it is unknown whether NR2B tyrosine phosphorylation contributes to HI brain injury.

To determine the functional significance of tyrosine phosphorylation of NR2B, we obtained mice with a knock-in mutation of the Tyr1472 site to phenylalanine (Y1472F). Neonatal HI was performed on postnatal day 7 wildtype (WT) or Y1472F mice and brain injury was examined at postnatal day 12 with cresyl violet and Perl's iron stain. Y1472F mice have decreased brain injury compared to WT mice (median=16.25, n=18 in WT mice and median=11, n=23 in Y1472F mice, p=0.034) with the largest decrease in brain injury in the cortex (p=0.013). There were no gender differences in brain injury. This study provides the first evidence that phosphorylation of the NR2B subunit at Tyr1472 contributes to HI brain injury. Our study suggests that therapies aimed at reducing NMDAR tyrosine phosphorylation could reduce injury in the human neonates with hypoxic-ischemic encephalopathy. Future studies will elucidate the molecular mechanisms by which Y1472 mediates cell death in the developing brain.
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Poster

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Support: 5R01NS060896

Title: Structural and functional cortical development following early cerebral hypoxia ischemia

Authors: *G. OR, S. RANASINGHE, A. IEVINS, P. S. MCQUILLEN
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Abstract: Neonatal cerebral hypoxia-ischemia (HI) disrupts both anatomic and functional cortical plasticity. Although impaired plasticity is accompanied by loss of parvalbumin expression, no evidence is found for loss of functional inhibition. Instead, early HI reduces the mature cortical response to an optimal visual stimulus suggesting disrupted development of excitatory circuits. Many aspects of structural and functional neuronal development are dependent upon neuronal activity. To understand abnormal cortical development following neonatal HI, we are correlating early EEG activity with glutamate receptor expression, dendritic structure and synapse formation following HI at postnatal day 2 (P2).
EEG is recorded from biparietal, epidural electrodes in awake, unrestrained rats from P3 - P21

and processed to measure amplitude-integrated EEG (aEEG), interburst interval (IBI) and to identify specific activity patterns as measures of brain maturation. Expression of synaptosomal glutamate receptor subunits and post-synaptic density proteins is quantified. Cortical pyramidal neuron morphology is visualized using a double adenoviral transduction to produce sparse, brightly labeled fluorescent neurons in all cortical layers. Selected cortical neurons are analyzed for dendritic length, volume and Sholl analysis using Imaris FilamentTracer. Synapse formation is quantified from synapsin immunofluorescence.
In control animals, background activity increases with development. In moderately injured animals following hypoxia ischemia at P2, the aEEG upper voltage margin was significantly lower from P3 to P11. Depressed activity in injured animals result from both decreased spectral power in all frequency bands ($P<0.01$) and a significantly prolonged population distribution of IBI's. Notably, by P14 the background activity in HI animals recovered to values comparable to the control group.
Consistent with these activity changes, there was decreased expression ($P<0.05$) of all the glutamate receptor subunits, PSD95 and CaM kinase at P3, following HI. Expression of glutamate receptors recovered to normal by P7, preceding the recovery of EEG background.
Layer V pyramidal neuron dendrites manifest markedly simplified branching with parvalbumin immunohistochemistry. Analysis of pyramidal neuron morphology and synapse counts is underway.
These observations suggest that neonatal HI impairs maturation of both excitatory and inhibitory circuits with delayed morphologic and functional neuronal development. Understanding early network development following neonatal brain injury will identify specific interventions to optimize recovery.

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Poster

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Title: Expression and function of Toll-like receptor 4 in hypoxic cerebellar microglia

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Abstract: Hypoxic injury damages cells including Purkinje neurons in the neonatal cerebellum. It was surmised that inflammatory mediators derived from microglia might contribute to this process. Toll-like receptors (TLRs) have been reported to induce microglial activation and production of proinflammatory cytokines in brain and spinal cord injuries. However, their role in the developing neural tissues following a hypoxic injury has remained uncertain. We report here TLR4 expression and its putative roles in hypoxic developing cerebellar microglia both *in vivo and in vitro*. Damage to Purkinje neurons and their dendrites was observed following exposure of 1-day old Wistar rats to hypoxia (5% oxygen +95% nitrogen) for 2h. TLR4 expression was detected in cerebellar microglial cells at different time points following hypoxic exposure. TLR4 immunofluorescence expression in microglia was markedly enhanced 3 days after hypoxia. Western blot analysis also showed a significant increase in TLR4 expression in the cerebellum after the hypoxic exposure. *In vitro*, by RT-PCR, western blot and immunofluorescence staining, TLR4 mRNA and protein expression levels were most drastically increased in primary cultures of microglia and BV-2 cells subjected to hypoxia. To further dissect the function of TLR4 in microglial activation and the associated molecular mechanism, we depleted TLR4 by small interfering RNA (siRNA) transfection and antibody neutralization. Knockdown of TLR4 effectively reduced hypoxia-induced upregulated expression of tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and inducible nitric oxide synthase (iNOS) in BV-2 cells. TLR4 knockdown-mediated inhibition of inflammatory cytokines was coupled by inhibition of NF-κB activation. Neutralization of TLR4 with its antibody also attenuated the hypoxia-induced expression of TNFα, IL1β and iNOS in primary cultured cerebellar microglia. We conclude that TLR4 expression in cerebellar microglia may be linked to regulation of inflammatory mediators via an NF-κB signaling pathway; hence, interference withTLR4 in activated microglia may reduce cerebellar damage caused by hypoxia. (Supported by grants R-181-000-140-592 and R-181-000-120-213, National University of Singapore).

Disclosures: L. Yao: None. **J. Lu:** None. **A. Hao:** None. **E. Ling:** None. **C. Kaur:** None.

Poster

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Support: Emmy Noether-Program of the DFG (Ha4466/3-1).

Title: Hippocampal theta bursts drive oscillatory entrainment of the neonatal prefrontal cortex under physiological conditions but not after hypoxic-ischemic injury

Authors: *M. D. BROCKMANN[1], M. KUKOVIC[1], M. SCHÖNFELD[2], J. SEDLACIK[2], I. L. HANGANU-OPATZ[1]
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Abstract: The coactivation of prefrontal and hippocampal networks in oscillatory rhythms is critical for precise information flow in mnemonic and executive tasks. We recently showed that even during early development the two brain regions are tightly coupled and their communication is directed via synaptic projections. Discontinuous theta-band activity in the neonatal hippocampus (HP) drives the entrainment of local prefrontal networks in oscillatory bursts with distinct spatial and temporal organization. It is very likely that this precise directed communication underlies the correct maturation of prefrontal-hippocampal networks and its disease-related impairment correlates with cognitive deficits at adulthood. To test this hypothesis, we assessed the effects of an early hypoxic-ischemic (H-I) injury on the functional prefrontal-hippocampal development. We focused on a mild/moderate peri-neonatal injury, which has been previously identified as cause of poorer cognitive performance in children at school age in the absence of massive morphological changes and gross behavioural deficits. The magnitude of injury in a rodent model of H-I [ligation of the common carotid artery followed by exposure to 5% oxygen at postnatal day (P)2], was assessed by T2 weighted MRI and post-mortem histology (Nissl and GFAP immunostaining).
To decide on the functional consequences of H-I, we performed extracellular multielectrode recordings in vivo in the prefrontal cortex (PFC) and CA1 area of the intermediate HP from neonatal (P6-8) rats with either severe or mild/moderate injury and compared them with sham-operated pups. Pups with mild/moderate H-I injury showed reduced discontinuous oscillatory activity of the neonatal PFC and prominent disruption of gamma-band entrainment of local networks. Additionally, the functional communication between the PFC and HP is impaired as shown by synchronization and causality analysis. The coherence between prefrontal and hippocampal oscillations was significantly lower in rats with mild/moderate H-I injury when compared with sham-operated animals. Moreover, the drive from HP to PFC was suppressed after mild/moderate H-I. These results indicate that even in the absence of major morphological insult, mild/moderate profoundly impairs the functional maturation of prefrontal-hippocampal networks and may account for cognitive deficits at adulthood.

Disclosures: M.D. Brockmann: None. **M. Kukovic:** None. **M. Schönfeld:** None. **J. Sedlacik:** None. **I.L. Hanganu-Opatz:** None.

Poster

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Topic: C.08. Ischemia

Support: The Baby Alex Foundation

Harvard Catalyst KL2 Award

Title: Altered FMRP/mTOR signaling and autism-like behavior in an animal model of EOP

Authors: *M. LECHPAMMER, P. M. KLEIN, M. R. WATTS, A. M. BACH, F. E. JENSEN
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Abstract: Studies of postmortem brain from infants with severe encephalopathy of prematurity (EOP) following perinatal hypoxic / ischemic (HI) injury show altered markers of excitotoxic and oxidative stress in neurons and glia. Premature brain injury is associated with later life neurobehavioral deficits and autism. Here we investigated whether HI may alter the neuronal expression of components of the fragile X mental retardation protein (FMRP) and mammalian target of rapamycin (mTOR) signaling pathways, both implicated in genetic causes of autism. We have previously shown these pathways to be dysregulated in postmortem human brain after premature brain injury. We hypothesized that their dysregulation during critical periods of cortical development may contribute to later life cognitive impairment and autism-like deficits. We used a rat model of selective white matter injury / EOP in P6 Long-Evans rat pups, induced by unilateral carotid artery ligation followed by severe hypoxia (6% O2 for 1 h). Healthy controls were age and sex matched littermates without treatment. Western blot analyses for total and phospho-FMRP(Ser^{499}) and mTOR downstream targets p70S6K and phospho-p70S6K(Thr^{389}) were performed. In the rat model of EOP (n=6) a suppression of FMRP and phospho-FMRP was observed 24 hours after HI brain injury compared to controls (n=5). A significant concomitant overexpression ($p<0.05$ vs. control) of both p70S6K and phospho-p70S6K was recorded in the same animals, mirroring expression pattern of FMRP/mTOR molecular components previously observed by our group in the patients with EOP. In addition, we assessed behavior in the three-chambered sociability test in young adult male Long Evans rats (P31-33; n=14) that were previously exposed to a severe HI brain injury at P6. While the post HI rats had normal interactions in the unfamiliar/novel rat/object behavioral test, when a second novel rat was introduced to them in the subsequent phase of the assay, they continued to spend significantly more time with the first, now-familiar animal, as opposed to the shifted

preference of control rats ($p<0.0001$). Observed resistance to change represents an important autistic-like behavioral outcome in young adult animals exposed to HI brain injury in their infancy. Presented results suggest that HI brain injury may cause its long term sequelae via dysregulation of FMRP/mTOR cascade as otherwise observed in common syndromes of inherited mental retardation (e.g. Fragile-X syndrome and tuberous sclerosis) and also propose this signaling pathway as a possible new molecular target for the treatment of this as yet untreatable cause of later life neurobehavioral deficits.

Disclosures: M. Lechpammer: None. **P.M. Klein:** None. **M.R. Watts:** None. **A.M. Bach:** None. **F.E. Jensen:** None.

Poster

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Topic: C.08. Ischemia

Support: NSC 100-2314-B-006-045-MY2

Title: IRS-1 in dietary restriction neuroprotection against neonatal HI brain injury

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Abstract: Introduction: Perinatal hypoxic-ischemia (HI) is a major cause of neonatal mortality and of subsequent neurological disabilities among infants who survive it. Recently, dietary restriction (DR) is known to protect against neonatal HI brain injury. Since insulin signaling pathway is the main regulator of metabolism, we hypothesis that rats raised on dietary restriction protect against neonatal hypoxic-ischemia brain injury through activating insulin signaling pathway.
Materials and methods: Rats were grouped from postnatal day (P) 1: normally fed (NL) rats (litter size: 12) and DR rats (litter size: 18). On P7, rats were subjected to HI induced by modified Rice-Vannucci model and then their brain tissues are removed for molecular biological assay.
Results: DR rats attenuated apoptosis and reduced brain infarction volume after induction of HI brain injury on P7. The DR rats kept the expression of insulin receptor substance (IRS-1) even after HI injury. In contrary, IRS-1 in NL rats decreased soon after HI injury. In addition, IRS-1 in DR rats after HI activated PI3K/Akt which further protect against HI injury. This in vivo

finding was also tested in vitro. Over-expression of IRS-1 increased cell viability after oxygen-glucose deprivation (OGD) in hippocampus neuron cell lines (H19-7) and knockdown of IRS-1 reduced the cell viability after OGD. The correlated decrease of Akt level after knockdown of IRS-1 were also noted in H19-7.
Conclusions: Our findings indicated DR can protect against neonatal brain HI injury through activation IRS-1/ PI3K/Akt pathway.

Disclosures: Y. Tu: None. **C. Huang:** None.

Poster

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Topic: C.08. Ischemia

Support: BAP 2009-2

Title: Investigation of mechanisms whereby uridine exhibits neuroprotective effects in a rat model of neonatal hypoxic-ischemic encephalopathy

Authors: *T. ALKAN[1], Z. MINBAY[2], M. CETINKAYA[3], B. GOREN[5], E. ORENLILI[4], M. CANSEV[4]
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Abstract: Hypoxic-ischemic brain damage is a major cause of neurological morbidity and mortality in neonates and results with sequelae such as cerebral palsy, mental retardation, epilepsy and learning disabilities.
We previously observed that uridine reduces brain damage in a rat model of neonatal hypoxic-ischemic encephalopathy. In this study, we aimed to investigate the mechanisms whereby uridine exhibits neuroprotective effects in this model.
Hypoxic-ischemic encephalopathy was achieved by ligation of right common carotid artery followed by subjection to hypoxia (92% nitrogen and 8% oxygen) for 2.5 h in 7-day-old Sprague-Dawley neonatal rats which were then arranged in control or uridine group. Uridine or saline was injected intraperitoneally immediately after hypoxia and 24 and 48 hours after the first dose. Possible mechanisms of neuroprotection exhibited by uridine were investigated 72 h after the hypoxic-ischemic insult by evaluating the hemispheres for cross-sectional area, infarct volume, apoptotic markers and matrix metalloproteinase expression.

Uridine treatment significantly reduced the infarct volume, number of apoptotic cells and number of matrix metalloproteinase-expressing cells in the brain. The results suggest that neuroprotection provided by uridine involves its actions on apopitosis and matrix metalloproteinase expression in a rat model of neonatal hypoxic ischemic encephalopathy.

Disclosures: T. Alkan: None. **Z. Minbay:** None. **M. Cetinkaya:** None. **B. Goren:** None. **E. Orenlili:** None. **M. Cansev:** None.

Poster

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Topic: C.08. Ischemia

Support: Swedish Research Council

Swedish Childhood Cancer Foundation

Title: Autophagy inhibition reduces neonatal mouse hypoxic-ischemic brain injury

Authors: *K. BLOMGREN[1,2], Y. SUN[2,4], H. LI[2,4], Q. LI[2,4], X. WANG[3], Y. UCHIYAMA[5], C. ZHU[2,4]
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Abstract: Autophagy is a strictly regulated process involving bulk degradation of cytoplasmic macromolecules and organelles via the lysosomal system. Autophagy is induced under stress to maintain cellular homeostasis, but this may be either beneficial or detrimental depending on the cell type and conditions. Lack of autophagy-related gene 7 (*atg7*) renders cells incapable of autophagy induction. *Atg7*$^{flox/flox}$ mice were bred with mice expressing cre recombinase under a nestin promoter to disrupt *atg7* expression in brain neurons. Hypoxia-ischemia (HI) was induced in postnatal day 9 (P9) mice using the Vannucci model and injury was evaluated on P17. The tissue loss was 55.1 ± 4.5 mm^3 in *atg7* +/- mice [n=28] and 32.0 ± 5.0 mm^3 in *atg7* -/- mice [n=38], i.e. 41.9 % reduction in *atg7* -/- brains (p=0.001). The overall neuropathological scores were reduced in the *atg7*-deficient mice (11.9 ± 0.8 [n=38] vs. 6.4 ± 1.0 [n=28] mm3, p=0.0002) The DEVDase activity (caspase-3-like activity) was 35.4 ± 4.6 pmol/min.mg protein in *atg7* +/- mice [n=6] and 21.7 ± 3.9 pmol/min.mg protein in *atg7* -/- mice [n=10] (p=0.038). The number of cells immunopositive for active caspase-3 were significantly lower in the dentate gyrus

(214,000 ± 14,700/mm^3 vs. 144,000 ± 23,940/mm^3, p=0.022) , the cornu ammonis (CA1 to 3) (41,810 ± 3,243/mm^3 vs. 22,860 ± 4,685/mm^3, p=0.003) and the cortex (20,410 ± 1,918/mm^3 vs. 12,800 ± 2,991/mm^3, p=0.004) of *atg7* -/- mice compared with *atg7* +/- mice (n=11 /group). These findings indicate that targeting autophagy may be fruitful in ameliorating perinatal brain injury.

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Poster

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Topic: C.08. Ischemia

Support: PO1-NS038475

RO1-NS31718

Title: Priming effects of hypoxic preconditioning on hypoxic/ischemic white matter injury through up-regulation AMPAR in the immature rats

Authors: *H.-K. PARK, L. JANTZIE, F. JENSEN
CLS 14060.3 Children's Hosp Boston, BOSTON, MA

Abstract: Hypoxia-ischemia(H/I) is a major factor in brain injury in premature infants and results in white matter injury, known as periventricular leukomalacia(PVL). Clinically, repeated H/I injuries are common. Experimental studies show a protective effect of recurrent H/I injury to neurons, termed “H/I preconditioning”. In cultured oligodendrocytes(OLs), sublethal oxygen glucose deprivation(OGD) results in an increase in expression of calcium-permeable GluR2 deficient AMPARs, with subsequently increased sensitization to later repetitive OGD(Deng et al. 2003). However, the effects of hypoxic preconditioning in vivo in white matter injury and specifically on OLs, is largely unknown. We examined the effect of hypoxic preconditioning prior to HI in a rodent model of PVL. Postnatal day(P) 5 Long-Evans rats were exposed to H/I(unilateral carotid ligation and hypoxia 1h 6% O_2) with or without hypoxic preconditioning at P3(1h 6% O_2), and were compared at P6 and P9 to control pups without hypoxia/ligation. White matter was evaluated at P6 or P9(n=10/group for each). Immunocytochemistry was performed for GluR4, and cell death (TUNEL staining) and double-labeled with cell specific markers for preOLs or mature OLs(O4, O1, MBP). Results indicate that H/I at P5 without prior hypoxia at

P3 did not induce significant injury, with no increase in TUNEL+cells or altered MBP at P6 or P9. However, rats that received hypoxia at P3 prior to P5 H/I demonstrated significant increases in TUNEL+cells in white matter at P6 compared to P5 H/I alone and sham controls. In these preconditioned pups, O1 cells and O1/O4 cell ratios were significantly decreased at P5(50±7, p=0.02) and P9(36±6, p=0.04), compared to P5 H/I alone(51±5 at P9) and controls(65±12 at P5, 55±8 at P9). In pups sacrificed at P5, 48 hours after exposure to hypoxia, OLs expressed increased GluR4 protein (219% of control, p<0.005). In-situ hybridization for GluR4 mRNA also showed increases in OLs in white matter (190% of control, p=0.03). Taken together, these results indicate that hypoxic preconditioning in the developing white matter may alter OL maturation and exacerbate OL susceptibility to secondary injury, in contrast to prior studies of neuronal protective preconditioning. Our results also suggest that hypoxia-induced increase in preOL GluR expression may all play a role in this increased susceptibility to white matter injury to recurrent H/I.

Disclosures: H. Park: None. **L. Jantzie:** None. **F. Jensen:** None.

Poster

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Heart and Stroke Foundation of Canada

Alberta Innovates Health Solutions

Title: Protective efficacy of the NKCC1 inhibitor bumetanide a rodent model of premature hypoxic-ischemic brain injury

Authors: *L. L. JANTZIE, M. Y. HU, H.-K. PARK, M. JACKSON, F. JENSEN
Neurol., Children's Hosp. Boston, Boston, MA

Abstract: Periventricular leukomalacia (PVL) is a major type of brain damage observed following hypoxia-ischemia (HI) in preterm infants and together with inflammation, is the most

common cause of cerebral palsy, neurodevelopmental delay, and epilepsy in this patient population. PVL is characterized by cerebral white matter injury and damage to immature oligodendrocytes (OLs), subplate, cortical neurons and deep gray matter structures. The Na+-K+-Cl- isoform 1 cotransporter (NKCC1) is highly expressed on neurons and astrocytes, and has been suggested to play a role in adult ischemia and neonatal seizures. As little is known about the expression of NKCC1 on OLs in the developing brain and whether NKCC1 expression is altered by HI in early life, we aimed to 1) investigate the developmental expression of NKCC1 in the immature rodent brain; 2) characterize the time course and cell-specific changes in NKCC1 expression following HI; and 3) investigate whether the NKCC1 inhibitor bumetanide protects against HI injury. We hypothesized NKCC1 is expressed on OLs during development and is upregulated following HI, and that bumetanide would attenuate HI-mediated injury in preterm equivalent rodents. NKCC1 expression was assessed in Long-Evans rats and PLP-EGFP transgenic mice from postnatal day 3 (P3)-P14 (n=6). A separate group of rats were subjected to unilateral carotid ligation and hypoxia at P6, and treated post-HI with 0.3mg/kg bumetanide q12 for 60h (n=7). Semiquantitative analyses reveal NKCC1 is expressed on OLs of all developmental stages and subplate neurons, throughout P3-P14, with a peak in expression from P5-P7. Following HI at P6, NKCC1 is significantly upregulated on cortical and white matter OLs at 6-24h ($p<0.05$). Similarly, NKCC1 is significantly upregulated on subplate neurons acutely following HI and on cortical neurons in and surrounding areas of HI injury at 6-72h ($p<0.05$). Post-HI administration of bumetanide significantly attenuates myelin basic protein injury scores (2.1 ± 0.4 vs. 1.0 ± 0.2, $p<0.05$) and reduces fluorojade cellular degeneration scores in the cortex (1.5 ± 0.3 vs. 0.6 ± 0.5) up to 7d post-HI. This is the first demonstration that NKCC1 is expressed on OLs during normal brain development and upregulated on OLs, cortical neurons and subplate neurons acutely following HI and that bumetanide protects against HI in the preterm equivalent rodent. These data suggest the relative overexpression of NKCC1 in the preterm window coincides with peak vulnerability to HI gray and white matter cell death. Further study is needed to understand the putative mechanisms of NKCC1-mediated injury in premature brain and the protective efficacy of bumetanide.

Disclosures: L.L. Jantzie: None. **M.Y. Hu:** None. **H. Park:** None. **M. Jackson:** None. **F. Jensen:** None.

Poster

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Topic: C.08. Ischemia

Support: CIHR

NSERC

Research Institute - McGill University Health Centre

Title: The effect of neonatal hypoxia-ischemia on the retinal structure and function of rats

Authors: *S. JUNG[1,2], A. POLOSA[2], A. JOHNSTONE[1], M. GAUVIN[2], P. LACHAPELLE[2], P. WINTERMARK[1]
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Abstract: **Background:** Birth asphyxia, i.e. insufficient delivery of oxygen and blood to the organs around the time of birth, leads to significant neurological complications including cerebral palsy, mental retardation and blindness. Although birth asphyxia has been extensively studied, relatively few studies explored the impact of neonatal HIE on the retinal tissue. Furthermore, eye examination is currently not part of the standard monitoring protocol of asphyxiated newborns.
Objective: The objective of the current study was to investigate the impact of neonatal hypoxia-ischemia (HI) on retinal function and cytoarchitecture.
Methods: Neonatal HI was induced in Long-Evans rat pups by left common carotid ligation followed by 2-hour hypoxia (8% oxygen) on postnatal day 10 (P10). At P60, electroretinograms (ERGs) were recorded in HI animals, as well as in control shams, in order to assess function of the retina. Animals were then sacrificed; their eyes were removed and fixed by immersion in 4% paraformaldehyde and subsequently embedded in resin. Sections of the retina were stained with toluidine blue, in order to investigate the cytoarchitecture of retinal cell layers.
Results: Compared to the control shams, ERGs of the HI animals demonstrated 30% reduction in the scotopic a-wave amplitude and 84% reduction in the scotopic and photopic b-wave amplitudes, suggesting that the function of the photoreceptors (where the a-wave is generated) was relatively preserved, whereas that of inner retinal neurons (where the b-wave is generated) was severely impaired. Supportive of the latter, retinal histology of the HI animals showed reduction in the thickness of the inner retina, including outer plexiform layer (none vs. 11.88±1.09 µm in controls), inner nuclear layer (17.44±4.29 µm vs. 26.66±3.40 µm), inner plexiform layer (18.46±26.10 µm vs. 50.69±0.16 µm) and ganglion cell layer (7.20±10.18 µm vs. 13.90±0.36 µm). In contrast, the photoreceptor outer nuclear layer remained intact in all HI animals (50.07±7.42 vs. 46.94±3.06 µm).
Conclusions: Our findings suggest that neonatal HI induces permanent damages to the structure and function of the inner retina (i.e. the layers of cells connecting the photoreceptors to the brain), but less so to the photoreceptors, which appear to be protected from HI insult. These findings may be explained by the fact that photoreceptors and inner retina have different blood

supplies (respectively choroid and retinal vasculature) and that these blood supplies may have different sensitivity to HI.

Disclosures: S. Jung: None. **A. Polosa:** None. **A. Johnstone:** None. **M. Gauvin:** None. **P. Lachapelle:** None. **P. Wintermark:** None.

Poster

863. Ischemia: Inflammation

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Topic: C.08. Ischemia

Support: BBSRC

Title: Optical imaging spectroscopy of early haemodynamic changes in a rat model of neuroinflammation

Authors: *N. BRAY, F. BURROWS, S. ALLAN, I. SCHIESSL
Univ. of Manchester, Manchester, United Kingdom

Abstract: Inflammation is a major driver of cell death in the brain after ischaemic stroke, seizure activity and traumatic injury. In order to open avenues for medical intervention we need to acquire a better understanding of the very early stages of neuroinflammation and its impact on cerebral blood flow responses to neuronal activity.
In an established model of excitotoxicity, striatal injection of AMPA at a toxic dose causes cell death in the striatum after 24 hours. However, co-injection of AMPA with interleukin-1β (IL-1β, a pro-inflammatory cytokine) leads to cortical cell death in addition to striatal damage. When injected alone, IL-1β leads to little or no cell death. In this study we image the changes of the haemodynamic response in the rat barrel cortex after injection of AMPA, IL-1β, AMPA+IL-1β or vehicle with two-dimensional optical imaging spectroscopy. The somatosensory cortex of the anaesthetised animals was stimulated by mechanical whisker manipulation for up to 6 hours after injection. Markers of neuroinflammation (immunoglobulin G and neutrophils) were analysed using immunohistochemistry.
Animals injected with AMPA+IL-1β or IL-1β alone showed a decrease in the size of oxygenated blood response in the active whisker barrel over time. Injection of AMPA alone or vehicle caused little or no trend change in oxygenated blood response. An increase of immunoglobulin G was found in the ipsilateral hemisphere of the brains injected with IL-1β (with or without AMPA), indicating an increase in blood-brain barrier permeability.
This continuing research aims to further investigate the mechanisms behind IL-1β's effects on

the blood flow response. Since the reduction of blood flow response precedes neuronal death, it may be instrumental in deciding the fate of neurones at later time points.

Disclosures: N. Bray: None. **F. Burrows:** None. **S. Allan:** None. **I. Schiessl:** None.

Poster

863. Ischemia: Inflammation

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Program#/Poster#:863.02/M5

Topic: C.08. Ischemia

Support: MRC

Title: 2D imaging spectroscopy of the dynamic changes in blood perfusion during ischaemic stroke

Authors: *F. BURROWS, A. DENES, N. BRAY, S. ALLAN, I. SCHIESSL
Univ. Of Manchester, Manchester, United Kingdom

Abstract: The aim of this study was to quantify the dynamic changes in cortical blood perfusion in response to focal cerebral ischaemia in anesthetised mice with high temporal and spatial resolution.
Middle cerebral artery occlusion (MCAo) was induced by advancing a filament along the internal carotid artery to occlude the middle cerebral artery. Generally this model does not allow real-time monitoring of vascular and metabolic changes in the brain at the onset of ischaemia due to the surgical approach, which is time-demanding. Here, we are using a remote filament approach which allows recording changes before, during and after focal cerebral ischaemia in a controlled manner.
During the 2D imaging spectroscopy, data at four different illumination wavelengths is collected. This allows us to calculate micromolar changes in the concentration of oxy- and deoxyhaemoglobin.
Our results show the expected decrease in the concentration of oxyhaemoglobin in the occluded MCA and supplying cortical regions. After removal of the filament, the MCA vessel shows an increase in oxyhaemoglobin concentration in large areas of the cortex which are insufficiently reperfused even several hours after the stroke.
Our results suggest that 2D imaging spectroscopy with the remote filament approach enables

precise mapping of vascular and metabolic changes in the mouse MCAo model, which can contribute to a better understanding ischaemia-induced changes in the brain.

Disclosures: F. Burrows: None. **A. Denes:** None. **N. Bray:** None. **S. Allan:** None. **I. Schiessl:** None.

Poster

863. Ischemia: Inflammation

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Support: Medical Research Council

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Title: Both central and haematopoietic-derived endogenous interleukin-1 contribute to brain injury after cerebral ischaemia in mice

Authors: A. DENES[1], F. L. WILKINSON[2], B. BIGGER[2], N. J. ROTHWELL[1], *S. M. ALLAN[3]
[1]Fac. of Life Sci., [2]Fac. of Med. and Human Sci., [3]Univ. of Manchester, Manchester, United Kingdom

Abstract: Interleukin-1 (IL-1) is a key regulator of inflammatory responses in the periphery and in the brain. Interventions against IL-1 markedly protect against ischaemic brain injury in experimental stroke. Although IL-1 can be produced by both resident brain cells and immune cells, the relative contribution of central and peripheral sources of IL-1 to brain injury is not well understood.
Here we show that haematopoietic-derived IL-1 is a key driver of ischaemic brain injury, as wild type (WT) mice transplanted with IL-1αβ-deficient bone marrow after lethal irradiation displayed a significant (40%) reduction in infarct size induced by focal cerebral ischaemia compared to WT mice transplanted with WT bone marrow. This was paralleled by improved neurological outcome and the almost complete absence of splenic, but not liver-derived IL-1α, in WT mice lacking haematopoietic-derived IL-1. IL-1αβ KO mice transplanted with IL-1αβ-deficient bone marrow show a 60% reduction in infarct size and significantly reduced blood brain barrier injury compared to WT mice receiving WT bone marrow.
These results indicate that both central and haematopoietic-derived IL-1 are important

contributors to brain injury induced by cerebral ischaemia. Identification of the cellular sources of IL-1 could allow targeted interventions against IL-1-mediated inflammation in brain diseases.

Disclosures: A. Denes: None. **F.L. Wilkinson:** None. **B. Bigger:** None. **N.J. Rothwell:** None. **S.M. Allan:** None.

Poster

863. Ischemia: Inflammation

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:863.04/M7

Topic: C.08. Ischemia

Support: Neuroscience Research Institute

Title: Systemic inflammation impairs cerebral blood flow after focal cerebral ischaemia

Authors: *K. MURRAY[1], W. M. HOLMES[4], K. DAVIES[2], S. WILLIAMS[2], A. PARRY-JONES[3], S. ALLAN[1]
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Abstract: Ischaemic stroke is characterised by an interruption in cerebral blood flow (CBF) and the extent of brain damage correlates with the time it takes to re-establish CBF. Neuroinflammation is a key dimension to cerebral ischaemia with evidence for an expansion of infarct core due to pre-existing infection or inflammation. Experimental work has shown that systemic inflammation exacerbates ischaemic damage via interleukin-1 (IL-1) and neutrophil dependent pathways. Preliminary data using magnetic resonance imaging (MRI) suggest that systemic IL-1 may influence CBF following reperfusion however the mechanisms through which systemic inflammation worsens stroke have yet to be elucidated. It was therefore the objective of the present study to investigate the effects of systemic IL-1 on CBF during early reperfusion in experimental stroke.
Transient focal cerebral ischaemia (60min) was induced in male wistar rats by intraluminal filament occlusion. Animals received intraperitoneal recombinant IL-1β (4μg/kg; n=8) or vehicle. Animals were transferred to a 7-Tesla magnet and remote filament reperfusion was performed. Repeated multi-slice diffusion weighted and perfusion weighted (arterial spin labelling) MRI was performed until 4h reperfusion. Perfusion maps were used to define hypoperfused tissue at risk of infarction and apparent diffusion coefficient (ADC) maps to define ischaemic damage. At 4h reperfusion animals were perfused, organs removed and brains fixed.

IL-1 caused a significant 2-fold increase in ischaemic volume at 3 and 4h reperfusion compared to vehicle-treated animals. IL-1 also led to a 3-fold increase in the volume and magnitude of tissue hypoperfusion on restoration of MCA patency at 15min, 1 and 2h reperfusion. This hypoperfused tissue progressed to infarction over 4h. Furthermore, immunohistochemistry highlighted a prominent role for platelet aggregates in clogging microvessels in IL-1 treated animals. These results reveal that IL-1 exacerbates ischaemic damage following stroke and suggests this injury is mediated by a platelet-dependent reduction in CBF due to microvessel obstruction.
Poor flow in the microcirculation due to systemic inflammation may lead to an accelerated collapse of salvageable tissue, thus reducing the time window and effectiveness of the only treatment available, thrombolysis. Ultimately, the goal of this research is to restore CBF after stroke by introducing treatment with IL-1 antagonists or platelet antibodies alongside thrombolysis.

Disclosures: K. Murray: None. **W.M. Holmes:** None. **K. Davies:** None. **S. Williams:** None. **A. Parry-Jones:** None. **S. Allan:** None.

Poster

863. Ischemia: Inflammation

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Program#/Poster#:863.05/M8

Topic: C.08. Ischemia

Support: MRC In Vivo Science Strategic Skills Awards

Title: Inflammatory changes in peripheral organs and the cerebrovasculature of obese mice may mediate enhanced responses to experimental stroke

Authors: *M. HALEY, N. GORENKOVA, R.-A. PIPE, C. B. LAWRENCE
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Abstract: Obesity is a risk factor for stroke and is associated with conditions such as atherosclerosis and diabetes that predispose to cerebrovascular disease. Inflammation may play a key role in mediating this interaction, since obesity features a raised inflammatory profile that has previously been shown to be detrimental to stroke outcome. The aim of this study was to assess whether there are changes in the inflammatory states of the cerebrovasculature and white adipose tissue of obese mice that could mediate the effects of obesity on stroke outcome.
Focal cerebral ischaemia was induced by intraluminal filament occlusion of the middle cerebral artery in 16-to-20-week-old obese ob/ob and control (ob/-) mice. Ischaemic damage was

assessed at 4 and 24 hours post-stroke, as were the cerebrovascular, adipose and systemic inflammatory responses. Expression of tight junction proteins and inflammatory molecules were analysed in isolated brain microvessels. Structural changes to the cerebrovasculature resulting from ischaemia were assessed in situ using electron microscopy. The adipose tissue response to cerebral ischaemia was also evaluated by ELISA for inflammatory mediators and immunohistochemistry for immune cell infiltration, and was similarly assessed in C57BL/6J mice fed a high fat diet for 16 weeks.
The incidence of haemorrhagic transformation (HT), blood-brain barrier (BBB) breakdown and extent of ischaemic damage were increased in obese mice. Furthermore, HT and BBB breakdown were prominent at 4 hours in obese mice only. Adipose tissue inflammation was also worsened by stroke in obese mice. These data suggest that obese mice suffer worse damage to their cerebrovasculature that may accelerate the development of ischaemic damage, in which the adipose tissue response to stroke may play a role.

Disclosures: M. Haley: None. **N. Gorenkova:** None. **R. Pipe:** None. **C.B. Lawrence:** None.

Poster

863. Ischemia: Inflammation

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Program#/Poster#:863.06/M9

Topic: C.08. Ischemia

Support: MRC Strategic Skills Award for In Vivo Science

Title: The effect of tumor necrosis factor-stimulated gene 6 on brain inflammation in experimental ischaemic stroke

Authors: *H. F. BUGGEY, A. J. DAY, A. DENES, S. M. ALLAN
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Abstract: Ischaemic stroke carries a huge socio-economic burden, and systemic inflammation is a major contributor to stroke damage. Pro-inflammatory cytokine interleukin-1 (IL-1) is a key mediator of systemic inflammation, and is able to exacerbate the outcome of experimental stroke. This effect is known to be dependent on neutrophils. Tumor necrosis factor-stimulated gene 6 (TSG-6) is expressed under inflammatory conditions, and has a number of anti-inflammatory properties including inhibition of neutrophil migration. The therapeutic value of TSG-6 has been demonstrated in a number of inflammatory conditions, but it has never been investigated in stroke. To test whether TSG-6 can protect against IL-1-induced exacerbation of brain injury and inflammation after stroke, C57BL/6J mice (n=12) were injected

intraperitoneally with vehicle, IL-1 (20ng/kg), TSG-6 (5µg) or IL-1 + TSG-6 (20ng/kg + 5µg), followed by 20 minutes middle cerebral artery occlusion (MCAo) and 24h reperfusion. No significant effects of TSG-6 were observed neurological outcome. In contrast, a significant reduction was seen in the number of neutrophils in the ischaemic lesion of animals treated with IL-1 + TSG-6 compared to mice treated with IL-1 alone. Immunohistological staining with IgG showed that TSG-6 also showed some benefit in limiting IL-1-induced blood-brain barrier breakdown. Furthermore, animals administered IL-1 + TSG-6 also displayed a trend for reduction in the presence of haemorrhagic transformation when compared to IL-1 treated mice. The results suggest that TSG-6 inhibits neutrophil migration into the brain, which might have some benefits for outcome and preservation of the blood-brain barrier, although it is insufficient for full protection against IL-1-induced exacerbation of brain injury. Interactions between IL-1 and TSG-6 need further investigation.

Disclosures: H.F. Buggey: None. **A.J. Day:** None. **A. Denes:** None. **S.M. Allan:** None.

Poster

863. Ischemia: Inflammation

Location: Hall F-J

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Program#/Poster#:863.07/M10

Topic: C.08. Ischemia

Title: Up-down regulation of HO-1 and iNOS gene expressions by ethyl pyruvate via recruiting p300 to Nrf2 and depriving it from p65

Authors: *H. LEE[1], I.-D. KIM[1], J.-H. SHIN[1], S.-W. KIM[2]
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Abstract: Ethyl pyruvate (EP), a simple ester of pyruvic acid, has been shown to exert robust neuroprotective effect in various neuropathological conditions, such as, cerebral ischemia and KA-induced seizure in animal models. The neuroprotective effect of EP is largely attributable to the anti-inflammatory effects of EP, which might be mediated by modulation of the NF-κB and p38 MAPK signaling pathways. In the present study, the authors reported a novel molecular mechanism underlying anti-inflammatory effects of EP. In normal BV2 cells, EP induced expression of hemeoxygenase 1 (HO-1) in a dose-dependent manner and nuclear factor E2-related factor 2 (Nrf2) was found to translocate from the cytosol to the nucleus beginning 30 min after EP-treatment, where it binds to the antioxidant response element (ARE) located on HO-1 promoter. EP dose-dependently suppressed nitrite production in LPS-treated BV2 cells, a

microglia cell line, wherein pre-treatment of EP was found to be more effective than co-treatment. As it has shown previously, I-kB degradation and p65 tanslocation to nucleus were conveyed normally in EP-treated cells, however, p65 binding to promoter was inhibited. We found that it is due to recruiting p300, a transcriptional co-activator of both Nrf2 and p65, to Nrf2 and depleting it from p65. EP-induced p300 squelching was confirmed by Nrf2 knockdown by Nrf2 siRNA. Together these results demonstrate that EP induced nuclear translocation of Nrf2, which binds to ARE along with p300, hampering iNOS expression via depleting p300 from p65. This is a novel anti-inflammatory mechanism conveyed by EP, which leads to enhanced protective effect by converging anti-inflammatory and anti-oxidative effects and might be applicable to various Nrf2-activating agents, such as phytochemicals.

Disclosures: H. Lee: None. **I. Kim:** None. **J. Shin:** None. **S. Kim:** None.

Poster

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Topic: C.08. Ischemia

Support: Stanford Graduate Fellowship

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Title: Astrocytic TGFbeta signaling limits the spread of immune responses and improves functional outcome after stroke

Authors: *E. CEKANAVICIUTE, N. FATHALI, J. HAN, K. P. DOYLE, M. S. BUCKWALTER
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Abstract: TGFβ is a master regulatory cytokine that modulates the immune responses to ischemia or injury in a context-dependent fashion. All brain cell types express TGFβ receptors, but stroke caused by distal middle cerebral artery occlusion (dMCAO) leads to a marked increase in TGFβ upregulation and signaling in astrocytes and monocyte-lineage cells. The TGFβ

downstream effector phosphorylated Smad2 was observed in astrocytes in both ipsilateral and contralateral cortex after dMCAO. To alter TGFβ signaling after stroke in an astrocyte-specific manner, we constructed transgenic mice that express a mutant dominant negative TGFβ receptor in reactive astrocytes (DN mice). DN mice express a dramatic, astrocyte-specific upregulation of transgene expression by 12h after stroke that continues for at least 28 days. Three days after stroke, astrocyte activation in DN mice was more widespread than in controls, as indicated by 200% larger area occupied by GFAP+ cells on the opposite side of the stroke. Multiplex luminex assay and ELISA were used to measure the levels of 26 cytokines and chemokines 2 days after stroke. We detected a 2-10 fold decrease in brain TGFβ of DN mice in ipsilateral and contralateral cortex and in ipsilateral striatum, but no genotype-dependent changes in other cytokines and chemokines. Thus, astrocytic TGFβ signaling is required for an autocrine, positive feedback loop that specifically upregulates brain TGFβ after injury. TGFβ signaling-dependent changes in astrocyte phenotype coincided with increased monocyte activation: three days after stroke, DN mice showed 39% more CD68+ cell infiltration than controls in the stroke border, and over 120% more on the opposite side. These effects were accompanied by infarct expansion in DN mice: while the transgene did not alter the initial infarct size, by 7 days DN mice had 32% larger strokes than controls. To study how astrocytic TGFβ signaling affects stroke functional outcomes, we subjected DN mice and their wildtype littermates to a battery of behavioral tests for 4 weeks after photothrombotic stroke. DN mice performed significantly worse in the beam balance test and the modified Garcia test, which together cover multiple aspects of motor behavior. We conclude that reducing astrocytic TGFβ signaling inhibits stroke-mediated increase in TGFβ secretion and leads to abnormal spread of astrogliosis and inflammation. This increases the infarct size within the first week, and correlates with worse motor performance for at least 4 weeks. Thus, astrocytic TGFβ signaling induces brain TGFβ production and limits the spread of the immune response, resulting in improved functional outcome after stroke.

Disclosures: E. Cekanaviciute: None. **N. Fathali:** None. **J. Han:** None. **K.P. Doyle:** None. **M.S. Buckwalter:** None.

Poster

863. Ischemia: Inflammation

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Program#/Poster#:863.09/M12

Topic: C.08. Ischemia

Title: NGAL (lipocalin 2) and microglial activation and function

Authors: *C. XING[1], E. H. LO[2]
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Abstract: Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2, is a 25 kDa protein belonging to the lipocalin superfamily. NGAL captures and transports iron particles to the inner cell by interacting with specific membrane receptors (24p3R or megalin). Traditionally, NGAL is released by activated neutrophils as part of innate immunity. More recently, it has been proposed that NGAL may also participate in inflammation and remodeling after ischemia. Here, we asked whether NGAL can directly modify microglia function, long considered a critical part of CNS pathophysiology. Primary microglial cultures were derived from rat cortex. Morphological changes were observed with phase-contrast microscopy. Cellular function was assessed with phagocytotic and migration assays. Gene expression levels were measured with RT-PCR. Cytokine and growth factor levels were quantified with ELISA. After NGAL treatment for 24 hrs, normally ramified "resting" microglia converted into a long-rod morphology with reductions in peripheral branching. mRNA levels of NAPDH oxidase and TLR2 increased by 2-fold after NGAL treatment for 4 hrs. NGAL treatment significantly increased TNFα and IL-10 release, and reduced IGF-1 production by microglia. No significant changes in IL-1β and VEGF levels were detected. NGAL-treated microglia also showed enhanced phagocytotic capacity. But there were no detectable effects on microglia migration. This study suggests that NGAL may regulate microglial activation and function. How NGAL-mediated microglial responses contribute to the pathophysiology of various CNS disorders warrants further investigation.
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Disclosures: C. Xing: None. **E.H. Lo:** None.

Poster

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Topic: C.08. Ischemia

Support: NIH/NINDS NS065849

McKnight Brain Institute Funds

Title: Post-ischemic treatment with BML-111, a lipoxin A_4 receptor agonist, reduces infarct size and neuroinflammation in a rat model of stroke

Authors: K. E. HAWKINS, H. S. CHO, J. FRANKOWSKI, *E. CANDELARIO-JALIL
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Abstract: Lipoxin A_4 (LXA_4) is an anti-inflammatory mediator derived enzymatically from arachidonic acid by the concerted action of lipooxygenases. Binding of LXA_4 to its receptor (FPR2/ALX) has pro-resolution effects on inflammation. Virtually nothing is known of the role of LXA_4 on neuroinflammation following cerebral ischemia. BML-111 is a FPR2/ALX receptor agonist and has been recognized to play a similar role as LXA_4 in anti-inflammatory responses. We hypothesized that treatment with BML-111 would reduce post-ischemic inflammation and provide neuroprotection in a rat model of ischemic stroke. Rats were subjected to 90 minutes of transient middle cerebral artery occlusion and sacrificed after 48 hours of reperfusion. Animals received BML-111 or the vehicle. The first dose (1 mg/kg) was given intravenously 10 minutes after the onset of reperfusion and another dose 24 hours after reperfusion. Pro-inflammatory markers such as myeloperoxidase (MPO), a neutrophil marker, and matrix metalloproteinases -2 and -9 (MMP-2 and MMP-9) were evaluated at 48 hours after reperfusion. Post-ischemic treatment with BML-111 significantly reduced infarct size and blood-brain barrier (BBB) damage as seen with 2,3,5-triphenyltetrazolium chloride (TTC) staining and brain IgG extravasation, respectively. BML-111-treated animals had significantly reduced neutrophil infiltration as seen by decreased MPO expression in the ipsilateral cerebral cortex compared to vehicle-treated animals as determined by western blotting. BML-111-treated animals also had significantly decreased levels of MMP-9 in the ipsilateral cortex and striatum compared to animals receiving the vehicle. However, levels of MMP-2 did not significantly decrease in the ipsilateral striatum and only approached significance in the ipsilateral cortex as seen with gelatin-substrate zymography. These data indicate that activation of the FPR2/ALX receptor with BML-111 results in the reduction of infarct size possibly through the attenuation of the inflammatory response following ischemic stroke.

Disclosures: K.E. Hawkins: None. **H.S. Cho:** None. **J. Frankowski:** None. **E. Candelario-Jalil:** None.

Poster

863. Ischemia: Inflammation

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Program#/Poster#:863.11/M14

Topic: C.08. Ischemia

Title: Toll-like receptor 2 provides protective effects on ischemic demyelination and oligodendrocyte death in a subcortical white matter stroke model.

Authors: *J. CHOI[1,2], J.-H. KIM[1], Y. KANG[1], B. KIM[1]
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Abstract: Ischemic lesion involving subcortical white matter constitutes more than 20% of all ischemic stroke subtypes. Furthermore, repeated ischemic subcortical strokes ultimately lead to vascular dementia, the second common cause of cognitive dysfunction in the elderly. Since demyelination and oligodendrocyte (OL) loss are prominent features of vascular dementia pathology, preventing demyelination and OL loss after ischemic subcortical white matter injury would be an important therapeutic strategy for the vascular dementia. We have developed a mouse model of white matter stroke in which endothelin-1 was stereotaxically injected into the posterior limb of internal capsule. In this model, a localized demyelinating lesion develops in the injection site, where activated macrophages are compacted. The macrophage infiltration was accompanied by the increased expression of proinflammatory cytokines. Toll-like receptor 2 (TLR2), the upstream modulator of innate immunity after ischemic stroke, was also increased in ischemic demyelinating lesion. Interestingly, the extent of demyelinating pathology was larger in TLR2 deficient mice than that of wild type animals. TLR2 (-/-) mice expressed more activated-caspase 3(+) OLs than wild type mice, although there was no difference of inflammatory cell infiltration into the ischemic lesion between TLR2 (-/-) and wild type mice. Phosphorylation of ERK 1/2, one of protective downstream signaling proteins of TLR2 was decreased in TLR2 (-/-) mice than wild type mice. We also found that cultured OLs obtained from TLR2 (-/-) animals were more vulnerable to oxygen-glucose deprivation (OGD) than that of wild type OLs *in vitro*. Applying Pam3CSK4, one of TLR2 agonist, after OGD in wild type OLs rescued OGD-induced OL death. These data demonstrated that TLR2 plays a protective role against ischemic demyelination and OL death, suggest modulation of TLR2 could be utilized as a novel therapeutic target to treat ischemic white matter stroke.

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Poster

863. Ischemia: Inflammation

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Topic: C.08. Ischemia

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Title: Endogenous interferon-β modulates hallmarks of the inflammatory and immune response during the first week after ischemic stroke

Authors: *A. R. INACIO[1], Y. LIU[2], K. KUCHARZ[1], T. STANKOVICH[1], S. ISSAZADEH-NAVIKAS[2], T. DEIERBORG[1]
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Abstract: Ischemic stroke leads to the activation of a dynamic inflammatory and immune response, in and outside the brain. This response has been shown to critically affect neuronal death and functional recovery, and identification of underlying molecular and cellular determinants may prompt the development of stroke treatment. Interferon-β (IFN-β) is a potent immunomodulator, used in the treatment of the multiple sclerosis, an autoimmune disorder of the central nervous system. We aimed at determining if endogenous IFN-β influences (1) hallmarks of the inflammatory/immune response, (2) the extent of sensory-motor deficits and (3) neuronal death after ischemic stroke.
We subjected wild type (WT) and IFN-β knockout (IFN-β KO) mice, males, to transient middle cerebral artery occlusion (tMCAo) or sham-surgery. Populations of inflammatory and immune cells in the brain, blood and spleen were characterized using flow cytometry. We quantified the protein concentrations of T helper (Th)1 and Th2 cytokines in blood plasma using a multiplex immunoassay. Sensory-motor deficits were assessed by a modified composite neuroscore, the rotating pole and grip strength tests, and cerebral infarct volumes were given by lack of neuronal nuclei immunoreactivity. Samples were collected 2 and 8 days after surgery.
Abrogation of IFN-β in mice resulted in a higher number of $CD11b^{+}/CD45.2^{high}$ cells (putative macrophages) within the injured cerebral hemisphere 2 days after tMCAo. Concomitantly, we found a higher number of circulating $B220^{+}/MHCII^{+}$ cells (B cells) in IFN-β KO mice than in WT controls. Increases in splenic $B220^{+}/MHCII^{+}$ and $CD8^{+}/CD122^{+}$ cells occurred between 2 and 8 days after tMCAo in WT, but not in IFN-β KO mice. In addition, deletion of *Ifnβ* in mice resulted in decrease in spleen weight, as well as in the number of splenocytes (as observed in sham-operated mice), and a stroke-induced decrease in the number of splenocytes was not verified in IFN-β KO mice. Beyond alterations in the inflammatory and immune response at the

cellular level, we found an increase in interleukin (IL)-1β and IL-5 in IFN-β KO mice, also 2 days after tMCAo. Importantly, IFN-β KO mice showed less neurological deficits 24 h after tMCAo (composite neuroscore). However, we did not observe a significant difference in behavioral performance 2 and 7 days after tMCAo, using the grip strength and rotating pole tests. No difference was obtained with respect to infarct volumes 8 days after stroke.
Our results indicate that endogenous IFN-β, while being a critical regulator of the inflammatory and immune response, may not significantly contribute to functional recovery and neuronal death post-stroke.

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Poster

863. Ischemia: Inflammation

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Program#/Poster#:863.13/M16

Topic: C.08. Ischemia

Title: HCA_2-mediated protective effect of nicotinic acid in cerebral ischemia

Authors: M. RAHMAN[1], S. MUHAMMAD[1], J. G. OKUN[2], S. OFFERMANNS[3], *M. SCHWANINGER[1]
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Abstract: Nicotinic acid has been used as a lipid modifying drug since decades. The anti-lipolytic effect of nicotinic acid in adipocytes is mediated by the hydroxy-carboxylic acid receptor HCA_2 (GPR109A), a $G\alpha_i$-coupled receptor. In addition to adipocytes, immune competent cells express HCA_2. Cannabinoid receptor 2 (CB_2) is another $G\alpha_i$-coupled receptor expressed in immune cells and has been reported to be protective in ischemic stroke by inhibiting neutrophil recruitment. Therefore, in this study we hypothesized that nicotinic acid and its receptor HCA_2 might have an important role in the context of cerebral ischemia. When we subjected mice to distal permanent middle cerebral artery occlusion (pMCAO), treatment with nicotinic acid for 48 hours reduced the infarct volume. However, this protective effect was lost in $HCA_2^{-/-}$ mice demonstrating that HCA_2 mediates neuroprotection. As HCA_2 is significantly expressed in immune cells, we depleted CD11b-positive cells using CD11bDTR mice and subjected them to pMCAO. We noticed a loss of neuroprotection upon nicotinic acid treatment

indicating the involvement of microglia/macrophages. Moreover, flow cytometry revealed reduced number of activated microglia/macrophages in the ischemic brain tissue when treated with nicotinic acid, which was confirmed by immunostaining. We generated chimeric mice by bone marrow transplantation to sort out whether central or peripheral macrophages are involved. Wild-type mice as well as $HCA_2^{-/-}$ mice that received wild-type bone marrow were protected against cerebral ischemia when treated with nicotinic acid whereas the effect of nicotinic acid treatment was abolished when $HCA_2^{-/-}$ bone marrow was transplanted to wild-type mice confirming that bone marrow derived $CD11b^+$ cells are mediating the protective effect of nicotinic acid. Thus, we conclude that nicotinic acid is protective in cerebral ischemia and this effect is mediated by HCA_2 receptor present in peripheral $CD11b^+$ cells.

Disclosures: M. Rahman: None. **S. Muhammad:** None. **J.G. Okun:** None. **S. Offermanns:** None. **M. Schwaninger:** None.

Poster

863. Ischemia: Inflammation

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Topic: C.08. Ischemia

Title: Intranasal delivery of HMGB1-binding heptamer peptide confers robust neuroprotection in the postischemic brain

Authors: *I.-D. KIM, J.-H. SHIN, Y.-C. JIN, L. LUO, H.-K. LEE, J.-K. LEE
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Abstract: Non-invasive intranasal drug administration enables direct drug delivery to the brain. In a previous study, we reported on the therapeutic efficacy of intranasal siRNA delivery in the postischemic rat brain. When siRNA for high mobility group box 1 (HMGB1), which functions as an endogenous danger molecule and aggravates inflammation, was delivered intranasally, expression of the target gene was found to be depleted in many brain regions and infarct volume to be suppressed by up to 42.8±5.6% at 48 hrs after 60 minutes of middle cerebral artery occlusion (MCAO). In the present study, we investigated the therapeutic efficacy of HMGB1 inhibitory heptamer peptide (HBHP; HMSKPVQ), a HMGB1-binding peptide selected using a phage display approach, in the same rat stroke model. Intranasal delivery of HBHP (300ng) at 60 minutes before MCAO suppressed infarct volume in the postischemic rat brain (maximal reduction to 58.8±4.5%) and ameliorated neurological and behavioral deficits. Furthermore, HBHP significantly suppressed HMGB1-mediated neuronal cell death in primary cortical

cultures. HBHP also dose-dependently suppressed HMGB1-mediated microglia activation. Binding experiments using biotin-labeled HBHP showed that HBHP binds directly to HMGB1, suggesting that this is responsible for inhibiting cytokine-like function of HMGB1. In contrast, a heptamer peptide composed of a scrambled sequence of the seven amino acids in HBHP had no protective effect or bond HMGB. Together these results suggest that intranasal delivery of HIHP ameliorates neuronal damage in the ischemic brain by inhibiting HMGB1.

Disclosures: I. Kim: None. **J. Shin:** None. **Y. Jin:** None. **L. Luo:** None. **H. Lee:** None. **J. Lee:** None.

Poster

863. Ischemia: Inflammation

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Topic: C.08. Ischemia

Title: Mesenchymal stem cells suppress infiltration of peripheral immune cells by downregulating production of MCP-1 in the ischemic rat brain

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Abstract: Mesenchymal stem cells (MSCs) have been shown to enhance the recovery of brain functions from the ischemic injury. In this study, we investigated the signaling molecules through which the grafted MSCs interact with damaged host brain and salvage the brain parenchyma from the secondary damages triggered by circulating immune cells. Transient occlusion of a middle cerebral artery induced the expression of proinflammatory cytokines such as MCP-1, TNFα, and IL-1β in the ischemic area. Transplantation of bone marrow-derived MSCs at postischemic day 3 reduced the expression of those cytokines and subsequent infiltration of invasive immune cells to the ischemic area. *In vitro* studies with anti-MCP-1 antibody indicated that MCP-1 in ischemic brain extracts was primary signaling molecule that chemoattracted peritoneal macrophages. The MSC-conditioned could also block both MCP-1 expression and immune cell migration. The results suggest that immunomodulatory functions of MSCs are mediated through downregulation of MCP-1 expression.

Disclosures: H.K. Suh-Kim: None. **S. Yoo:** None. **D. Chang:** None. **H. Lee:** None. **B. Ryu:** None. **E. Joe:** None. **S. Hong:** None. **Y. Lee:** None. **S. Kim:** None.

Poster

863. Ischemia: Inflammation

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:863.16/M19

Topic: C.08. Ischemia

Support: NINDS

VA Merit

Title: Temporal window of protection by splenectomy against experimental stroke

Authors: *M. A. YENARI[1], X. TANG[1], C. HSIEH[2], M. NAKAMURA[2]
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Abstract: Immune responses play a pivotal role in protecting and worsening ischemic injury. The spleen is the largest lymphoid organ of the body, and is the site where many acquired immune responses occur, such as antigen presentation, complement opsonization and other functions. Prior work has shown dramatic splenic atrophy and protective effects of splenectomy on outcome from experimental stroke. Here, we attempt to define the temporal therapeutic window for splenectomy and neuroprotection against brain ischema. We used an intraluminal filament model to occlude the middle cerebral artery (MCAO) for 1h followed by 22h reperfusion. 38 male C57BL/6 mice were randomly divided into 5 groups: sham splenectomy+MCAO, spelenectomy performed 1, 3, 7 or 14d prior to MCAO. Compared to mice receiving sham splenectomy, infarct size was decreased among mice receiving spelenectomy 1d prior to MCAO (130.00±7.43 mm3 vs 80.10±9.79 mm3, $p<0.05$), while mice receiving splenectomy 3 or 7d prior showed a trend towards increased infarct size (splenectomy 3d before MCAO: 130.00±7.43 mm3 vs 162.45±15.39 mm3; splenectomy 7d before MCAO: 130.00±7.43 mm3 vs 160.79±7.1690 mm3) and no effect was observed when splenectomy was performed 14d earlier. A pilot group of 3 mice were also studied where splenectomy was carried out post MCAO and at the time of reperfusion, but all animals died before 24h. We then studied an additional 9 mice to determine leukocyte counts and phenotype in the brain for MCAO only, sham splenectomy plus MCAO, and splenectomy carried out 1d prior to MCAO, and splenectomy but no MCAO (n=3/ group). Leukocytes from the brain parenchyma were harvest using a density-gradient centrifugation technique. Cells were stained with the following

fluorescence-tagged antibodies: anti-CD45 APC (clone Ly5) (eBioscience), anti-CD11b (clone M1/70) PE (Invitrogen) or PE-Cy5 (eBioscience), anti-Ly6G (clone 1A8) PE-Cy7 (BD Biosciences) and sytox blue. After incubation, cells were sorted by FACS machine. There was no statistical difference between MCAO only and sham splenectomy plus MCAO, but numbers of circulating monocytes was decreased by ~50% in animals receiving splenectomy 1d prior to MCAO. We show that splenectomy protects only when carried out 1d prior to MCAO, and is associated with reduced monocyte infiltration into the ischemia brain. These observations expand prior studies showing the neuroprotective effects of splenectomy. The defined time window for such an effect may provide insight into potential mechanisms.

Disclosures: M.A. Yenari: None. **X. Tang:** None. **C. Hsieh:** None. **M. Nakamura:** None.

Poster

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Topic: C.08. Ischemia

Support: CIHR

Title: Live imaging of innate immune response: Chronic estrogen deprivation alters microglia activation profiles after stroke.

Authors: *P. CORDEAU JR[1], Y. WENG[2], J. KRIZ*[2]
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Abstract: Background: Our previous study employing live imaging analysis of neuroinflammatory signals revealed a marked gender difference in astrocyte response to ischemic injury. However, the effects of estrogens (gender) on microglia activation profiles after stroke remain unclear. Microglia are critical for proper innate immune responses in the brain since they are responsible for orchestration of the injury-induced inflammation, the processes that may significantly affect evolution of the ischemic injury. To date, the role of estrogens in the modulation of the post stroke innate immunity is far from being elucidated. On those bases, the in vivo study of the microglial responses/TLR2 signal induction in ovariectomized and control mice could bring some light on the mechanism affecting the stroke evolution.
Methods: The TLR2-Luc-GFP, TLR2-Luc-GFP x ErαKO and TLR2 x ErβKO mice were subjected to middle cerebral artery occlusion (MCAO) after a deprivation of estrogens of 14 or 40 days and compared to adequate controls. The pattern and the intensity of the TLR2 signals were longitudinally monitored using in vivo biophotonic/bioluminescence imaging.

Results: In all groups a marked upregulation of the TLR2 signal was detected post MCAO. Interestingly, we did not observed significant difference in TLR2 induction between control male and female mice. However, our results revealed a significant difference in the signals measured before and after the MCAO in the group chronically deprived of estrogens. The chronic estrogen deprivation was associated with significant increase in post-ischemic microglial activation and larger infarct lesions. The increase in TLR2 signals was blunted in ErαKO.
Conclusions: The TLR2 reporter mouse represents a valid model to study microglia activation in ischemic brain of live animals after chronic or acute estrogens deprivation. Furthermore, the receptor Erα seems to be needed for TLR2 activation after MCAO. Further studies are needed to better understand a complex relationship between the inflammation, estrogens and brain recovery.

Disclosures: P. Cordeau Jr: None. **Y. Weng:** None. **J. Kriz*:** None.

Poster

863. Ischemia: Inflammation

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Topic: C.08. Ischemia

Support: Swedish Research Council

European Commission

Title: Chemokine receptor CXCR4 antagonism improves functional recovery after stroke

Authors: K. RUSCHER[1], E. KURIC[1], A. INACIO[1], Y. LIU[2], S. ISSAZADEH-NAVIKAS[2], E. ENGLUND[1], *T. W. WIELOCH[1]
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Abstract: Inflammation in the ischemic hemisphere hampers brain tissue reorganization and functional recovery after stroke. Here, we show that rats housed in enriched environment (EE) dramatically improves recovery of lost neurological functions after experimental stroke induced by permanent occlusion of the middle cerebral artery (pMCAO). Enriched environment profoundly attenuated levels of pro-inflammatory cytokines in the ischemic core and the surrounding peri-infarct area. We also observed a significant reduction in the stroke-induced chemokine receptor CXCR4 and its natural ligand stromal cell-derived factor-1 (CXCL12). The receptor was also increased in the brains of stroke patients. To mimic the beneficial effects of EE, we studied the impact of inhibiting CXCL12/CXCR4 on functional recovery after transient

MCAO (tMCAO). Rats treated with the specific CXCR4 antagonist 1-[4-(1,4,8,11-Tetrazacyclotetradec-1-ylmethyl)phenyl]methyl]-1,4,8,11-tetrazacyclo-tetradecan (AMD3100) showed improved recovery compared to saline-treated rats after tMCAO. Enhanced recovery was accompanied by a reduction in infiltrating immune cells in the ischemic territory, particularly CD3+ and CD3+/CD4+ T cells. Splenatrophy and delayed death of splenocytes, seen after tMCAO, was prevented by AMD3100 treatment. However, AMD3100 treatment did not affect the number of circulating lymphocyte and the number of circulating CD34 positive progenitor cells 6 days after tMCAO. We conclude that the CXCL12/CXCR4 pathway contributes to depression of neurological function after stroke and that its inhibition is beneficial and may become an attractive stroke therapy.

Disclosures: K. Ruscher: None. **E. Kuric:** None. **A. Inacio:** None. **Y. Liu:** None. **S. Issazadeh-Navikas:** None. **E. Englund:** None. **T.W. Wieloch:** None.

Poster

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Topic: C.08. Ischemia

Support: Swedish Research Council No 2011-2684

ESN grant No 201024

Title: Levodopa treatment attenuates inflammation and improves functional recovery after experimental stroke

Authors: E. KURIC, T. WIELOCH, *K. RUSCHER
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Abstract: Delayed treatment of stroke patients with levodopa/benserazide contributes to enhanced functional recovery, but the mechanisms involved are poorly understood. The present study was designed to investigate if L-Dopa/benserazide treatment improves recovery of lost neurological function in rats by affecting the inflammatory response in the ischemic hemisphere. We subjected male Wistar rats to transient occlusion of the middle cerebral artery (tMCAO, 120min) and treated animals with levodopa (1, 5 and 20 mg/kg)/benserazide (15mg/kg) or saline for 12 consecutive days starting on day two after tMCAO. Thereafter, infarct volume was determined and sensorimotor function was assessed using the cylinder test, the rotating pole test and a 28 points neuroscore on day 2, 7 and 14 after tMCAO. The ischemic core and adjacent

peri-infarct area were dissected and pro-inflammatory cytokines were measured in different areas of the ischemic hemisphere to estimate the poststroke inflammatory response.
Our data show that treatment with levodopa/benserazide significantly improves recovery of sensorimotor function following tMCAO assessed by multiple behavioral tests without affecting the infarct volume. We also observed that levodopa significantly reduced the levels of interleukin-1beta and interferon-gamma in the infarct core and adjacent peri-infarct area on day 14 after tMCAO. Concomitantly, expression of dopamine receptors on inflammatory cells such as microglia/macrophages indicate their susceptibility to treatment.
After stroke, levodopa treatment affects cellular and molecular processes which improve recovery of lost neurological function. The present study corroborates that levodopa contributes to the inhibition of inflammatory processes in the ischemic hemisphere.

Disclosures: E. Kuric: None. **T. Wieloch:** Research Grant; Swedish Research Council. **K. Ruscher:** Research Grant; Swedish Research Council.

Poster

863. Ischemia: Inflammation

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Title: $CD4^+$ T helper cell infiltration in the AV3V region and frontal cortex is associated with hypertension in response to placental ischemia in pregnant rats

Authors: *K. L. WALLACE, M. DARBY, J. MOSELEY, B. LAMARCA
Univ. MS Med. Ctr., JACKSON, MS

Abstract: INTRODUCTION. We have previously shown that a reduction in uterine perfusion pressure (RUPP) causes hypertension, elevations in circulating inflammatory cytokines and in agonistic autoantibodies to the angiotensin II receptor and activation of $CD4^+$ T helper cells in the circulation of Sprague Dawley rats. Furthermore, we have also shown that adoptive transfer of $CD4^+$ T cells from RUPP rats into normal pregnant (NP) rats also causes hypertension and increases these same inflammatory mediators. The central nervous system plays an important

role in the pathogenesis of hypertension and neuronal ischemia and inflammation is associated with the development of hypertension during preeclampsia. Therefore we hypothesize that hypertension in response to chronic placental ischemia stimulates $CD4^+$ T cells infiltration into the central nervous system.
METHODS. Sprague Dawley rats were anesthetized on gestational day (GD) 14 of pregnancy and underwent either examination under anesthesia (NP) or had the lower abdominal aorta above the iliac bifurcation chronically constricted (0.203mm ID clip) along with both ovarian arteries (0.100mm ID clip). On GD18 rats were instrumented with a carotid catheter for arterial pressure measurement (MAP). We measured MAP and circulating T cells on GD19 in NP and placental ischemic pregnant RUPP rats. We utilized flow cytometry to analyze $CD4^+$ T cells from RUPP and NP plasma. Maternal brains were collected at the time of sacrifice and processed for histological analysis for CD4 expression.
RESULTS: MAP increased from 96.2 $\pm$ 3.6mmHg (n=5) in NP rats to 127.8 $\pm$ 3.8mmHg (n=5; p=0.0003) in RUPP rats. $CD4^+$ T cells increased to 48.69 $\pm$ 2.8% gated (n=3) in RUPP rats compared to 24.35 $\pm$ 2.5% gated (n=4; p=0.001) in NP rats. After staining with cresyl violet, brains from RUPP rats had darker neurons, representative of neuron injury and analysis revealed a disorganized cytoarchitecture when compared to brains from NP rats. Additionally, there were more infiltrated $CD4^+$ T cells in the AV3V region and along the corpus callosum in RUPP brains compared to NP rat brains.
CONCLUSION.These data indicate that $CD4^+$ T cells are increased in response to placental ischemia in both the circulation and in regions of the brain and may therefore be one potential link in the neuropathophysiology of hypertension associated with preeclampsia.

Disclosures: K.L. Wallace: None. **M. Darby:** None. **J. Moseley:** None. **B. LaMarca:** None.

Poster

863. Ischemia: Inflammation

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Topic: C.08. Ischemia

Support: CIHR

NSERC

Title: Regulation of Macrophage migration inhibitory factor (MIF) expression by NF-κB signaling

Authors: *S. F. ZHANG, O. ZIS, W. SONG
Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Macrophage migration inhibition factor (MIF) is a pleiotropic protein expressed in various cell types and involved in many cellular activities. Our lab has previously demonstrated that MIF gene expression was elevated following cerebral ischemia through the hypoxia signaling pathway. Although the role of MIF during ischemic insult remains exclusive, it is of interest to explore other signaling pathways that can regulate MIF gene expression. NF-κB, a transcriptional factor, has been shown to be activated after stroke. Analysis of the promoter region of MIF gene reveals several putative NF-κB consensus binding sites. Therefore, we investigated whether NF-κB could also regulate the transcription of MIF. Using luciferase as the reporter gene, we demonstrated that MIF promoter activity was upregulated by NF-κB signaling activation. In addition, using gel shift and super shift assay, we demonstrated the binding between NF-κB and the putative consensus binding sites of NF-κB on MIF promoter. Moreover, we observed upregulation of MIF mRNA upon NF-κB signaling activation. Simultaneous upregulation of MIF and NF-κB was also observed in the protein level. Finally, we showed that MIF level in mouse brain was elevated after ischemic insult. The results of the current results suggested NF-κB can enhance the expression of MIF gene under ischemic insult.

Disclosures: S.F. Zhang: None. **O. Zis:** None. **W. Song:** None.

Poster

864. Brain Trauma

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DoD award #W81XWH-11-2-0057

Title: Cell adhesions affect neuronal injury in a model of mild traumatic brain injury.

Authors: *M. A. HEMPHILL, J. A. GOSS, B. E. DABIRI, K. K. PARKER
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Abstract: Blast-induced Traumatic Brain Injury has been called the signature wound of the Afghanistan and Iraq wars and has underscored the current limitations in treating this disease. Mild Traumatic Brain Injury (mTBI) has presented a unique challenge in that there is a lack of

mechanistic understanding of the processes which initiate neuronal injury. Previous reports have shown that a mechanical insult can rupture the cell membrane and initiate injurious signaling cascades, but this mechanism cannot account for all injured neurons. Cells can transduce extracellular mechanical signals directed through adhesion complexes into normal remodeling signals. We hypothesize that injury forces transmitted through adhesion complexes in the cell membrane may initiate remodeling pathways in a pathological manner which can account for injury in neurons following mTBI

To study this, we built two in vitro devices which allowed us to deliver mechanical stimuli to a population of neurons through specific cell adhesions. First, primary cortical neurons from neonatal sprague-dawley rats were seeded on stretchable membranes coated with adhesion proteins poly-l-lysine (PLL) or fibronectin (Fn) and cultured for 5 days in 10% serum supplemented media. A single, high speed stretch (1 % per ms) to a specific magnitude was administered to mimic mTBI. In the second model, 4.5 μm ferromagnetic beads were coated with the same adhesion proteins and bound to neurons seeded on PLL coated substrates. A brief magnetic pulse was applied generating a force on the adhesion between neuron and bead

We found that neurons subjected to a single rapid stretch exhibited neurite focal swelling, similar to that observed in axons following TBI, that was strain magnitude dependent. Furthermore, the extent of injury at a given magnitude was found to be dependent upon the adhesion protein at 10 minutes following injury, but not at 60 minutes. Neurons which were subjected to bead twisting forces also exhibited magnitude and adhesion coating dependent injury levels. Interestingly, in these experiments we observed that neurons coated with FN coated beads exhibited injury onset at earlier time points than neurons coated with PLL, however, by 60 minutes the extent of injury was similar.

These results indicate that the type of adhesion though which a mechanical stimulus is transmitted may affect the extent of neuronal injury. Since the adhesion type appears to alter the injury initiation time, it is possible that it also affects the mechanism of injury. Future studies are necessary to further elucidate the injury mechanisms underlying neuronal injury following mTBI.

Disclosures: M.A. Hemphill: None. **J.A. Goss:** None. **B.E. Dabiri:** None. **K.K. Parker:** None.

Poster

864. Brain Trauma

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KUMC Biomedical Research Training Program.

Title: Altered expression of injury response mediators after TBI in juvenile rats with decreased brain omega-3 DHA content

Authors: *K. L. RUSSELL[1], N. E. J. BERMAN[2], B. LEVANT[3]
[1]Pharmacology, Toxicology & Therapeut., [2]Anat. & Cell Biol., [3]Pharmacology, Toxicology, & Therapeut., Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Children under 5 years of age are at high risk for sustaining traumatic brain injuries (TBI) and tend to have poorer outcomes despite the greater neuroplasticity in children. N-3 (omega-3) polyunsaturated fatty acids (PUFA) are a major component of neural membranes and have multiple neuroprotective and anti-inflammatory activities. These fatty acids, of which docosahexaenoic acid (DHA) is the major species in brain, accumulate in the brain during late gestation and early childhood. Low dietary N-3 PUFA content results in decreased DHA accumulation in the developing brain. We have previously shown that juvenile rats with a large decrease in brain DHA level (-54%) have poorer sensorimotor outcomes after TBI. Accordingly, this study examined the effects of dietary modulation of brain DHA content on molecular outcomes after TBI in a juvenile rat model. Two sequential litters of male, Long-Evans rats (n=5-13/group) were raised from conception on diets containing adequate n-3 PUFA (N-3) or low in n-3 PUFA (Low N-3). First and second litter rats raised on the Low N-3 diet had decreases in brain DHA of 25% and 54%, respectively, compared to rats raised on the N-3 diet. On postnatal day 17, rats received a unilateral controlled cortical impact injury to the sensorimotor cortex or a sham surgery. The sham surgery consisted of scalpel incision but no craniotomy. Expression of genes associated with inflammation and blood brain barrier disruption were measured via real-time PCR 1 and 7 days after surgery. Monocyte chemotactic protein-1 (Mcp-1) and glial fibrillary acidic protein (Gfap), markers of inflammation and microglial activation, and matrix metalloproteinase-9 (Mmp-9), a mediator of blood-brain barrier disruption, were increased after TBI regardless of brain DHA level. In contrast, tissue inhibitor of metalloproteinase-1 (Timp-1), had increased expression after TBI, but the increase in Timp-1, was smaller in rats with lower brain DHA levels. Besides inhibiting MMP-9, TIMP-1 also acts as an anti-apoptotic and survival factor, indicating altered expression of this gene may play an important role in the extent of secondary damage after TBI. This suggests that altered expression

of injury response mediators in rats with low brain DHA rats may contribute to the previously observed worsened behavioral outcomes after TBI.

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Poster

864. Brain Trauma

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Topic: C.10. Trauma

Support: Alzheimer's Association Grant IIRG-11-202064

NIH Grant R01 CA133216

Title: Long-term effects of traumatic brain injury on the prefrontal cortex: tau alterations and cognitive dysfunction

Authors: *L. NAVARRIA, JR[1,2], J. MORGANTI[1], A. ALLEN[1], K. BELARBI[1], E. CUELLAR[1], T. JOPSON[1], J. FIKE[1], S. ROSI[1]
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Abstract: Traumatic brain injury (TBI) is a major risk factor for the development of multiple neurodegenerative diseases, such as Alzheimer's disease and frontotemporal dementia. Patients with TBI often suffer from deficits in working memory function related to prefrontal cortex (PFC) and show macroscopic abnormalities in this region. Additionally, human studies have shown aggregation of the neuronal microtubule-associated protein tau in the post mortem brains from long-term survivors of a single TBI. Tau stabilizes and promotes microtubule polymerization and its alteration is involved in multiple neurodegenerative disorders. Post-translational modifications of tau such as hyperphosphorylation and/or cleavage reduce its ability to bind microtubules, leading to a loss of function and its progressive pathological accumulation. In the present study we examined changes tau (phosphorilation and cleavage) and cognition in the PFC long time following TBI in young (2 month) and old (16 month) C57BL/6J male mice. Briefly, mice received a unilateral cortical contusion to the right parietal cortex using a controlled cortical impact. Two months after TBI or sham surgery, PFC-related cognitive function was assessed using reversal learning radial arm water maze (RAWM). The test revealed a significant impairment in young animals that received TBI relative to the respective sham operated animals. Quantitative western blot analysis for tau and its multiple phosphorylated sites

was performed using AT8, pSer396 and tau-1 antibodies. TBI induced significant hyperphosphorylation of tau in the ipsilateral PFC of young mice relative to sham. Furtheremore, TBI induced the appearance of a small c-terminal derived tau fragment in the PFC of both young and old mice not apparent in sham mice. Interestingly, there was a direct correlation between the level of phorphorilated tau and the cognitive performances.
Our data demonstrate for the first time that TBI can induce post-translational modifications of tau and suggests these changes could have a crucial role in the development of TBI-induced cognitive dysfunctions associated with the PFC. Further characterization of the alterations of tau following TBI may provide a basis for pharmacological interventions as a viable treatment to prevent cognitive decline.

Disclosures: **L. Navarria:** Employment; University of California San Francisco. Other Research Support; Italian Ministry of Education. **J. Morganti:** Employment; University of California San Francisco. **A. Allen:** Employment; University of California San Francisco. **K. Belarbi:** Employment; University of California San Francisco. **E. Cuellar:** Employment; University of California San Francisco. **T. Jopson:** Employment; University of California San Francisco. **J. Fike:** Employment; University of California San Francisco. **S. Rosi:** Employment; University of California San Francisco.

Poster

864. Brain Trauma

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Title: Conditional knock out of beta-catenin in GFAP-expressing cells reduces GFAP-positive cell proliferation and immature neuron numbers after traumatic brain injury

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Abstract: β-catenin, known for its role in mediating the canonical Wnt signaling pathway and cell-cell interaction, can influence cell proliferation and differentiation. Astrocyte and neural progenitor cell (NPC) division rely on β-catenin, as does NPC differentiation into additional NPCs, new neurons, or new astrocytes. These cell populations are profoundly affected by traumatic brain injury (TBI) induced using a controlled cortical impact (CCI) model. CCI initially results in a dramatic loss of immature neurons while stimulating a delayed proliferation of NPCs and astrocytes, both of which stain positively for glial fibrillary acidic protein (GFAP). We hypothesize that β-catenin signaling is critical for both astrocyte and NPC proliferation following CCI. The present experiments utilize a novel transgenic mouse (cat-cKO) in which β-catenin signaling is conditionally knocked out in GFAP-expressing cells using the Cre-lox system, thereby affecting NPCs and astrocytes. Adult mice were injected with bromodeoxyuridine (BrdU) 4h prior to euthanasia at 72h post-injury, to label dividing cells at a time point of peak hippocampal proliferation. Dual immunofluorescent labeling for BrdU and cell-specific markers was used to identify cell phenotype. GFAP-positive (GFAP+) cell proliferation typically increases by 72h following CCI, while immature neuron numbers decrease. Our results confirm increased GFAP+ cell proliferation in wild-type (WT) mice (n=5) at 72h, while cat-cKO mice (n=6) show significantly less GFAP+ proliferation. The total number of immature neurons is also less in cat-cKO mice compared to WT mice at 72h, presumably due to decreased NPC proliferation and differentiation. Because microglial lineage is distinct from GFAP+ NPCs and astrocytes, microglial proliferation was examined as a control in the cat-cKO mice. Unexpectedly, compared to WT, cat-cKO mice show a decrease in microglial proliferation 72h post-CCI. We therefore examined a second cohort of TBI-injured animals, injected with BrdU 4h prior to euthanasia at 48h, a time at which there is minimal GFAP+ cell proliferation, but robust microglial proliferation. At 48h post-CCI, there is no effect of the cat-cKO (n=4) on microglial proliferation compared to WT mice (n=5), confirming the specificity of the GFAP-linked deletion, while also suggesting that proliferating GFAP+ cells participate in a feedback loop to enhance microglial proliferation. Future studies will explore downstream effects of β-catenin-dependent GFAP+ cell proliferation on neurodegeneration, synaptic/cognitive alterations, and blood-brain barrier integrity following trauma.

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Poster

864. Brain Trauma

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Program#/Poster#:864.05/N9

Topic: C.10. Trauma

Title: Tracking neuroprogenitor cells and mesenchymal stem cells following traumatic brain injury.

Authors: *A. DARKAZALLI[1,2], T. J. MORGAN[2], J. T. ROSENBERG[3], M. MUROSKI[4], G. STROUSE[4], S. C. GRANT[3], C. W. LEVENSON[2]
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Abstract: Stem cell-mediated therapies have a great deal of potential for the treatment of traumatic brain injury (TBI). We are developing novel magnetic resonance imaging (MRI)-based techniques to track the migration and fate of both endogenous stem cells that arise from the subventricular zone (SVZ) as well as mesenchymal stem cells (MSC) administered after TBI. Previous work has shown that neuroprogenitor cells residing in the SVZ are capable of endocytosing superparamagnetic iron oxide particles (SPIO) that can be tracked by MRI in vivo as they migrate along the rostral migratory stream (RMS). We have now shown that a similar approach can be used to monitor the migration of these cells to the site of TBI. Furthermore, our ability to track these cells at high resolution is enhanced by our use of ultra-high field MRI (900, MHz, 21.1Tesla) in vivo. Using this technology to track rodent and human bone marrow-derived MSC is facilitated by the intrinsic ability of these stem cells to migrate to lesions resulting from a wide variety of brain injury and disease. After receiving a TBI via controlled cortical impact, rats received an intra-carotid injection of 3x106 SPIO-labeled MSC which were localized by in vivo MRI to the penumbral region 48 h post-injury. No labeled cells were detected outside of this region of the brain. We have further refined our tracking ability by modification of fluorescent quantum dots with cell penetrating peptides to facilitate uptake by MSC as well as gene constructs designed to enhance both survival and tracking. Histological analysis confirmed MRI data showing migration of MSC to the penumbral region. Because this region that surrounds the site of injury is susceptible to secondary cell death, we are tracking the fate of these cells both temporally and phenotypically. Thus, these advances constitute a valuable tool for assessing the behavior of stem cells in preclinical models of brain injury and other models of neurodegenerative disease.

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Poster

864. Brain Trauma

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:864.06/N10

Topic: C.10. Trauma

Support: NIH-NINDS- R01NS069629

NICHD- R01HD059288

NINDS- 5T32NS007413-13

Title: Alterations in neuronal-glial metabolic coupling following lateral fluid percussion injury

Authors: *J. M. SEE[1], J. ELKIND[1], O. HORYN[2], I. NISSIM[2], E. DAIKHIN[2], I. NISSIM[2], A. COHEN[1]
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Abstract: Traumatic brain injury (TBI) often causes enduring disabilities including cognitive impairment and memory dysfunction. These functional deficits result from changes in hippocampal network excitability due to imbalances between excitatory and inhibitory synaptic activity, including decreased network excitability in CA1 and increased net synaptic efficacy in the dentate gyrus. Furthermore, neuronal and astrocyte metabolism following injury are altered, including increased accumulation of lactate and an increase in intracellular glutamate, but the interaction between astrocyte-derived metabolites and neuronal metabolism following injury is unclear. Thus, the purpose of this study is to investigate the changes in metabolic interactions between glia and neurons following injury. We have investigated the effects of fluid percussion injury (FPI) on cellular metabolism in hippocampus by treating hippocampal slices from injured and sham mice with 13C-labeled metabolic substrates, using 13C-acetate to specifically examine changes in astrocyte metabolism and 13C-glucose to investigate non-cell type specific metabolic changes. Samples were then analyzed to determine whether injury alters neurotransmitter production, amino acid metabolism or organic acid production. Previous data have shown that treatment with branched-chain amino acids (BCAAs) leads to improved functional recovery following injury, so we have also investigated the effects of BCAA treatment on hippocampal metabolism. Total neurotransmitter and amino acid concentrations are unchanged by injury, and acetate-derived 13C enrichment of aspartate and glutamate was also unchanged following FPI. Interestingly, acetate-derived 13C GABA and 13C glutamine enrichment are increased following FPI, suggesting that neuronal metabolism from glial-derived metabolites is augmented following injury. Addition of BCAAs returns GABA and glutamine enrichment to normal. To investigate the effects of hippocampal FPI on glucose metabolism, we incubated slices with 13C glucose and determined amino acid and neurotransmitter enrichment, finding that BCAA treatment alters glucose derived 13C incorporation into glutamate, aspartate, and alanine. These experiments suggest that changes in coupling of neuronal and glial metabolism may underlie and contribute to changes in excitability following TBI and that BCAA treatment mitigates these changes thus

indicating a possible mechanism underlying previously observed improvements in functional recovery.

Disclosures: J.M. See: None. **J. Elkind:** None. **O. Horyn:** None. **I. Nissim:** None. **E. Daikhin:** None. **I. Nissim:** None. **A. Cohen:** None.

Poster

864. Brain Trauma

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Support: Department of Veterans Affairs 003-40-4111

NIH R01 NS061934

Title: Chronic Pain after TBI: Thalamic abnormalities and prophylaxis with glyburide

Authors: *A. S. PARIHAR[1,2], M. J. SIMARD[2], K. KELEDJIAN[2], V. GERZANICH[2], A. KELLER[1]
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Abstract: In the US alone, about 1.4 million people suffer from traumatic brain injury (TBI) every year. TBI is an important health issue in both military and civilian life, and is very common among war-fighters. About one half of these individuals suffer not only from cognitive and motor deficits, but also from excruciating, unrelenting and chronic pain. In the current study, we aim to understand the mechanisms that lead from brain trauma to the development of chronic pain. We also explore the prophylactic potential of glyburide (GLY) to prevent the development of this devastating condition. The mechanisms of chronic headaches in general, and post-TBI pain in particular, are largely unknown. Considerable evidence, from both human and animal studies, indicates that this is the result of “central sensitization”, a process by which brain structures undergo maladaptive plasticity, resulting in abnormal activity of brain neurons. We have recently demonstrated that after spinal cord injury (SCI), such central sensitization occurs in the posterior thalamus (PO). Here, we test the hypothesis that TBI-Pain results in changes in PO similar to those after SCI. Testing for mechanical hyperalgesia revealed that TBI-Pain rats have a significantly lower tolerance to pain compared to the control group, and to TBI-Pain rats that received GLY. GLY (infusion of 200 ng/h of GLY yields plasma concentrations ~ 5 ng/ml, this has a minimal effect on serum glucose) was administered through mini-osmotic pumps implanted subcutaneously immediately after TBI surgery. Consistent with the behavioral data,

single unit electrophysiological recordings from the PO showed an increase in the spontaneous firing rate of neurons from TBI-Pain rats, compared to sham. Furthermore, histology from TBI-Pain rats revealed upregulation of SUR-1-regulated NCCA-ATP channel (molecular target for GLY), GFAP (astrocytic marker), and Iba1 (marker for microglial activation) in the thalamus of TBI-Pain rats. These data support the hypothesis that TBI-Pain is associated with maladaptive plasticity in the PO thalamus and that prophylactic treatment with GLY prevents TBI-Pain.

Disclosures: A.S. Parihar: None. **M.J. Simard:** None. **K. Keledjian:** None. **V. Gerzanich:** None. **A. Keller:** None.

Poster

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NIH R01 NS064247

Title: Detrimental neuroinflammatory response following TBI is mediated by microglia via p38α MAPK dependent mechanism

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Abstract: Neuropathologic damage following traumatic brain injury (TBI) is the result of both the immediate impact injury and secondary injury mechanisms. Post-traumatic glial activation with increased production of inflammatory molecules such as proinflammatory cytokines is a secondary injury mechanism that contributes to pathology progression in both animal models and human head injury patients. Moreover, following TBI the inflammatory response can result in persistently sensitized, or “primed” microglia, which can contribute to a chronic state of neuroinflammation. We recently demonstrated using in vitro models that p38α MAPK signaling in microglia is a key event in promoting cytokine production in response to diverse disease-

relevant stressors and subsequent inflammatory neuronal dysfunction. From these findings, we hypothesized that the p38α MAPK signaling pathway in microglia could be contributing to the secondary neuropathologic sequelae following a diffuse TBI. To test this hypothesis, we used a midline fluid percussion model to induce a diffuse brain injury in young adult wild-type (WT) mice or in conditional knockout mice genetically deficient in microglia p38α MAPK (p38α KO). From day 1-7 post-injury, motor coordination and balance were tested using a rotarod task. Injured WT mice showed a significant impairment on the rotarod task compared to sham-injured mice, while p38α MAPK deficiency in microglia prevented this TBI-induced impairment. Microglial activation was assessed by immunohistochemistry for three different microglial markers: IBA-1, CD45 and CD68. Mechanistically, we found that p38α MAPK was critical for microglia activation, as the p38α KO mice showed a dramatically reduced microglial activation response to the injury compared to the WT mice. The results suggest that stressor-induced activation of p38α MAPK signaling in microglia is a key event that primes microglia and leads to motor deficits following a diffuse TBI.

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Poster

864. Brain Trauma

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Topic: C.10. Trauma

Support: NIH-NINDS- R01NS069629

NICHD- R01HD059288

Title: Theta burst filtering by the dentate gyrus is diminished by brain injury and reinstated following treatment with branched chain amino acids.

Authors: J. WASSERMAN[1,2], B. N. JOHNSON[1], J. A. ELKIND[1], G. XIONG[1], *A. S. COHEN[1,2]
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Abstract: The dentate gyrus (DG) filters afferent input from the entorhinal cortex, and plays a pivotal role in preventing the spread of hyperactivity to area CA3. The ability of the DG to function as a filter requires particular and localized inhibition across microcircuits of the

hippocampal network, and is not well understood (Hsu 2007), but could provide new therapeutic targets for the reduced seizure threshold caused by traumatic brain injury (TBI). One of the ways in which TBI decreases inhibitory efficacy within dentate granule cells is by eroding the chloride gradient necessary for robust inhibition (Bonislawski et al., 2007). The resulting decrease in the driving force for GABAergic currents may explain why the increased frequency of mEPSCs and action potentials of GABAergic hilar inhibitory neurons after TBI has little effect on the overall excitability of granule cells (Hunt et al., 2011).
Systemic and *in vitro [[Unsupported Character - Codename]]*treatment with branched chain amino acids, which are precursors for *de-novo[[Unsupported Character - Codename]]* synthesis of glutamate and GABA, restores DG network excitability in slices from brain injured animals to levels recorded in slices from sham animals. Using later Fluid Percussion Injury (lFPI) together with voltage sensitive dye imaging we assessed the spatial and temporal dynamics of filtering efficacy in the dentate gyrus seven days post injury. We found that treatment with BCAAs restored normal excitability of the molecular layer and normal filtering of the propagated activity to the hilus following theta burst stimulation of the perforant path. Interestingly, depolarization in the hilus reached a plateau after the first theta burst event. Further experiments will aim to characterize the origin of this restored inhibitory efficacy following BCAA treatment and perhaps give insight into hilar area-mediated inhibition of cortical input into the dentate gyrus.

Disclosures: J. Wasserman: None. **B.N. Johnson:** None. **J.A. Elkind:** None. **G. Xiong:** None. **A.S. Cohen:** None.

Poster

864. Brain Trauma

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Topic: C.10. Trauma

Support: Swedish Research Council

Swedish Brain Foundation

Title: Regulation of antigen presention in cerebral cortex following traumatic brain injury

Authors: *C. ISRAELSSON, A. KYLBERG, L. T. HILLERED, T. EBENDAL
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Abstract: A unique and significant increase in the histocompatibility complex type II *H2-Aa* transcript was identified by unbiased analysis of cerebral cortex in response to traumatic brain injury using a mouse controlled cortical impact model (CCI). The indicated increase in MHC II antigen presentation made us turn to isolation of dendritic cell populations from neocortex three days after injury using anti-CD11c and anti-PDCA-1 antibodies coupled to magnetic beads. The results from qRT-PCR examination of this material directly link *H2-Aa* expression to CD11c/*Itgax*-positive conventional dendritic cells in the injured mouse brain. In order to suppress the increase of plasmacytoid dendritic cells in the injured mouse brain, we also administered antibodies directed against PDCA-1/*Bst2* positive cells. A significant reduction of the *Cxcl10* transcript was seen in the injured neocortex in mice receiving the anti-PDCA-1 antibodies compared to mice given equal doses of normal rat immunoglobulin. The results indicate that expression of the *Cxcl10* chemokine induced by injury, directly or indirectly depends on the presence of plasmacytoid dendritic cells. In contrast, expression of the *H2-Aa* transcript was induced to the same extent in the injured neocortex irrespective of the injected antibodies. This shows that the *H2-Aa* is not associated with the level of plasmacytoid PDCA-1 dendritic cells. Further cell types involved in injury responses were detected by *in situ* hybridization, demonstrating *Lyz2* expression in large phagocytic cells that also exhibited the cell surface marker isolectin B4 (IB4). In addition, small activated microglia were positive for IB4 in the injured neocortex and hippocampus three days post-injury but did not express *Lyz2*. The injury-induced cortical cavity formation was significantly reduced in *Ccr2*-/- mice seven days after injury in accordance with a reduction of *Lyz2*, adding support to the notion that *Ccr2* is a key receptor in recruitment of different invading peripheral cells. The importance of leukocyte invasion and activation in the development of antigen-presentation post-injury was confirmed by administration of cyclophosphamide to injured mice resulting in strong and selective reduction in several inflammatory-related transcripts e.g. *Ccr2*, *H2-Aa*, *Itgax* and *Bst2*. In contrast, *Cx3cr1* and *Ccl3* transcripts associated with resident microglia, were not affected. The data clearly demonstrate distinct functions of invading inflammatory cells of monocytic lineage separated from the roles of activated resident microglia.

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Poster

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Support: NIH-NINDS- R01NS069629

NICHD- R01HD059288

Title: Inhibitory neurons in specific regions of CA1 are affected differently by injury and vary in their sensitivity to Branched Chain Amino Acids

Authors: *B. N. JOHNSON[1], E. BOURGEOIS[1], J. WASSERMAN[1,2], J. A. ELKIND[1], G. XIONG[1], A. S. COHEN[1,2]
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Abstract: Neurological impairments associated with TBI include deficits in learning and memory and a reduction in seizure threshold. The hippocampus is critically involved in both of these phenomena, and is susceptible to damage from traumatic brain injury. In TBI, as in epilepsy and other CNS disorders, the balance between excitation and inhibition is disrupted. In the hippocampus, area CA1 becomes less excitable, while the DG becomes more excitable. The balance between inhibition and excitation in a network of neurons is set through a combination of synaptic and non-synaptic means. We focus here on the synaptic component, and on the decreased network excitability in area CA1. Previous work in our laboratory showed increased inhibition within CA1 after injury, which could be reduced to normal levels by treatment with branched chain amino acids (BCAAs), key precursors in the *de novo* synthesis of glutamate and GABA.

In order to identify the source of inhibition we characterized the spatial and temporal dynamics within the circuit by combining voltage sensitive dye imaging with simultaneous field recordings. Although we did find injury-induced decreases in both field potential and VSD signals when stimulating the Schaffer Collaterals in control aCSF, these diminished signals were NOT restored to normal by treatment with BCAAs. We also did not observe any obvious changes in inhibition, either with Schaffer collateral stimulation, or when stimulating in the alveus to directly activate interneurons in the oriens and cell body layer. We repeated these experiments in APV and CNQX in order to isolate inhibitory responses, and found a significant increase in hyperpolarization in the oriens when the stratum radiatum was stimulated, but no differences in oriens hyperpolarization when the alveus was stimulated. These findings are consistent with the hypothesis that interneurons with excitable elements in the distal radiatum, and synapses in the oriens, increase the strength of their projection to the oriens after injury. This inhibition in the oriens, however, was also insensitive to bath application of BCAAs.

Although the Schaffer collaterals provide the primary input to CA1, temporoammonic (TA) pathway fibers from the entorhinal cortex also synapse in CA1. We stimulated the TA pathway to test for changes in TA signaling, and preliminary evidence suggests that inhibition in SR is increased after injury, in a frequency-dependent manner, and restored to normal by BCAAs. We conclude that the BCAA mediated restoration of normal CA1 inhibition after injury occurs primarily via changes in the TA pathway, and not the Schaffer Collaterals.

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Poster

864. Brain Trauma

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Title: Lithium reduces bace1 overexpression, beta amyloid accumulation and spatial learning deficits in mice with traumatic brain injury

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Abstract: Traumatic brain injury (TBI) leads to both acute injury and long-term neurodegeneration, and is a major risk factor for developing Alzheimer's disease (AD). Beta amyloid (Aβ) peptide deposits in the brain are one of the pathological hallmarks of AD. Aβ levels increase after TBI in animal models and in patients with head trauma, and reducing Aβ levels after TBI has beneficial effects. Lithium is known to be neuroprotective in various models of neurodegenerative diseases and can reduce Aβ generation by modulating glycogen synthase kinase-3 (GSK-3) activity. This study explored whether lithium would reduce Aβ load after TBI and improve learning and memory in a mouse TBI model. Lithium chloride (1.5 mEq/kg, i.p.) was administered 15 minutes after TBI and once daily thereafter for up to three weeks. Western blotting analysis of Aβ and β-APP-cleaving enzyme-1 (BACE1) in the corpus callosum and hippocampus were performed at three days after injury. Immunostaining of Aβ, amyloid precursor protein (APP) and phospho-Tau were also carried out at the same time point. Enzyme-linked immunosorbent assay was further employed to evaluate Aβ42 levels in the corpus callosum and hippocampus after TBI. Results showed that lithium attenuated TBI-induced Aβ load increases, APP accumulation, and BACE1 overexpression in the corpus callosum and hippocampus at three days post-injury. Increased Tau protein phosphorylation in the thalamus

was also attenuated after lithium treatment following TBI at the same time point. Notably, lithium treatment significantly improved spatial learning and memory in the Y-maze test conducted 10 days after TBI, and in the Morris water maze test performed 17 to 20 days post-TBI, in association with increased hippocampal preservation. Thus, post-insult treatment with lithium appears to alleviate TBI-induced Aβ load through blockade of TBI-induced BACE1 overexpression and consequently improve spatial memory. The findings suggest that lithium is a potentially useful agent for managing memory impairments after TBI or other head trauma.

Disclosures: F. Yu: None. **Y. Zhang:** None. **D. Chuang:** None.

Poster

864. Brain Trauma

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Topic: C.10. Trauma

Title: Heat acclimation changes beta catenin fate after traumatic brain injury

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Abstract: Traumatic brain injury (TBI) triggers depressive behavior. Depression was earlier shown to be attenuated by inhibitors of glycogen synthase kinase-3 (GSK-3) which is also a potent regulator of cell apoptosis. TBI was shown to increase phosphorylation of GSK-3β and accumulation of its downstream target β -catenin in the hippocampus. Heat acclimation (HA, 30d at 34 ±1°C), a well studied pre-conditioning model, offers benefits against various types of stressors, including TBI (Shein et al 2007) where it reduces depressive behavior. We have observed that in HA mice the pre-TBI levels of brain derived neurotrophic factor (BDNF) are elevated and that Akt phosphorylation levels are increased immediately after TBI (SHein et al., 2008). HA also dramatically reduces apoptosis and caspase 3 activity (Umschwief et al., 2010). In the present study HA mice and their normothermic (NT) controls were subjected to TBI and 24h later we evaluated the changes in GSK3β, a downstream target which is inhibited by pAkt and in turn stabilizes β -catenin, preventing its phosphorylation and degradation. Using Western blot analysis, we found in HA mice elevated levels of pGSK3 β ($p<0.05$ vs NT). However, unexpectedly levels of p- β -catenin were higher in HA mice ($p<0.05$ vs NT), with no further degradation or reduced nucleus accumulation. To reconcile this apparent conflict, we

investigated the fate of β -catenin in NT and HA mice 24h after TBI. We found induction of β -catenin cleavage in NT mice and reduction in its cytosolic levels. On the other hand, co-immunopercipitation showed weaker interaction between β -catenin and membranal N-cadherin in HA mice ($p<0.05$ vs NT) possibly allowing β -catenin to participate in neuro-regeneration process. Elevated tyrosine phosphorylation of β -catenin, is a process downstream to BDNF activity. BDNF, which is elevated in HA mice after TBI, may disrupt β -catenin/N-cadherin interactions in HA mice, and reduce degradation of the cytosolic protein.

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Poster

864. Brain Trauma

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Topic: C.10. Trauma

Title: 12/15-LOX inhibition reduces neuronal cell death *in vitro* and secondary brain damage after experimental traumatic brain injury

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Abstract: Excitotoxicity and oxidative stress are important mechanisms contributing to posttraumatic brain damage. Our recent in vitro studies suggested a key role for lipooxygenases (LOX) in intrinsic death pathways involving mitochondrial demise and translocation of apoptosis inducing factor (AIF) to the nucleus. The aim of the present study was to differentiate the effects of 12/15- and 5-LOX inhibition in models of neuronal damage in vitro and in a model of traumatic brain injury (TBI) in vivo.
We used the specific inhibitors PD146176 (12/15-LOX) and YS121 (5-LOX) in different models of excitotoxicity and oxygen glucose deprivation (OGD) in HT-22 cells as well as in primary cortical neurons. In vivo, male C57bl/ 6 mice received different doses of each inhibitor 10 minutes before Controlled Cortical Impact trauma (impact velocity 8m/s, impact depth 1mm). Male 12/15-LOX null mice were used for knock out studies. Lesion volume, brain edema formation, neurological function, and ICP were assessed 24 hours after TBI.

12/15-LOX inhibition protected HT-22 cells and primary cultured neurons from glutamate- and OGD-induced cell death, respectively, while 5-LOX inhibition alone did not have any protective effect. In vivo, 12/15-LOX inhibitor PD146176 (0.4mg/kg) reduced lesion volume 24h after TBI and 12/15 LOX knock out mice showed a tendency towards neuroprotection. In keeping with the in vitro data, 5-LOX inhibition did not influence secondary brain damage after TBI.
In conclusion, 12/15-LOX but not 5-LOX inhibition attenuated neuronal cell death in vitro and posttraumatic brain damage in vivo. Therefore 12/15 LOX may represent a novel therapeutic target for the prevention of secondary brain damage following TBI.

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Poster

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Title: mRNA translation is altered in an *in vitro* model of traumatic brain injury.

Authors: *R. MOTT, D. F. MEANEY
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Abstract: Developing effective therapies for traumatic brain injury (TBI)-impaired learning and memory is challenging because the underlying pathology is not fully understood. Neuronal apoptosis and necrosis are well documented mechanisms for dysfunction, but many studies indicate cognitive deficiencies in the absence of neuronal cell death. Changes in receptor expression after TBI suggest the existence of an injury induced ‘pathological plasticity’ that alters the function of the surviving cells. A key calcium-dependent, mechanism implicated in plasticity is dendritic mRNA translation. Previous fluid percussion injury studies by others (Atkins et al., JCBFM, 2006 and Chen et. al, JCBFM, 2007) found CaMKII, CPEB and mTor pathways to be activated in the cortical and hippocampal tissue of injured rats. This in vitro study measures the impact of moderate stretch (55-70%) injury on all three pathways, which are key regulators for both dendritic and global mRNA translation. We find the S6 ribosomal protein, a downstream effector of mTor, and CaMKIIα pathways to be rapidly activated by stretch injury of rat cortical cultures. S6 activation was significant within 30 min of stretch injury (163±17% of

control, n=5, p<.01) and remained elevated for at least 1 hour post injury (166±32% of control, n=5, p<.05). CamKIIα was significantly elevated within 10 min of stretch (122±9.8% of control, n=4, p<.05). The S6 activation was both NMDAR and mTor-dependent, as it could be blocked by pretreatment with APV or rapamycin, but the voltage-gated calcium channel blocker, nimodipine, failed to suppress S6 activation. The impact of stretch injury on mRNA translation was also directly measured using an in vitro imaging assay; changes in translation of dendritically localized mWasabi-tagged proteins were found within minutes of stretch injury. Overall translation rate was measured as the change in fluorescence over time, and increased approximately 5-fold with injury (13.1% vs. 2.6% increase in fluorescence over a ten minute period, n=10 cells, p<.05). Interestingly, the impact of moderate stretch had a negative effect on the activation of CPEB; the phosphorylation of CPEB at Thr171 decreased to 82% of control values within 10 min of injury (n=4, p<.05) under an NMDAR-dependent mechanism. The results of this study suggest that mechanical injury can alter the expression of dendritic proteins via modulation of translational pathways; future studies will aim to determine the impact on individual mRNAs under the regulation of various *cis*-elements including the CPEB regulated CPE.

Disclosures: R. Mott: None. **D.F. Meaney:** None.

Poster

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Title: Nrf2 activator induced improvement after mild traumatic brain injury in mice may involve HSP70

Authors: *B. A. CITRON[1,2], L. RACHMANY[3], H. HATIC[1], A. SHAER[3], V. RUBOVITCH[3], C. G. PICK[3], J. N. CHANG[1,2]
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Florida Morsani Col. of Med., Tampa, FL; [3]Anat. and Anthrop., Sackler Sch. of Medicine, Tel Aviv Univ., Tel Aviv, Israel

Abstract: Traumatic brain injury (TBI) is currently untreatable and is the signature affliction of modern deployed forces. Over 300,000 military personnel have received these injuries and the worldwide prevalence is about 0.5% with persistent effects on patients, families, and our healthcare system. We have investigated traumatic brain injury models in vivo and in vitro with the goal of enhancing our understanding of the mechanisms that contribute to TBI induced neurodegeneration. The model systems for these experiments included a closed head, weight drop injury with mice and a rapid, biaxial stretch (mimicking TBI torsional insult) with model neurons in culture. We have previously shown that multiple administrations of tert-butylhydroquinone (tBHQ), an activator of transcription factor Nrf2, result in improved cognitive performance after mild traumatic brain injury (mTBI) and upregulation of HSP70. Here we describe the improvement seen with a single administration of the Nrf2 activator at 30 minutes after injury and that model neurons exposed to mTBI in culture are also protected by tBHQ and that this neuroprotection is blocked by an inhibitor of HSP70. These results may facilitate the identification of therapeutic approaches that could positively affect biological processes occurring during injury-induced loss of neuronal function in the brain and prevent the lasting consequences of these injuries.

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Poster

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Topic: C.10. Trauma

Title: The adaptive arm of ER stress - a therapeutic target in traumatic brain injury.

Authors: V. RUBOVITCH, R. BARATZ-GOLDSTEIN, A. SHACHAR, S. EDUT, *C. G. PICK
Tel Aviv Univ., Tel Aviv, Israel

Abstract: We have previously reported that mild traumatic brain injury (mTBI) induced various short and long term cognitive deficits as well as apoptosis in the mice brains. Apoptosis may be caused by prolonged and severe accumulation of misfolded proteins and protein aggregation in the endoplasmic reticulum (ER). ER stress enables the cell to adapt and survive by upregulating

translation of stress response proteins and attenuating global protein synthesis. Insulin like growth factor (IGF-1) is known to be neuroprotective. Hence, the present study was aimed at establishing a novel mechanism for IGF-1 neuroprotection, which involves the adaptive arm of the ER stress. As expected, IGF-1 administration significantly improved memory deficits following mTBI (as assessed by the Y-maze and the Novel Object recognition tests). In addition, mTBI induced a time-dependant elevation of the ER stress sensor protein ATF6 (Activating transcription factor 6). IGF-1 administration had a significant effect on CHOP (C/EBP homologous protein) and phospho-eIF2-alpha (elongation initiation factor alpha) expression following mTBI as was seen by western blot analysis. In addition, immunofluorescent examinations revealed that under IGF-1 administration CHOP was significantly co-localized with phospho-eIF2-alpha in the cortices and hippocampi of mTBI mice. These results confirmed our working hypothesis that IGF-1 exerts at least part of its neuroprotection via the adaptive pathway of the ER stress. A direct activation of eIF2-alpha by the selective phosphatase inhibitor Salubrinal, completely abolished the mTBI-induced damage to the cognitive deficits. Altogether, these results reveal a novel neuroprotective mechanism for IGF-1 and suggest the adaptive eIF2-Alpha pathway as a possible target for pharmacological therapy.

Disclosures: V. Rubovitch: None. **R. Baratz-Goldstein:** None. **A. Shachar:** None. **S. Edut:** None. **C.G. Pick:** None.

Poster

864. Brain Trauma

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:864.18/O2

Topic: C.10. Trauma

Support: Neurotrauma Research Program WA

NHMRC 572550

NHMRC 1002347

Title: Myelin sheath decompaction, axon swelling and functional loss during chronic secondary degeneration in rat optic nerve

Authors: *S. A. DUNLOP[1], S. C. PAYNE[2], C. A. BARTLETT[2], A. R. HARVEY[3], M. FITZGERALD[2]
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Abstract: Purpose: To examine disruptions in optic nerve (ON), axons, myelin and visual function during the chronic phase of secondary degeneration, up to 6 months after partial injury.
Methods: Dorsal ON axons were transected, leaving ventral ON as the area vulnerable to secondary degeneration. At 3 and 6 months following partial ON transection, toluidine blue stained sections were used to assess dimensions of the ON injury site. Transmission electron microscopy (TEM) images of ventral ON were used to quantify numbers, diameter, area and myelin thickness of axons with normal and decompacted myelin. Immunohistochemistry (MBP, β-III tubulin) and fluoromyelin staining were used to semi-quantitatively assess myelin protein, lipids in ventral ON, and RGCs in mid-ventral retina. Visuomotor function was assessed using the optokinetic nystagmus test.
Results: Ventral ON and axons, vulnerable to secondary degeneration, as well as visual function remained disrupted 6 months following partial ON transection. Although ventral ON swelling observed at 3 months ($p \leq 0.05$) was no longer seen at 6 months, at the ultra-structural level, myelinated axons remained swollen ($p \geq 0.05$) and myelin thickness had increased ($p \leq 0.05$) due to loosening of lamellae. Axons with decompacted myelin persisted and were distinguished as having large axonal calibres. Progressive loss of myelin lipids was also seen at 6 months. Despite no further loss of ventral axons between 3 and 6 months ($p \geq 0.05$), visuomotor function had progressively declined by 6 months following partial ON transection ($p \leq 0.05$).
Conclusion: Decompaction of myelin and the swelling of myelinated axons are persistent features of secondary degeneration and likely contribute to progressive loss of visual function.

Disclosures: S.A. Dunlop: None. **S.C. Payne:** None. **C.A. Bartlett:** None. **A.R. Harvey:** None. **M. Fitzgerald:** None.

Poster

864. Brain Trauma

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Topic: C.10. Trauma

Support: NJCBIR 09-0003-BIR1 to VS, 11-0003-BIR2 to VS and BJP

Title: Similar enhancement in dentate network excitability despite marked differences in early cellular injury following fast- and standard-rate concussive brain trauma.

Authors: *E. J. NEUBERGER[1], R. A. WAHAB[2], A. JAYAKUMAR[1], B. J. PFISTER[2], V. SANTHAKUMAR[1]
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Abstract: The primary cause of concussive brain injury can include a wide spectrum of insults ranging from explosions in combat to falls by the elderly. Accordingly, the kinematics of the trauma can vary in key features including the rate and magnitude of the insult. In the fluid percussion injury (FPI) model, the effect of peak injury pressure on neurological outcome has been well examined. However, it is unknown whether differences in the rate of rise of the injury waveform can lead to distinct cellular and physiological changes in the hippocampus. We created a novel programmable voice-coil driven FPI device capable of producing pressure waves with different rise times to address this question.
Young adult male rats (25 day) were subject to lateral FPI at a constant peak pressure (2 atm) with a rise time of either 3 ms (fast-rate) or 10 ms (standard-rate of conventional FPI). Histological and physiological studies were conducted in hippocampal slices obtained 4-6 hours and 7-10 days after FPI and in sham-injured controls. Fast-rate FPI resulted in immediate behavioral seizures and fatalities in significantly fewer rats than after standard-rate FPI. Numerous hilar neuronal profiles were labeled by the Gallyas stain indicating extensive mechanical injury after standard-rate FPI. In contrast, only a few hilar neurons were labeled after fast-rate FPI. Similarly, FluroJade staining immediately after fast-rate FPI revealed fewer degenerating neurons in the dentate hilus and CA1 compared to standard-rate FPI. One week after injury, hippocampal slices from rats subject to both fast- and standard-rate FPI showed a significant enhancement of the perforant path-evoked granule cell field responses compared to sham controls. Notably, despite the early differences in cellular injury, the afferent-evoked dentate population spike amplitude measured one week after fast- and standard-rate FPI was not different.
These data demonstrate the rate of rise to peak pressure of the injury waveform plays a crucial role in determining the extent and subtype specificity of regional cell loss. Our data indicate that reduced cellular damage and improved immediate neurological outcome, may obscure the severity of neurophysiological changes after fast-rate injury. Our findings suggest that strategies used to assess and manage patients with relatively slower civilian TBI may not directly apply while evaluating patients with fast-rate, blast-related TBI.

Disclosures: E.J. Neuberger: None. **R.A. Wahab:** None. **A. Jayakumar:** None. **B.J. Pfister:** None. **V. Santhakumar:** None.

Poster

864. Brain Trauma

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Topic: C.10. Trauma

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Chinese Natural Science Foundation 81020108021, 81171149, 81150110494, 81000497

Title: Scriptaid, a new histone decetylase inhibitor protects against tramatic brain injury via modulation of PTEN and the AKT pathway

Authors: *G. WANG[1,2,3], H. PU[1], X. JIANG[1], W. ZHANG[1], X. HU[2], A. LIOU[2], Y. GAO[1,2], J. CHEN[2,1,4]

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Abstract: Traumatic brain injury (TBI) is a leading cause of motor and cognitive deficits in young adults without an effective therapeutic agent to date. Treatment with histone deacetylase inhibitors (HDI) limiting deacetylase activities have shown to preserve motor and cognitive function after TBI. In this study, we characterized the protective capacity and mechanism of a new inhibitor of class I and II histone deacetylase, *Scriptaid*, on injury induced by controlled cortical impact (CCI).

Scriptaid when introduced at doses between 1.5 to 5.5 mg/kg, 30 minutes post-injury, elicited a dose-dependent decrease in lesion size as well as a concomitant attenuation in motor and cognitive deficits as determined by wire-hanging, cylinder, foot fault and Morris water maze tests. Furthermore, protection against neuronal degenration and behavioral deficits from *Scriptaid* was observed 35 days post-injury and when its administration was delayed to 12 hr post-injury demonstrating its long term efficacy with relative wide therapeutic window for this HDI. The improvement in neurobehavioral functions conferred by *Scriptaid* was also mirrored by a corresponding increase in surviving neurons within the CA3 region of the hippocampus. To ascertain the protective mechanism of *Scriptaid*, we looked for the activation of various protective pathways. Immunohistochemical staining and Western blot analysis revealed a decrease in phopho-AKT (pAKT) and phosphorylated phosphatase and tensin homolog deleted on chromosome 10 (pPTEN) after TBI in the cortex and CA3 region with no detectable change in the total AKT and PTEN protein levels. Mechanistically, the decrease in pPTEN stabilizes PTEN which in turn prevents the phosphorylation of AKT. However, this decrease in pAKT and

pPTEN were reversed after treatment with *Scriptaid*. As expected, further addition of LY294002 (an inhibitor of the AKT pathway) abolished the protection conferred by *Scriptaid* suggesting its main protection was derived from the activation of the AKT pathway. In summary, our study has shown that *Scriptaid* is an effective pharmacological agent that modulates PTEN and the AKT pathway to protect against TBI injury.

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Poster

864. Brain Trauma

Location: Hall F-J

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Program#/Poster#:864.21/O5

Topic: C.10. Trauma

Title: Cognitive amelioration of mild traumatic brain injury by exendin-4 and PFTα

Authors: *L. RACHMANY RABER[1], D. TWEEDIE[2], V. RUBOVITCH[1], Q.-S. YU[2], N. H. GREIG[2], C. G. PICK[1]
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Abstract: Traumatic Brain Injury (TBI) annually impacts 1.7 million Americans, causing considerable suffering, with no effective current treatment. Mild TBI (mTBI) accounts for 80% of TBI events and can have enduring consequences. Prior studies have shown that mTBI may lead to long-term cognitive, emotional, and behavioral deficits. Additionally, mTBI-triggered changes in the gene expression, biochemical cascades and apoptotic neuronal degeneration, particularly in the cortex, hippocampus and forebrain, provides a potential gateway to late-term disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Two different therapeutic approaches were assessed to halt mTBI neuronal apoptosis and trigger reparative processes: exendin-4 (Ex-4), a long-acting glucagon-like peptide-1 (GLP-1) receptor agonist approved for type 2 diabetes treatment, and the experimental anti-apoptotic tetrahydrobenzothiazole analogue, pifithrin-α (PFTα). Both agents readily enter the brain, and whereas Ex-4 has demonstrated neurotrophic/neuroprotective actions in cellular and animal

models of AD and PD, and is in current clinical assessment for these disorders, PFTα, which inhibits p53-mediated translocation and lowers transcription, has been reported to prevent neuronal loss in cellular and animal models of stroke, PD and AD. mTBI was induced by a non-invasive closed-head weight drop device and 30 g weight, and behavioral and cognitive exams were conducted either 7 or 30 days post trauma. Mice subjected to mTBI demonstrated a deficit in visual memory compared to sham control mice at both assessment times. Administration of either Ex-4 or PFTα post-mTBI proved well tolerated, fully ameliorated this impairment, and may provide a clinically translatable treatment strategy.

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Poster

864. Brain Trauma

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Program#/Poster#:864.22/O6

Topic: C.10. Trauma

Support: New Jersey Commission on Brain Injury Research

Title: Inhibition of p75 pathway improves outcome following traumatic brain injury

Authors: *J. ALDER, W. FUJIOKA, A. GIARRATANA, B. PATEL, T. CHAKRABORTY, A. PARIKH, H. GIRN, S. THAKKER-VARIA
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Abstract: Following traumatic brain injury (TBI), changes in metabolic, cytotoxic and apoptotic processes lead to pathophysiological alterations in the cortex and hippocampus. Along with mature neurotrophins' well-established role of regulation in neural development and adult synaptic plasticity in the hippocampus and cortex, the precursor form of neurotrophins, the pro-neurotrophins, are known to control neuronal apoptosis and long-term depression. Studies indicate that Brain-Derived Neurotrophic Factor (BDNF) is transiently elevated after TBI. However, the increase in expression of the trophic factor in the brain following TBI is not sufficient to support complete regeneration, suggesting that the role of BDNF in pathological conditions is not entirely understood. As it is not yet known whether the mature BDNF and its receptor TrkB which supports cell survival or the proBDNF isoform and its receptor p75 which causes cell death are upregulated following TBI, the consequences of the increased BDNF levels for recovery processes remain to be elucidated. We hypothesize that the toxic proBDNF/p75 pathway is the predominant one upregulated after injury, suggesting that it may be partly

responsible for the deleterious consequences of brain injury. We are using lateral fluid percussion injury (LFP) to model moderate TBI and examine whether there is increased signaling through p75 receptor either through upregulated proBDNF and/or p75 expression. Our results indicate that p75 is upregulated and trkB is downregulated after LFP in the hippocampus and cortex. To reveal the identity of the cells expressing the specific BDNF receptors, immunohistochemistry was performed to co-localize trkB or p75 to specific cell types. p75 receptors were found to be associated predominantly with astrocytes after TBI and trkB with neurons. To determine whether lack of p75 signaling confers protection against neuronal damage following TBI, we have used activated caspase-3 and Flouro-Jade C to quantitate neural cells undergoing cell death and neurodegeneration in wild type and p75 null mice. Our ultimate goal is to study whether the impairment of cognitive and motor function following TBI is reduced in the absence of p75 signaling. Behavioral experiments demonstrate that there is improved response in the vestibulomotor rotarod test and in the spatial learning Morris Water Maze test in mice with reduced p75 following LFP when compared to wild type mice indicating that p75 signaling pathway may contribute to the damage following TBI. We are currently using small molecules to inhibit the p75 pathway and activate the TrkB pathway to measure histological and behavioral outcomes following injury.

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Poster

864. Brain Trauma

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Topic: C.10. Trauma

Support: CNRM grant [G1703O] to ZG

Career development grant [G1708X] to MKJ

Title: *In vivo* and *in vitro* neuronal and astro-vasculature correlates in the somatosensory cortex after traumatic brain injury

Authors: *M. K. JAISWAL[1,2], F. W. LISCHKA[1,2], Z. GALDZICKI[1,2]
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Abstract: Clinical evidence indicates that TBI is linked to the development of dementia and cognitive dysfunction, suggesting that TBI may damage nearby neurons, astrocytes and associated neuro-astro-vasculature coupling. In addition previous in vitro work has shown that a balance between excitation/inhibition networks has been disrupted in major neurological disorders like epilepsy, schizophrenia and autism. We hypothesize that TBI impacts the balance of excitatory/inhibitory sensory circuits with vascular defects exacerbating neuronal recovery. In this study we focused on the assessment of network activity by 2-photon in vivo imaging to monitor activity of excitatory/inhibitory neurons in GAD67-GFP knock-in mice. In order to control for the impact of vascular injury on neuronal activity, we monitor vascular changes caused by damage to the integrity of the BBB with dye specific for blood vessels/arteries with simultaneous recordings of calcium transients in neurons and astrocytes by 2-photon imaging in cortical tissue followed by TBI.
2-photon calcium imaging revealed a decrease in neuronal activity (higher number of hypoactive neurons) in excitatory/inhibitory layer 2/3 cortical neurons within 300-500µm from injury epicenter in TBI mice. At the same time some of the neurons displayed an unexpected increase in their calcium transients (hyperactivity) in the frequency of spontaneous Ca2+ transients in TBI neurons in comparison to sham littermates. Electrophysiological characterization of the neurons in the vicinity of the injury in TBI mice shows altered spontaneous activity in comparison to the sham control. In excitatory neurons of TBI mice we have observed a shift of cumulative probability towards the higher amplitude of spontaneous EPSCs in comparison to sham control, whereas opposite trend have been seen in fast-spiking inhibitory neurons. Overall, our results suggest desynchronization of neocortical activity following TBI with an impaired inhibition underlying hyperactivity/hypoactivity of excitatory neurons possibly due to disruption of feedback inhibition. In order to preserve intact circuitries and maintaining blood supplies to cortex in vivo approach has been initiated in combination with electrophysiology in a mouse model of TBI. The 2-photon recordings will be done in parallel with vascular assessment to monitor multi-network calcium imaging to identify alterations in network dynamics produced by TBI in correlations to vascular damage. In summary, our results suggest that dysfunctional cortical circuit likely contributes to the pathophysiology of TBI and the vascular damage plays an important role in pathophysiology of TBI.

Disclosures: M.K. Jaiswal: None. **F.W. Lischka:** None. **Z. Galdzicki:** None.

Poster

864. Brain Trauma

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Topic: C.10. Trauma

Support: NIH-NINDS- R01NS069629

NICHD- R01HD059288

Title: Mild Traumatic Brain Injury affects excitatory synaptic transmission in the medial prefrontal cortex, an area distant from injury site

Authors: *C. SMITH[1,2], C. P. PALMER[2], G. XIONG[1], J. A. ELKIND[1], O. VENEGAS[2], B. PUTNAM[2], A. S. COHEN[1,2]
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Abstract: Traumatic Brain Injury (TBI) affects more than 1.5 million people in the United States each year and even mild TBI can lead to a vast array of long-lasting neurological impairments (Rutland-Brown et al., 2006). Despite its prevalence, currently no therapy exists to mitigate or treat the underlying causes of cognitive impairments suffered by TBI patients. TBI predominantly causes damage to the frontal and temporal lobes of the cerebral cortex; this damage causes significant neuropsychological sequelae, including persistent memory deficits that disrupt the lives of TBI survivors (Stuss, 2011). The medial prefrontal cortex (mPFC) is critically involved in higher cognitive functions, including memory, that are disturbed by mild traumatic brain injury (mTBI). In order to model mTBI in mice we employed lateral fluid percussion injury (LFPI). LFPI is a commonly used rodent model of brain injury that reproduces many key features of human TBI including neuronal cell loss, gliosis, ionic perturbation and memory deficits (Dixon et al., 1987, McIntosh, 1987, 1989, Smith et al., 1991). Following an LFPI designed to mimic an mTBI we investigated alterations to synaptic transmission in the prelimbic cortex, the functional homolog of human mPFC. After mTBI, amplitude of presynaptic fiber volleys from layer 2/3 stimulation as well amplitude of field excitatory postsynaptic potentials recorded in layer 5 were reduced in brain-injured mice. When fiber volley size was normalized, fEPSPs were significantly reduced in slices derived from brain-injured mice. Furthermore, whole-cell patch clamp recordings revealed that the frequency and amplitude of spontaneous and miniature excitatory postsynaptic currents (sEPSCs, mEPSCs) were increased in layer 2/3 neurons in brain slices derived from brain-injured animals. Investigations regarding excitatory currents in layer 5 neurons, as well as anatomical analyses of both layer 2/3 and layer 5 neurons are ongoing. These results demonstrate that excitatory synaptic transmission in the mPFC is functionally altered by mTBI and may contribute to observed behavioral and cognitive deficits.

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Poster

864. Brain Trauma

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Support: Aresty Foundation

UMDNJ-RWJMS Start-Up Funds

NIH grant NS065183

Title: Structural and molecular adaptions induced by traumatic brain injury (TBI) in mice

Authors: *D. P. CROCKETT, K. P. BALDEV, K. K. PATEL, R. R. BHAVSAR, V. L. DIBONA, M. R. RASIN, H. ZHANG
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Abstract: At least 1.4 million people each year suffer from traumatic brain injury (TBI) with the cost to the economy estimated to be in excess of $60 billion according to the CDC. Even mild TBI can cause a wide range of pathologies including diffuse axonal injury, secondary cell death (including oligodendrocytes) and inflammation, which may be progressive, affecting the deep white matter and disrupt local circuitry distant from the focal injury site. These consequences can lead to continued loss of function. We have been studying the biology of TBI in mice subjected to either lateral fluid percussion injury (LFPI) or controlled cortical impact injury (CCI). We have found plastic changes of the homologous region of the contralesional neocortex 7 and 30 days post-TBI. In particular, we observed altered dendritic structure revealed by decreased SMI32 (hypo-phosphorylated neurofilament) immunoreactivity in projection neurons of layers 2 and 3. Our data suggest that cell death does not seem to account for these observations in the contralesional cortex. However, evidence of apoptosis was observed in the ipsilateral hemisphere. In order to elucidate the underlying mechanisms responsible of TBI induced changes in projection cell polarity, we stained for importin 13 (IPO13) and eukaryotic initiation factor 4e (eIF4e), which were reported to be disrupted in the contralesional neocortices after a hemisection spinal cord injury (SCI) (Thompson et al., 2010). Surprisingly, immunostaining levels for IPO13 and eIF4e were unchanged in the contralesional cortex at 7 days post TBI. These data suggest that TBI induces acute adaptive changes in contralesional neocortices that are different than those induced by hemisection SCI. Indeed, we found an increased expression of histone deacetylase 1 (HDAC1) and β-amyloid precursor protein within the corpus callosum that are indicative of axonal pathology and likely disruption of inter-hemispheric communication following TBI. We plan to further explore the mechanisms that underlie the reorganization of

cortical microcircuitry following TBI using transgenic mice expressing fluorescent markers (e.g., Thy1-YFP expressing mice) and 2-photon microscopy. This will allow us to further define injury-induced alterations (both structural and molecular) in cortical microcircuitry.

Disclosures: D.P. Crockett: None. **K.P. Baldev:** Research Grant; Aresty Foundaton, Rutgers University. **K.K. Patel:** None. **R.R. Bhavsar:** None. **V.L. DiBona:** None. **M.R. Rasin:** Other Research Support; UMDNJ-RWJMS Start-Up Funds. **H. Zhang:** Research Grant; NIH grant NS065183. Other Research Support; UMDNJ-RWJMS Start-Up Funds.

Poster

864. Brain Trauma

Location: Hall F-J

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Program#/Poster#:864.26/O10

Topic: C.10. Trauma

Title: Cellular alterations in human traumatic brain injury: Characterization of astrocyte early and late morphologic changes within the first week post Injury

Authors: I. BALAN[1], A. SALADINO[2,3], R. CASTELLANI[2], B. AARABI[4], H. EISENBERG[5], C. WADE[6], D. STEIN[6], *D. SCUTELLA[1], G. FISKUM[1]
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Abstract: Astrocytes are thought to play a critical role in the survival of other cellular cortical tissue elements, and their response to a significantly altered cellular environment is often reflected in morphologic changes. Our study is the first to characterize time-based astrocytic changes after human TBI and to compare these changes with human control tissue removed in the course of surgical treatment of temporal lobe epilepsy.
Cerebral cortex samples were surgically resected from 17 patients with a clinical diagnosis of blunt TBI and from one epilepsy patient with partial onset seizures. All procedures were approved by our Institutional Review Boards.
The astrocytes of the control cerebral tissue were characterized by round to oval nuclei with a thin rim of condensed chromatin at the nuclear membrane. Peripheralized nucleoli rarely were present. Perinuclear cytoplasm was scanty and often not visible. The astrocytic perivascular foot processes surrounding capillaries were thin profiles containing few cytoplasmic organelles.
At 6 hours post-injury, there was swelling of perivascular astrocytic end-feet as well as some astrocytic foot processes surrounding neuronal cell bodies Perikarya of those neurons showed a

reciprocal decrease in the cross sectional area of their cytoplasm.
At 38 hours post-injury, there was increased expansion of astrocytic cytoplasm in perikarya and proximal processes, the astrocytic nuclei were larger and paler as compared with those of TBI cerebral tissue resected at 6 hours post-injury. The perivascular end-feet were remarkably swollen and many of them were degenerated. The swollen astrocytic end-feet compressed the microvessels and appeared to induced microvascular obstruction as evidenced by red blood cell stasis. There was an increased number of advanced swollen and degenerated astrocytic end-feet surrounding neuronal cell bodies. Some of these neurons showed advanced levels of degeneration. Paired astrocytic nuclei occasionally were seen.
By 7 days post-injury, the majority of swollen astrocyte cell bodies and their foot processes had become necrotic; and microvascular compression as evidenced by stasis had almost disappeared. Astrocyte proliferation and hypertrophic astrocytes with multiple nuclei (2 and more) were frequently seen.
This study shows that over the course of the first seven days after human TBI the astrocytic population undergoes a variety of both degenerative and proliferative changes while in contact with the microvasculature and with neurons.

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Poster

865. Neuroprotection: Natural Products

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:865.01/O11

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: USDA-Intramural

Title: The effects of blueberry supplementation in middle aged mice consuming a high fat diet.

Authors: *A. N. CAREY, B. SHUKITT-HALE
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Abstract: Consuming a high fat diet may result in behavioral deficits that are similar to those observed in aging animals, possibly because of increased brain inflammation and oxidative stress. Our lab has demonstrated that diets supplemented with polyphenolic-rich berries, such as blueberries, can allay behavioral deficits associated with aging. Thus, it stands to reason that supplementation of a high fat diet with blueberry may offer protection against these putative high fat diet-related declines. Nine month old C57Bl/6 mice were maintained on one of four diets for

a five month period: low fat (Harlan, TD.08806, 10% calories from fat), low fat + 4% blueberry (freeze-dried extract, provided by the U.S. Highbush Blueberry Council), high fat (TD.06414, lard-based, 60% calories from fat), and high fat + 4% blueberry. Mice were tested with the novel object recognition memory task after 2, 3, and 4 months on the diets. Mice were also tested in the Morris water maze for spatial memory after 5 months on the diets. Studies are ongoing, but preliminary data suggest that recognition memory performance was impaired by high fat diet and blueberry-supplementation prevented some of the recognition memory deficits. The goal of the study is to begin to determine if making small changes to a poor diet, like incorporating more nutrient dense foods, may allay or prevent cognitive dysfunction associated with consumption of a high fat diet.

Disclosures: A.N. Carey: Employment; USDA-Intramural. **B. Shukitt-Hale:** Employment; USDA-Intramural.

Poster

865. Neuroprotection: Natural Products

Location: Hall F-J

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Program#/Poster#:865.02/O12

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: USDA intramural grants from agreements between USDA-ARS and California Walnut Commission

Title: Serum metabolites from walnut-fed aged rats attenuate stress-induced neurotoxicity in brain cells *in vitro*

Authors: S. M. POULOSE, D. R. FISHER, *D. F. BIELINSKI, B. SHUKITT-HALE
Neurosci. & Aging Lab., USDA Human Nutr. Res. Ctr, Tufts Univ., BOSTON, MA

Abstract: The shift in equilibrium towards excess reactive oxygen or nitrogen species production from innate antioxidant defense in brain is a critical factor in the declining neural functions and cognitive deficits accompanying age. In aging, there are noticeable alterations in the membrane microenvironment, with increased oxidized proteins and lipids, altered calcium buffering, accumulation of damaged organelles and protein structures, weak neurotransmitter release, and dystrophy and death of neurons. Previous studies from our laboratory have reported that walnuts, rich in polyphenols, antioxidants, and omega fatty acids such as alpha linoleic acid (ALA) and linolenic (LA), improve the age-associated declines in cognitive and neural deficits in rats. Possible mechanisms of action of these effects include enhancing protective signaling, altering membrane microstructures, decreasing inflammation, and preventing accumulation of

polyubiquitinated protein aggregates in critical regions of the brain. In the current study, we investigated whether the serum collected from aged animals fed with walnut diets (0, 6% and 9%) would enhance protection on stressed BV2 microglial and HT22 hippocampal neurons, in vitro. In the growth medium, fetal bovine serum (FBS) was substituted with the serum collected from 22 month old rats fed with either walnut or control diets for 12 weeks. Walnut diet serum (6 and 9%) significantly attenuated dopamine-(DA-)induced neurotoxicity, as measured by recovery from calcium influx in potassium chloride-(KCl-)induced depolarized hippocampal neurons. Additionally, walnut diet serum (6 and 9%) significantly attenuated lipopolysaccharide-(LPS-)induced nitrite release in the microglial cells. Neither control serum nor FBS alone (negative control) elicited similar rescuing effects on either type of brain cell. While identification of the individual metabolites, as well as the measurement of oxidant/antioxidant enzymes in these sera are being done, preliminary results strongly suggest protection or enhancement of membrane-associated functions in brain cells by walnut serum metabolites.

Disclosures: S.M. Poulose: None. **D.R. Fisher:** None. **D.F. Bielinski:** None. **B. Shukitt-Hale:** None.

Poster

865. Neuroprotection: Natural Products

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Program#/Poster#:865.03/O13

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: USDA Intramural

Title: Human cognition and mobility in aging: A model for berry fruit interventions

Authors: *M. G. MILLER[1], B. SHUKITT-HALE[2]
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Abstract: Changes in motor function in aging, in both animals and humans, include decrements in balance, strength, and coordination, even in the absence of specific movement disorders such as Parkinson's disease. In humans, these alterations can increase fall risk, often leading to injury and premature nursing home admissions. Parallel to alterations in motor function, changes in cognitive function in aging include decrements in learning, memory, and spatial cognition, even in the absence of specific neurodegenerative diseases such as Alzheimer's disease. In humans, these alterations can lead to decreased productivity, independence, and quality of life. In the present study, men and women between the ages of 21 and 75 years were recruited to investigate parallel declines in cognition and mobility. Balance and gait were measured using a treadmill

instrumented with an array of high-density pressure sensors. Balance was assessed during both eyes-open and -closed conditions. Gait was assessed at both 1.5 mph and preferred walking speeds. Spatial learning and working memory were measured using a virtual version of the Morris water maze (vMWM) task and executive function was measured using the trail-making test (TMT). Results show that increased age was associated with increased postural sway during quiet standing and declines in preferred walking speed. Increased age was also associated with declines in spatial navigation ability, characterized by increasing acquisition latencies and impaired probe trial performance in the vMWM. Similarly, increased age was associated with declines in psychomotor speed and executive function as measured by the TMT. Dietary interventions with berry fruit are able to rescue cognitive and motor performance in animal models; the test battery employed in the present study may therefore present a useful model for assessing the effect of dietary interventions in older adults.

Disclosures: M.G. Miller: None. **B. Shukitt-Hale:** Employment; USDA Intramural.

Poster

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Program#/Poster#:865.04/O14

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: USDA Intramural

AIBMR Life Sciences

Title: Acai fruit improves motor and cognitive function in aged rats

Authors: *B. SHUKITT-HALE, A. N. CAREY, M. G. MILLER, S. M. POULOSE
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Abstract: Aged rats show impaired performance on motor and cognitive tasks that require the use of spatial learning and memory. In previous studies, we have shown the beneficial effects of various berry fruits (blueberries, strawberries, and blackberries) in reversing age-related deficits in behavioral and neuronal function when fed to rats from 19-21 months of age. These effects may be the result of increasing antioxidant and/or anti-inflammatory levels, or by direct effects on signaling and autophagy, in the brain. Acai is a black-purple fruit (genus *Euterpe*) cultivated in the Amazon delta and in Brazil (*Euterpe oleracea* Mart. -EO), as well as southern Central America and Columbia (*Euterpe precatoria* Mart. - EP), and it is known to be rich in polyphenolics that may affect cell-to-cell signaling, receptor sensitivity, inflammatory enzyme

activity or gene regulation. Thus, the present studies were carried out to determine if EO or EP, fed in the rat diet at 2% for 8 weeks, would be efficacious in reversing the deleterious effects of aging on motor and cognitive behavior in 19 mo Fischer 344 rats. Results for the motor testing showed that the EO diet improved performance on wire suspension, while the EP rats turned more on the planks, leading to improved balance performance. Additionally, the EO diet improved reference and working memory in the Morris water maze compared to control, but not the EP, diet. We are currently assessing whether alterations in signaling and autophagy may be involved in the mechanisms of action through which the acai polyphenols could be producing their effects.

Disclosures: B. Shukitt-Hale: Employment; USDA Intramural. Research Grant; AIBMR. **A.N. Carey:** None. **M.G. Miller:** None. **S.M. Poulose:** None.

Poster

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Program#/Poster#:865.05/O15

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Investigating the neuroprotective effects of American skullcap (Scutellaria lateriflora)

Authors: M. LOHANI[1], M. AHUJA[2], D. SHANNON[3], A. RIMANDO[4], D. SCHWARTZ[1], B. KEMPPAINEN[1], *M. DHANASEKARAN[2]
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Abstract: Introduction: Oxidative stress plays an important role in the pathogenesis of neuronal diseases, resulting in cellular damage and possibly cell death. Bioactive compounds (e.g., phytochemicals) present in medicinal plants neutralize or scavenge unstable/toxic free radicals thus preventing them from attacking vital components of cells. American skullcap, a native plant of North America has been used by Americans and Europeans as a nerve tonic, sedative and anticonvulsant for more than 200 years. However, the neuroprotective effects of American skullcap are not fully understood. Therefore, the objective of this study is to investigate the antioxidant effects of American skullcap.
Methods: The antioxidant potential of aqueous or alcoholic extracts of American skullcap was determined in rat brain tissue. In-vitro and quantitative PCR assays were used to determine the antioxidant effects of American skullcap.
Results and Conclusion: Both ethanolic and aqueous extracts of American skullcap expressed

antioxidant activities. The ethanolic and aqueous extracts dose-dependently scavenged the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. Only the ethanolic extract scavenged the hydroxyl radicals (3-fold greater activity compared to positive control, ascorbic acid) and reduced the lipid peroxidation (16% decrease compared to negative control) in the mouse brain homogenate. Protein (5.9 ug/mg vs. 0.00021 ug/mg) and glutathione content (0.275 ug/mg vs. 0.191 ug/mg) were greater in ethanolic than aqueous extracts of American skullcap when determined using colorimetric and fluorometric methods, respectively. However, incubation of the alcoholic extract with the hippocampal cells did not affect the gene expression of catalase and superoxide dismutase. In conclusion, due to the presence of glutathione and other polyphenols, American skullcap exhibits antioxidant activities and prevents neuronal damage. Hence, American skullcap has good potential as a therapeutic agent in the future for various neuronal disorders.

Disclosures: M. Lohani: None. **M. Ahuja:** None. **D. Shannon:** None. **A. Rimando:** None. **D. Schwartz:** None. **B. Kemppainen:** None. **M. Dhanasekaran:** None.

Poster

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Program#/Poster#:865.06/O16

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Natural Sciences and Engineering Research Council

Canada Foundation for Innovation

Title: Berry extracts protect cortical cells from mechanically induced trauma

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Abstract: Oxidative stress is believed to contribute to aging, as well as cell damage and death in neuropathologies such as stroke and traumatic brain injury. Foods containing particularly high amounts of antioxidants (e.g. polyphenols in berries) may offer a potential means of protection against traumatic insults. We have previously found that oxyresveratrol, a potent antioxidant derived from mulberry wood, protects cortical neurons from traumatic injury in vitro. Chemical analysis of extracts from bilberries and lingonberries demonstrates that these berries contain several anthocyanins and flavonols known to have antioxidant capacity. Therefore, we tested the potential of these extracts to provide protection against traumatic injury. Primary cortical cell

cultures were produced from neonatal rats (P1-3). An in vitro model of traumatic injury was used to produce mild linear mechanical strain. Cells were analyzed using immunohistochemical techniques, including DAPI, which labels the nuclei of all cells, as well as antibodies for MAP-2 and NeuN, which label only neurons. Lactate was measured in culture media using a multi-assay biochemical analyzer. Mild injury caused a significant loss of neurons when measured 24 hr post-injury, but there was no significant loss of glia. Bilberry extracts were added to cultures 15 min before injury, which decreased the extent of neuronal loss at 24 hr. A high level of lactate was observed in media 15 min after injury, indicating that cells were unhealthy, and the addition of bilberry extracts significantly inhibited the trauma-induced rise in lactate. Addition of lingonberry extracts 15 min pre-injury significantly protected against cell loss at 24 hr. Trauma also appeared to increase the expression of microtubule-associated protein light chain 3 (LC3), a marker of autophagy, and lingonberry treatment decreased LC3 expression after trauma. Although further experimentation is necessary, these results suggest that compounds derived from natural products may offer protection from insults such as mild traumatic brain injury, which likely involves oxidative stress.

Disclosures: L. Chibrikova: None. **E. Kelly:** None. **E. Shea:** None. **M.Z. Hossain:** None. **J.T. Weber:** None.

Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R21AT005246

Title: Resveratrol promotes neuron survival in primary postnatal cultures

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Abstract: Primary dissociated neuronal cultures remain an important mainstay of neuroscience research. Various media have been optimized for neuronal survival and development in cell culture. Serum free media necessitate a number of supplements to support enriched neuronal cultures. The standardized B27 supplement with neurobasal medium has been used to facilitate neuronal growth and attenuates glial proliferation. Our goal is to identify supplements that can improve neuron survival of postnatal neuronal cultures. We have documented that resveratrol

(3,4’5-trihydroxystilbene), a natural polyphenol and a bioactive component of red wine, have numerous neuropropective properties. We have postulated that this stilbene would not likely act as a direct antioxidant but act as a bioactive agent capable (even in small amount) to induce an intracellular cytoprotective cascade. Postnatal cultures of cortical neurons were derived from 0- to 1-day-old C57BL/6 mice. Neurons were plated at a density of 5 million cells per well in three different media: neurobasal medium without any supplement (NBM), neurobasal medium without any supplement but containing resveratrol 10μM (NBM+R10μM), and neurobasal medium with B27 supplement (NBM+B27). Cells were maintained in growth medium at 37°C in a 95% air/5% CO_2 humidified atmosphere. In order to assess neuronal morphology, pictures were acquired from inverted microscope at 24, 48 and 72h after plating for resveratrol induced neuronal attachment. Significant increase in live cells (52.9+/-5.5% and 53.6+/-4.5%, $p<0.0001$) was recorded in cultures incubated in NBM+R10μM and NBM+B27 respectively, when compare to NBM by calcein-AM assay and increase in viability (140.8+/-8.5% and 134.3+/-6.5%) by MTT assay. Significant decrease in dead cells (73.9+/-6.3% and 74.7+/-5.3%, $p<0.0001$) was recorded in cultures incubated in NBM+R10μM and NBM+B27 respectively, when compare to NBM by EthD-1 assay and decrease in cytotoxicity (17.9+/-2.5% and 19.4+/-2.8) by LDH assay. Results indicated that growth of primary neurons in resveratrol containing medium was as good as comparable to B27 supplement medium revealing that resveratrol itself is sufficient to enhance neuronal attachment and survival during plating and can potentially be a substitute of growth supplement. Efforts are currently being conducted to characterize the mechanism by which resveratrol can limit cell death and increase neuron survival.

Disclosures: M. Agrawal: None. **S. Dore:** None.

Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS Grant-in-Aid for Scientific Research (C) (21590225)

Title: PC12 cells are influenced differently by resveratrol depending on their differentiation

Authors: N. HAYAKAWA[1,2], M. SHIOZAKI[3], M. SHIBATA[4], M. KOIKE[5], Y. UCHIYAMA[5], *T. GOTOW[1]
[1]Lab. of Cell Biol., Koshien University, Col. of Nutr., Takarazuka/Hyogo, Japan; [2]Lab. Mol. Pathol., Osaka Univ. Grad. Sch. Med. Hlth. Sci., Suita, Japan; [3]Dept. Cardiovasc. Surg., Osaka Univ. Grad. Sch. Med., Suita, Japan; [4]Div. Gross Anat. Morphogen., Niigata Univ. Grad. Sch. Med. Dent. Sci., Niigata, Japan; [5]Dept. Cell Biol. Neurosci., Juntendo Univ. Grad. Sch. Med., Tokyo, Japan

Abstract: Resveratrol (RSV), a polyphenol found in grape skin and red wine, has attracted great interest due to its diverse health benefits including neuroprotection and antitumor effect. We analyzed how RSV (1, 10 and 100 μM) influenced undifferentiated (naive) and differentiated PC12 cells, because naive PC12 cells were tumorigenic in nature while differentiated ones, pretreated with NGF (50ng/ml for 24hr), were neuronal, both of which were from the same cell line. Morphological, immunocytochemical and biochemical methods were used to clarify especially the difference of mitochondrial and autophagy functions between two types of cells. Compared to RSV-untreated (control) PC12 cells, the cells treated with RSV for 48hr showed the following results: Mitochondrial number and ATP synthase β subunit expression were almost unchanged until 10 μM RSV and significantly reduced at 100 μM RSV in naive cells, but significantly increased and unaltered, respectively, in any doses of RSV in differentiated cells. Autophagosomes, sometimes with autolysosomes, were, as in the control, visible in any RSV doses but more conspicuous in 10 and 100 μM RSV-treated differentiated cells. LC3-II, a maker for autophagy, was gradually reduced in expression in a RSV dose-dependent manner in naive cells, but constantly expressed until 10 μM RSV and significantly higher at 100 μM RSV in differentiated cells. At 100 μM RSV, most naive cells were dead without any sign for neurite outgrowth, indicating that they were killed by high dose of RSV. In NGF-treated cells, some cells were also dying or dead at 100 μM RSV, suggesting that the cells, which are not yet differentiated without neurites, are killed by high dose of RSV, but differentiated cells are protected even by such RSV dose, possibly due to the activation of mitochondria and autophagy. Phosphorylated form of AMP-activated kinase (AMPK), a bioenergetic sensor, was more expressed in both cells in a dose-dependent manner, suggesting that RSV-activated AMPK is harmful for naive cells but beneficial to differentiated ones. SIRT1 was more reduced in expression in naive cells especially at 100 μM RSV, but SIRT3 was enhanced slightly in differentiated cells at 100 μM RSV. However, expression ratio of Bcl-xL/Bax at 100 μM RSV was more enhanced in naive cells than in differentiated ones, meaning that naive cells may be

killed by RSV through a cascade different from the Bax-related apoptosis. In conclusion, RSV kills PC12 cells when they are undifferentiated or tumorigenic, but protects them when differentiated or neuronal with extensive neurites, possibly through a mechanism related with mitochondria and autophagy including AMPK activation pathway.

Disclosures: N. Hayakawa: None. **M. Shiozaki:** None. **M. Shibata:** None. **M. Koike:** None. **Y. Uchiyama:** None. **T. Gotow:** None.

Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CNPq Grant

CAPES Grant

Title: Mangiferin, a natural xanthone ameliorates 6-hydroxydopamine-induced neurocytotoxicity and oxidative stress in ketamine model of schizophrenia

Authors: P. A. RODRIGUES[1], G. M. ANDRADE[1], M. T. S. TREVISAN[2], V. S. RAO[1], *F. SANTOS[1]
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Abstract: Accumulating evidence indicates that mangiferin (MGF), a natural xanthone by virtue of its antioxidant and anti-inflammatory properties is neuroprotective. Here we sought to verify the neurocytoprotective role of MGF on cultured rat primary mesencephalic cells exposed to 6-hydroxydopamine (6-OHDA) *in vitro*, and the MGFs anti-inflammatory potential in mouse model of ketamine-induced schizophrenia *in vivo*. MGF was extracted and isolated from the bark of *Mangifera indica* L**.** (Anacardiaceae) as per standard procedures. MTT (3[4,5-dimethylthiazole-2il]-2,5-diphenyltetrazolium bromide)-assay was performed to measure cell viability in mesencephalic cell cultures exposed to neurotoxin (6-OHDA, 40 µM). Schizophrenia was induced in mice by ketamine (50 mg/kg, i.p., twice a day, for 7 days). The treatment effects of MGF (50 mg/kg, p.o., for 7 days) were verified on locomotor behavioral changes in open-field test, and on the oxidant stress-related increase in lipid peroxidation (malondialdehyde) and interleukin-6 (IL-6) levels in brain tissues. MGF (10-100 µM) produced no *per se* effect on cell viability as measured by MTT assay, but significantly prevented the 6-OHDA-induced cell death

in a concentration-dependant manner. Acridine orange/ethidium bromide (AO/EtBr) staining confirmed the absence of 6-OHDA-induced morphological changes characteristic of apoptosis/necrosis. In open-field test, ketamine-induced impaired locomotor activity and behavioral changes such as grooming and stereotyped but not rearing were effectively ameliorated by MGF pretreatment. Also, ketamine-associated increase in brain tissue levels of IL-6 and MDA were significantly lowered in MGF pretreated mice. Mangiferin has a neurocytoprotective role related, at least in part, to an antioxidant and anti-inflammatory mechanism, which could be explored for more effective therapies of schizophrenia and other neurodegenerative diseases.

Disclosures: P.A. Rodrigues: None. **G.M. Andrade:** None. **M.T.S. Trevisan:** None. **V.S. Rao:** None. **F. Santos:** None.

Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: PAPIIT Grant IN201910

CONACYT Grant 129838

Title: Protective effect of curcumin against hemin-induced neurotoxicity in primary cultures of cerebellar granule neurons of rats

Authors: *S. GONZALEZ[1], S. GUZMAN-BELTRAN[2], J. PEDRAZA-CHAVERRI[1]
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Abstract: Curcumin, a yellow pigment derived from Curcuma longa Linn, has attracted great interest during the past decades. Extensive studies documented that curcumin has diverse biological activities as anti-inflammatory, anticarcinogenic and antioxidant, among others. This work was designed to determinate the potential protective effect of curcumin on neurons in culture.
Primary cultures of cerebellar granule neurons (CGNs) from rats were treated with curcumin (10-50 μM) to evaluate its cytotoxicity by MTT reduction method. Then, we evaluated if this compound has protective effect. CGNs were pretreated with curcumin and subsequently exposed to hemin as a generator of oxidative stress and cell viability was monitored.

It was found that curcumin (1-30 μM) was unable to modify cell viability significantly. In contrast, hemin alone (30 μM) decreased viability to 50% but when CGNs were pretreated with curcumin (5, 10 and 15 μM) before the addition of hemin, the viability diminished 12% compared with curcumin or control. In addition, it was found that curcumin induced heme oxygenase-1 (HO-1) in a concentration and time-dependent way. Furthermore, HO activity was directly correlated with the amount of intracellular protein. Curcumin protection was blocked by the HO inhibitor tin mesoporphyrin. Moreover, the content of reduced glutathione (GSH) was increased when the cells were incubated with curcumin alone and this increase was diminished by buthionine sulfoximine, an inhibitor of GSH synthesis. Hemin decreased GSH content and this decrease was blocked when CGNs were exposed to curcumin and hemin simultaneously, It is concluded that curcumin has a protective role against hemin toxicity and that HO activity, HO-1 expression and GSH are involved in this protective effect.

Disclosures: S. Gonzalez: None. **S. Guzman-Beltran:** None. **J. Pedraza-Chaverri:** None.

Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: FAPERGS

CNPq

Centro Universitário Metodista, do IPA

IBRAVIN

Title: Neuroprotective effect of organic and conventional white grape juices on oxidative stress induced by sodium azide in cerebral cortex of rats

Authors: *C. DANI[1], B. R. ONGARATTI[2], F. D. MACHADO[2], N. D. MEDEIROS[2], D. C. DE ALMEIDA[2], C. D. DESTRI[3], E. R. DA SILVA[3], A. QUINCOZES-SANTOS[5], C. D. FUNCHAL[4]
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Abstract: Oxidative damage induced by reactive species (RS) has a relevant pathophysiological role in several types of human diseases. In order to neutralize these RS, the cells have developed a complex biochemical redox mechanism, consisting of both enzymatic and non-enzymatic components. Moreover, foods, particularly fruits and vegetables, also have an important role in maintaining physiological redox equilibrium. In this context, grapes are rich in phenolic compounds, such as flavonoids. The objective of this study was to investigate the *in vitro* neuroprotective effect of conventional and organic white grape juices on oxidative stress induced by sodium azide in cerebral cortex of rats of 10-day-old-rats. Cerebral cortex was homogenized and preincubated with 40% (w/v) conventional or organic white grape juices, furthermore the brain was incubated for 1 h with 5 mM sodium azide the presence or absence of 40% (w/v) conventional or organic white grape juice. The assays of acid reactive species (TBARS), sulfhydryl content and the activity of the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) were performed. Sodium azide was able to enhance lipid peroxidation (TBARS) and to reduce the non-enzymatic antioxidants defenses (sulphydryl) in cerebral cortex of rats. Moreover sodium azide inhibited the activity of CAT and SOD. Conventional and organic white grape juices were able to prevent the effects caused by sodium azide in all assays, except the SOD activity. These results indicate that sodium azide induces oxidative stress in cerebral cortex of young rats and that conventional and organic white grape juices exhibit antioxidant properties capable of preventing the oxidative damage caused by this compound.
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Disclosures: C. Dani: None. **B.R. Ongaratti:** None. **F.D. Machado:** None. **N.D. Medeiros:** None. **D.C. de Almeida:** None. **C.D. Destri:** None. **E.R. da Silva:** None. **A. Quincozes-Santos:** None. **C.D. Funchal:** None.

Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: FAPERGS

CNPq

Title: Antioxidant and neuroprotective effect of purple grape juice in rats supplemented with high fat diet

Authors: *C. S. FUNCHAL[1], M. G. CARDOSO[1], N. MEDEIROS[1], D. DOS SANTOS LACERDA[1], D. C. DE ALMEIDA[1], C. D. DESTRI[1], E. R. DA SILVA[1], J. P. HENRIQUES[2], R. GOMEZ[2], C. DANI[1]
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Abstract: The consumption of foods and beverages containing phenolic compounds have been reported due to the benefits they produce on human health. Among these, stands out the grape juice. The Serra Gaucha is described as the most important wine region of Brazil. Regarding cultivars widespread in the Serra Gaucha, about 90% of the area is occupied by vines of *Vitis labrusca* cultivars, with a predominance of Isabel, Bordô and Concord that are more rustic and productive in relation to *Vitis vinifera*. The objective of this study was to investigate the antioxidant and neuroprotective effect of chronic use of purple grape juice (organic and conventional) from Bordô variety on oxidative stress in different brain regions of rats supplemented with high fat diet (HFD) for three months. A total of 40 male rats (21-day-old) were randomly divided into four groups. Group 1 received a standard diet and water, group 2 HFD and water, group 3 HFD and conventional grape juice (CGJ), and group 4 HFD and organic grape juice (OGJ). All groups had free access to food and drink and after three months of treatment the rats were killed by decapitation and the cerebral cortex, hippocampus and cerebellum isolated and homogenized on ice for oxidative stress analysis. The results demonstrated that HDF enhanced lipid peroxidation (TBARS) in cerebral cortex and hippocampus and that only OGJ was able to prevent this increase. HDF also increased protein damage measured by the carbonyl assay in cerebral cortex and hippocampus and that CGJ and OGJ were able to prevent this enhance. On the other hand, HDF reduced the non-enzymatic antioxidants defenses (sulphydryl) in cerebral cortex and cerebellum and both CGJ and OGJ were able to prevent this inhibition. Therefore, we conclude that the HFD induced oxidative stress in the brain of rats and that the chronic use of purple grape juice was able to prevent these oxidative alterations.

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Poster

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Program#/Poster#:865.13/P3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: The effect of proton particle radiation and pomegranate treatment on behavior in male and female wildtype mice

Authors: *M. S. DULCICH, E. THORNDYKE, J. RUBINSTEIN, N. MISTRY, R. E. HARTMAN
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Abstract: Astronauts have traveled outside Earth's atmosphere into lower earth orbit (LEO) and beyond for decades. However, these individuals are constantly exposed to ionizing radiation that may have deleterious effects on physical and mental health. With plans to extend space travel beyond LEO for longer durations (i.e., manned expeditions to Mars), the radiation exposure will increase. Because of the complex behaviors and cognitive functions required for mission completion and survival, the long-term effects of ionizing radiation on the brain and behavior for both male and female astronauts must be explored. Previous research indicates that males and females may respond differently to radiation exposure. Furthermore, some evidence suggests that antioxidants may offer some protection against the cellular effects of radiation, and research from our lab suggests that dietary sources of antioxidant polyphenols (such as pomegranate juice) may provide significant neuroprotection against Alzheimer's neuropathology, stroke, and traumatic brain injury. A diet enriched with polyphenols may confer enough protection from the effects of radiation to maintain the complex cognitive and fine motor skills required by astronauts. Ninety-six C57/BL6 mice (half receiving dilute pomegranate juice and half receiving dilute sugar water as a control) were irradiated with 2 Gy proton radiation (1-2 Gy/min), and post-radiation behaviors (activity, motor skills, spatial learning, anxiety, and learned helplessness) were assessed two months later. Proton irradiation was associated with more learned helplessness in the tail suspension test and the pomegranate diet significantly ameliorated those deficits. The pomegranate diet also ameliorated radiation-induced relational learning deficits in the spatial water maze task for females, but not males. Finally, control diet males, but not pomegranate diet males displayed worse coordination and balance on the rotarod than females. Thus, a pomegranate-enriched diet conferred protection from the effects of proton radiation in terms of learned helplessness (depression-like behaviors), and spatial learning in the water maze test (females only). Analysis of the effects of pomegranate juice supplementation on hippocampal neurogenesis after proton irradiation, as well as behavioral performance on the balance beam and openfield activity tests is currently underway. Overall, it appears that proton irradiation, such as that which may occur during long space flights, may cause a different pattern of behavioral deficits in males than females, and that a pomegranate enriched diet may confer protection against some of those effects.

Disclosures: M.S. Dulcich: None. **E. Thorndyke:** None. **J. Rubinstein:** None. **N. Mistry:** None. **R.E. Hartman:** None.

Poster

866. Neuroinflammation: Neurodegeneration

Location: Hall F-J

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Program#/Poster#:866.01/P4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Office of Dietary Supplement OD-Y2-OD-2005-01

National Institutes of Health Intramural Research Program

Title: Dietary omega-6 polyunsaturated fatty acid deficiency reduces lipopolysaccharide-induced changes in behavior sickness and brain arachidonic acid inflammatory response

Authors: *A. TAHA, Y. CHEON, H. C. BLANCHARD, V. KELESHIAN, E. RAMADAN, M. CHEN, L. CHANG, S. I. RAPOPORT, J. S. RAO
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Abstract: The omega-6 polyunsaturated fatty acid (n-6 PUFA), arachidonic acid (AA, 20:4n-6), is a precursor of prostaglandins, leukotrienes, and related compounds that have important roles in mediating the brain's innate inflammatory response to cytokines, which have been implicated in neuropsychiatric and neurodegenerative disorders. AA is released from the *stereospecifically numbered*-2 position of membrane phospholipids by AA-selective calcium-dependent cytosolic phospholipase A_2 ($cPLA_2$). The AA cascade can be activated in rodents with the bacterial endotoxin, lipopolysaccharide (LPS), which causes neuroinflammation and a characteristic depressive-like sickness behavioral profile. This study tested the hypothesis that dietary n-6 PUFA deprivation, which limits the supply of AA to the brain, would reduce LPS-induced AA-mediated neuroinflammation and associated changes in behavioral sickness. Male Fischer rats were fed an n-6 PUFA adequate diet (control, 6% of energy) or an n-6 PUFA deficient diet (0.5 % of energy) for 15 weeks, after which, they were infused with LPS (250 ng / hr) intracerebroventricularly into the 4th ventricle for 2 days using a mini-osmotic pump. Two days after infusion, behavioral sickness was scored, and the incorporation rate of intravenously infused unesterified ^{14}C-AA into brain phospholipids (an established marker of neuroinflammation). Dietary n-6 PUFA deprivation attenuated LPS-induced increases in the rate of ^{14}C-AA incorporation into brain phospholipid ($P < 0.05$ for main effect of LPS and diet by two-way ANOVA). This was associated with a significant decrease in behavioral sickness scores, which were increased by LPS infusion in control rats ($P < 0.05$). Because the AA incorporation rate is a quantitative marker of neuroinflammation, our results suggest a direct anti-inflammatory effect of dietary n-6 PUFA deprivation, in association with reduced behavioral

sickness. Thus, lowering dietary n-6 PUFA consumption in humans from estimated current intakes of 7% of energy to clinically achievable levels of 0.5~1% of energy, might increase resistance against pro-inflammatory insults to the brain or reduce the pathological neuroinflammation reported in neuropsychiatric and neurodegenerative diseases.

Disclosures: A. Taha: None. **Y. Cheon:** None. **H.C. Blanchard:** None. **V. Keleshian:** None. **E. Ramadan:** None. **M. Chen:** None. **L. Chang:** None. **S.I. Rapoport:** None. **J.S. Rao:** None.

Poster

866. Neuroinflammation: Neurodegeneration

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS063878

NIH Grant P30 MH062261

Title: Microglia ctivation induces axonal injury in corpus callosum

Authors: J. ZHANG, J. LIU, J. KRUCHOWSKY, H. S. FOX, *H. (. XIONG
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Abstract: Cumulative evidence from both autopsy and animal models of white matter tract disorders such as multiple sclerosis and amyotrophic lateral sclerosis suggest an involvement of activated microglia in nerve fiber injury. The activated microglia was also observed in subcortical white matter of both HIV-infected human subjects and SIV-infected monkeys. Whether the activated microglia induces axonal injury in corpus callosum (CC) remains to be determined. In this study, we examined if lipopolysaccharide (LPS)-activated microglia produce axonal injury by Western blot detection of β-amyloid precursor protein (β-APP) and electrophysiological analysis of compound action potentials (CAPs) in rat CC. For Western blotting, the CC slices were treated with different doses of LPS in oxygenated artificial cerebrospinal fluid for 12 h before CC dissection and protein extraction. Our results showed that treatment of the CC slices with LPS resulted in microglia activation and β-APP accumulation as illustrated by a significant increase of β-APP density when compared with the β-APP density obtained from untreated control CC slices, suggesting that LPS-activated microglia produced axonal injury in the CC. In parallel, electrophysiological studies revealed that treatment of CC slices with LPS significantly reduced input-output (I/O) responses as determined by measurement and analysis of CAP amplitudes. This LPS-associated reduction of I/O responses in

the CC was attenuated by RS-LPS, a toll-like receptor 4 (TLR-4) competitive antagonists, indicating activation of microglia by LPS resulted in a reduction of I/O responses. In all cases, LPS activation of microglia was judged by morphological changes visualized with fluorescent microscope by using Iba-1 immuno-fluorescent staining. Taken together, these results demonstrated that activated microglia induces axonal injury in the CC.
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Poster

866. Neuroinflammation: Neurodegeneration

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5P60-DK020572

Title: Role of exercise in the reduction of inflammatory mediators in painful diabetic neuropathy

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Abstract: Painful neuropathy is a significant complication in diabetic patients. Previous evidence suggests that proinflammatory cytokines may play a role in pain perception. We found in this study that regular exercise decreased painful neuropathy in the Type 1 model of diabetic animals by reducing inflammatory markers in the nervous system that are responsible for the alterations in pain perception in diabetic animals. Type 1 model of streptozotocin-diabetic animals were placed in motorized running wheels for sixty minutes per day, five days a week, starting at one week of diabetes and up to six weeks after diabetes. Behavioral measures of Hargreaves test to assess the thermal pain threshold, acetone spray to access cold allodynia and mechanical stimulation by the Randall-Selitto method to access nocifensive responsiveness were performed at two-week intervals after the onset of diabetes. Finally, animals were euthanized for collection of tissues and for further analysis of inflammatory mediators. After analysis of the results, we confirmed our hypothesis that exercise alleviated the pain related behaviors. There were significant changes in the mechanical hyperaglesia testing. The pain threshold of the control animals were 101 ± 5.3 g, whereas it was decreased in the diabetic sedentary group: 57 ± 1.8 g and the threshold improved in the diabetic exercise group: 87 ± 4.95 g. The latency to

withdraw from the cold stimulus was significantly decreased in diabetic sedentary group compared to control sedentary animals (diabetic 3.89 ± 1.89 sec; control 14.29 ± 1.63 sec) whereas diabetic exercise group showed improved latency to withdraw from the stimulus (17.44 ± 8.1 sec) but there was no change in the thermal hyperalgesia between control (12.14 ± .75 sec), diabetic sedentary (12.13 ± 1.56 sec) and diabetic exercise (12.60 ± 0.5 sec). On the basis of biochemical analyses of the tissues, exercise reduced the up-regulation of proinflammatory cytokines, which may directly correlate with the pain in the diabetic animals. Overall, this research suggest that exercise may provide an alternate route of treatment for painful neuropathy in Type 1 diabetic animals by decreasing the use of pain medication and provide a more useful and efficient way of management of pain.

Disclosures: J. Meyers: None. **C. Galloway:** None. **M. Chattopadhyay:** None.

Poster

866. Neuroinflammation: Neurodegeneration

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Program#/Poster#:866.04/P7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Effect of calcium-independant phospholipase A2 group VIA (iPLA2β) deficiency on enzymatic markers of arachidonic cascade and inflammation in old mice.

Authors: *H. BLANCHARD[1], A. Y. TAHA[1], Y. CHEON[1], H. KIM[1], J. TURK[2], S. I. RAPOPORT[1]
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Abstract: Calcium-independant phospholipase A2 group VIA (iPLA2β) participates in the remodeling of phospholipids by releasing polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA) from the sn-2 position of membrane phospholipids. iPLA2β mutations occur in infantile neuroaxonal dystrophy (INAD) and dystonia-parkinsonism. We reported in 4-5 month-old iPLA2β-/- mice with minimal neuropathology compared to wild type that brain DHA metabolism was reduced while markers of arachidonic acid (ARA) metabolism were elevated. DHA metabolites have anti-inflammatory properties in the brain, whereas ARA is a precursor of pro-inflammatory eicosanoids. We hypothesized that iPLA2β deficiency triggers an imbalance between DHA and ARA metabolism in aged mice, and would promote inflammation and neuronal loss in the brain. Male iPLA2β-/- and wildtype mice were killed at 15-19 months of age and RT PCR and Western blotting were performed on whole brain homogenate. Results showed that phosphorylated ARA-releasing cytosolic PLA2 was increased

significantly in iPLA2β-/- mice, whereas other ARA cascade markers were unchanged (secretory PLA2, COX-2, mPGES). We found no significant change in pro-inflammatory cytokines expression (TNFα, Il-1β) or in synaptic markers (drebrin, synaptophysin). This study showed that iPLA2β deficiency in old mice leads to an elevation of ARA metabolism but does not trigger neuroinflammation or synaptic loss, suggesting that the neurodegeneration observed in these mice is not associated with neuroinflammation. It would also be of interest to assess localized synaptic integrity and to quantify downstream DHA and ARA metabolites. This research was supported by the Intramural Research Program of the NIH, National Institute on Aging.
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Disclosures: H. Blanchard: None. **A.Y. Taha:** None. **Y. Cheon:** None. **H. Kim:** None. **J. Turk:** None. **S.I. Rapoport:** None.

Poster

866. Neuroinflammation: Neurodegeneration

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Program#/Poster#:866.05/P8

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Intravenous injection of the anti-inflammatory protein TSG-6 reduces neural damage after traumatic brain injury in mice

Authors: *J. WATANABE, D.-K. KIM, D. PROCKOP
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Abstract: Administrations of mesenchymal stem/stromal cells (MSCs) were previously reported to produce beneficial effects in a series of disease models, including ischemic and traumatic injury to the central nervous system. In several models, the beneficial effects were in part explained by the MSCs being activated in vivo to express the anti-inflammatory protein TNF-α-stimulated gene/protein 6 (TSG-6). Here, recombinant TSG-6 protein was intravenously administrated in an experimental mouse model of traumatic brain injury. TSG-6 decreased the blood brain barrier leakage and the loss of tissue from the cerebrum and hippocampus. TSG-6 also decreased neutrophil extravasation, and significantly reduced expression of matrix metalloproteinase 9 (MMP9) by the infiltrated neutrophils and endothelial cells of the brain. The

data suggested that TSG-6, an anti-inflammatory protein produced by MSCs in response to injury signals, can provide an effective therapy for TBI.

Disclosures: J. Watanabe: None. **D. Kim:** None. **D. Prockop:** Consultant/Advisory Board; Member advisory board of Temple Therapeutics, LLC. Less than 5% equity after dilution..

Poster

866. Neuroinflammation: Neurodegeneration

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

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Research to Prevent Blindness Career Development Award (RMS)

NEI Core Grant P30EY008126 (Vanderbilt Vision Research Center)

Title: Differential signaling of CCL5 in retinal ganglion cells in the microbead occulsion model of glaucoma

Authors: *D. S. DUNCAN[1], A. C. REHORN[1], G. L. DAVIS[2], R. M. SAPPINGTON[1]
[1]Vanderbilt Eye Inst., [2]Interdepartmental Grad. Program, Vanderbilt Univ., Nashville, TN

Abstract: Glaucoma is one of the leading causes of blindness worldwide, where increased intraocular pressure in the eye results in retinal ganglion cell (RGC) death. To date, therapies developed lower elevated intraocular pressure; however these therapies do not directly prevent the death of RGCs. Inflammation of the eye, the release of inflammatory cytokines and chemokines by retinal glial cells, also contributes to the ensuing neuroinflammation and neurodegeneration in glaucoma. Thus, understanding the inflammatory mechanisms that influence RGC function and interactions with retinal glial cells may provide insight into glaucomatous pathology and the development of more effective therapies. Our laboratory has previously shown that retinal microglia upregulate CCL5 in response to elevated hydrostatic pressure. Therefore in this study, we examined the expression and localization of CCL5 and its receptors CCR3 and CCR5 in an acute murine model of glaucoma. We performed immunohistochemistry to assess the expression and localization of CCL5, CCR3 and CCR5 in retinas of 4 and 8 month old C57BL/6 mice in the acute microbead occlusion model. We co-labeled vertical retinal paraffin sections and whole mount retinas with CCL5, CCR3 and CCR5,

in addition to a cell-type specific marker for RGCs (SMI-31) to assess expression in RGCs and label intensity was used to quantify expression in both the central and peripheral retina. CCL5, CCR3 and CCR5 expression was detected in multiple layers of the retina in normal C57BL/6 mice. Furthermore, we observed that CCL5 signaling molecules are constitutively expressed in the ganglion cell and nerve fiber layers. Using SMI-31 co-labeling for anatomical localization, CCL5 and CCR5 were restricted to RGC somas and axons. In microbead-injected eyes, CCL5 intensity increased in both the ganglion cell and nerve fiber layers in the inner and outer retina of microbead-injected eyes compared to saline-injected eyes of 4 and 8 month old mice. CCR3 expression was significantly decreased in the inner retina of microbead-injected eyes, as compared to saline-injected eyes. This decrease was primarily associated with RGCs, as evidenced by a reduction in CCR3 in the ganglion and nerve fiber layers. Similar to CCR3, CCR5 expression also decreased in RGCs following elevated intraocular pressure in microbead-injected eyes. These data support a functional role for constitutive and inducible CCL5 signaling in RGCs. Additionally, the differential effects of elevated intraocular pressure further provide evidence that CCL5 is relevant to glaucomatous pathology and the inflammatory microenvironment relating to RGC dysfunction and RGC-glial interactions.

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Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant AG020569 EY1234

NIH grant AG028367 EY1234

Title: P38MAPK, a mediator of hypoxia-induced cerebrovascular inflammation

Authors: D. TRIPATHY, A. SANCHEZ, X. YIN, J. LUO, J. MARTINEZ, *B. B. MILLER, P. GRAMMAS
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Abstract: Vascular perturbations and hypoxia are increasingly implicated in Alzheimer’s disease (AD) pathogenesis. Cerebral hypoxia induces a large number of inflammatory proteins in brain endothelial cells via signaling pathways that have not been defined. The p38 mitogen-activated

protein kinase (MAPK) signaling system has been implicated in endothelial injury and inflammation. The objective of this study is to determine the role of p38 MAPK signaling in hypoxia-mediated effects on brain endothelial cells. Exposure of brain endothelial cells to hypoxia (2-6 hours) shows a time-dependent increase in the phosphorylated (active) form of p38 MAPK (pp38 MAPK). Examination of these cultures at 6 hours hypoxia shows that inflammatory proteins such as monocyte chemoattractant protein-1, interleukin-6, and thrombin are significantly elevated and that the selective p38 MAPK inhibitor SB203580 significantly reduces the hypoxia-mediated increase in their expression. Expression of pp38 MAPK is also elevated in the cerebromicrovasculature of rats treated with 10 mg/kg $CoCl_2$ which induces hypoxia in vivo. Finally, western blot analysis of both human samples and tissues from AD transgenic mice show that pp38 MAPK is increased isolated brain vessels derived from AD brains compared to control-derived vessels. These data demonstrate that pp38MAPK is a key regulator of hypoxia in the cerebrovasculature and suggest that control of this signaling pathway could have therapeutic value in AD and other disorders where hypoxia is involved. Supported in part by NIH AG020569 and AG028367

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Poster

866. Neuroinflammation: Neurodegeneration

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Grey matter pathology during the course of EAE in Dark Agouti rats

Authors: D. MORSALI[1], *J. O'BRIEN[2], W. LEE[3]
[1]Neurol., Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [2]Univ. Texas Hlth. Sci. Cntr Houston, HOUSTON, TX; [3]Neuroinflam., Univ. Col. London, London, United Kingdom

Abstract: Objectives
Recent quantitative magnetic resonance imaging studies in multiple sclerosis (MS), a progressive disease of the central nervous system involving inflammatory processes, demyelination and irreversible degeneration of the axons of neurons, indicate that grey matter atrophy occurs from the earliest stages of disease. It develops faster than white matter atrophy, and is more related to physical disability and cognitive impairment than T2 and T1 white matter lesion volumes. However, the majority of studies on the experimental models of MS, experimental autoimmune

encephalomyelitis (EAE), have largely overlooked the events occurring in the grey matter. In this study, we have explored grey matter pathology at five stages throughout the course of EAE in Dark Agouti rats.

Methods

EAE in DA rats was induced by subcutaneous immunisation with recombinant myelin oligodendrocyte glycoprotein (rMOG). Animals were culled at five different time points along the disease course ('Preinduction (naïve)', 'Predisease', 'Peak of disease', 'Remission', and 'Relapse') and the tissue was processed immunohistochemically for motor neurons, astrocytes, and perivascular macrophages. The neurological deficit was assessed daily for seven days on a 10 point scale. Terminal electrophysiological examination was performed under ketamine (90mg/kg)/xylazine (10mg/kg) anaesthesia to record motor neuron excitability (F wave), followed by a cardiac perfusion for histological analysis.

Results

The number of activated astrocytes and perivascular macrophages was significantly higher at the 'Peak of disease' compared to 'relapse' or other time points along the disease course in animals with EAE. This occurred despite EAE animals exhibiting the same intensity of neurological deficits at both 'Peak of disease' and 'relapse'. Interestingly, the number of intact motor neurons significantly decreased from 'Peak of disease' onwards. The latter occurred in conjunction with declining motor neuron excitability, further confirming motor neuron loss of function.

Conclusion

Differences in grey matter pathology between 'Peak of disease' and 'relapse' in EAE indicate that the underlying mechanism(s) of pathology at these two critical stages are different. Our results emphasize an important role played by grey matter pathology in EAE.

Disclosures: D. Morsali: None. **J. O'Brien:** None. **W. Lee:** None.

Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NIGMS (2SO6 GM08016-39)

NIH-RCMI 2 G12 RR003048

NIMH-COR (MH 16580-28)

Title: Protective effects of low alcohol concentrations against inflammatory-mediated toxicity in neuroblastoma-derived cells

Authors: Y. CHIN, D. O. BROWN, R. E. TAYLOR, *Y. TIZABI
Pharmacol., Howard Univ. Col. Med., Washington, DC

Abstract: Detrimental effects of alcoholism and neurotoxic effects of higher alcohol concentrations in in-vitro models are well documented. In contrast, epidemiological and preclinical studies suggest positive effects of low alcohol concentrations. Thus, protective effects of alcohol in cultured neuroblastoma-derived (SH-SY5Y) cells that were exposed to salsolinol, an endogenous neurotoxin resembling MPTP, have been reported. Since inflammatory processes may be an important contributory factor in neurodegenerative diseases, we were curious to determine whether low alcohol concentration may also provide protection against inflammatory-mediated toxicity. For this purpose we used two inflammatory cytokines (TNF-alpha and IL1-beta) as well as lipopolysaccharide (LPS), a chemical expressed on the outer membrane of gram-negative bacteria to induce cellular toxicity. Exposure of SH-SY5Y cells to 30 ug/ml of either TNF-alpha or IL1-beta for 72 h resulted in approximately 35% toxicity as determined by MTT (3,[4,5-dimethyliazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Similar exposure to 300 ug/ml LPS resulted in approximately 40% toxicity. Combining the two cytokines alone or together with LPS did not result in any additional toxicity. Pre-treatment of cells with ethanol (0.1-50 mM) resulted in dose-dependent protection against all 3 toxicants with maximal protection at 50 mM ethanol. None of the alcohol concentrations on their own resulted in any cellular toxicity. These preliminary results suggest protective effects of low alcohol concentrations against inflammatory mediated neurotoxicity. Further verification of the anti-inflammatory effects of ethanol in other in-vitro and in-vivo models and elucidation of the protective mechanism may provide therapeutic potential for low alcohol concentrations in neuroinflammatory-mediated diseases.
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Poster

866. Neuroinflammation: Neurodegeneration

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Tau hyper-phosphorylation alters CX3CL1 levels and induces autophagic defects and 4E-BP signaling: a possible link between inflammation and autophagy

Authors: *M. HEBRON[1], I. LONSKAYA[2], N. ALGARZAE[2], N. DESFORGES[2], C. MOUSSA[2]
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Abstract: Several neurodegenerative diseases are characterized by accumulation of hyper-phosphorylated Tau (p-Tau) and neuro-inflammation. To measure inflammatory markers and p-Tau clearance, our lab generated lentiviral gene transfer animals by expressing $A\beta_{1-42,}$ wild type Tau, and P301L in the cortex of two-month-old male Sprague-Dawley rats. P301L resulted in significantly higher levels of p-Tau, compared to wild type Tau injected animals. A significantly higher level of TNF-α and IL-6 were detected in Tau and P301L expressing animals compared to LacZ, but CX3CL1 levels were significantly higher in Tau expressing animals compared to P301L. Lentiviral expression of $A\beta_{1-42}$ remarkably increased p-Tau, TNF-α levels, and caspase-3 activation at four weeks compared to two weeks post-injection, suggesting that the additive effects of $A\beta_{1-42}$ and p-Tau accelerate cell death. Tau resulted in increased levels of ADAM-10, ADAM-17, and soluble CX3CL1 levels. In correlation, CX3CL1 levels were also significantly increased in $A\beta_{1-42}$ expressing animals at four weeks post-injection, when p-Tau was detected. The increase in CX3CL1 levels, at four weeks post injection in $A\beta_{1-42}$ expressing animals, was associated with increased microglial activation, independent of astrocytes. Assessing the overall effects of autophagy revealed an increase in HDAC6 levels and activation of LC3, without autophagic clearance. Subcellular fractionation confirmed the accumulation of p-tau in autophagosomes (AV-20), which resulted in autophagic vacuole accumulation in the cytosol. Tau increased mTOR activity and resulted in the activation of 4E-BP signaling. Accumulation of p-Tau alters normal autophagy but inhibits autophagic clearance, perhaps due to the destabilization of microtubules and impairement of axonal transport. Accumulation of p-Tau is linked to changes in inflammatory responses, including CX3CL1 signaling, suggesting a possible cross-talk between amyloid-stressed neurons and microglia.

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Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA I10001_04_RC_C

DTRA W911NF-07-D-001

Title: Electroencephalograph (EEG) implantation with isoflurane anesthesia 7 days prior to brain injury induced by soman significantly reduces neuropathology and inflammation in rats.

Authors: *E. A. JOHNSON, T. L. DAO, J. F. IRWIN, J. A. LEUSCHNER, S. W. KASKI, R. K. KAN
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Abstract: Status epilepticus (SE) can cause significant neuronal cell death as a consequence of acute exposure to potent acetylcholinesterase inhibitors such as soman (GD). The monitoring of SE is traditionally accomplished by implantation of cortical electrode probes under isoflurane anesthesia for electroencephalographic (EEG) recording with post-operative buprenorphine pain management. Anesthetics, such as isoflurane but not buprenorphine, can be neuroprotective when given prior to CNS insult, though these effects greatly diminish beyond 2 days. Therefore, to avoid unintended experimental effects from isoflurane preconditioning, a 7-day delay between surgery and experimental injury has been typically used. Here we describe a significant reduction in several inflammatory factors both in the brain and in circulation and a reduction in neuropathology following seizurogenic exposure to GD after a 7-day recovery period from EEG surgery in which isoflurane was used as the anesthetic. Levels of central and circulating inflammatory proteins were quantified using Luminex multiplex bead immunoassays up to 24 hours following SE onset in rats that either did or did not receive EEG surgery. Various factors involved with neuropathology were also compared between these groups. Significant differences were found in the magnitude and temporal expression of inflammatory and neuropathology associated proteins between EEG surgery and no surgery groups. These data show that even brief exposures (<20 min) to anesthetic amounts of isoflurane or the surgery itself 7 days prior to experimentation can significantly reduce the central and peripheral inflammatory response to brain injury. Therefore, longer recovery times following surgery may be necessary to reduce unintended secondary surgery effects on neuroinflammation studies.
Disclaimer: The views expressed in this abstract are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. This research was supported by the Defense Threat Reduction Agency - Joint Science and Technology Office, Medical S&T Division.

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Poster

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: ECOS-CONICYT C10S01

Title: Cannabinoids prevent the amyloid β-induced hemichannel activity in neurons and glial cells

Authors: *J. A. ORELLANA[1], K. F. SHOJI[2], J. C. SÁEZ[2], C. GIAUME[3]
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Abstract: The mechanisms involved in Alzheimer's disease (AD) are not completely understood and how glial cells contribute to this neurodegenerative disease remains to be elucidated. Recently we showed that amyloid-β peptide (Aβ) increase hemichannel activity in microglia, astrocytes and neurons, affecting neuronal viability. Because cannabinoids (CB) have anti-inflammatory properties and their receptors (CB-R) are largely expressed in microglia and astrocytes, we investigated upon primary glial cell cultures and hippocampal slices, the effects of CBs (WIN, 2-arachidonyglycerol, and methanandamide) on (i) Aβ-induced hemichannel activity in brain cells; (ii) Aβ-induced release of glutamate and ATP from glial cells and (iii) Aβ-induced neuronal death. Cultures of microglia, astrocytes or neurons as well as acute hippocampal slices were treated with 10 μM $A\beta_{25-35}$ for 72 h or 3 h, respectively. Hemichannel activity was monitored by single channel recordings and by time-lapse ethidium uptake while neuronal death was assessed by Fluoro-Jade C staining. We found that WIN, 2-arachidonyglycerol and methanandamide strongly inhibited the $A\beta_{25-35}$-induced hemichannel activity in glial cells, but not in neuronal cultures. Importantly, the $A\beta_{25-35}$-induced release of glutamate and ATP via glial cell hemichannels was inhibited by CBs, reducing neuronal death. Pharmacological characterizations of the CBs actions on $A\beta_{25-35}$-induced changes in glial hemichannel activity, glial glutamate/ATP release and neuronal revealed that these effects were mainly mediated through the CB1-R activation, although some partial action was observed via CB2-R activation. Altogether these data support the idea that under pro-inflammatory conditions, cannabinoids exert, through activation of different sub-types of glial CB-Rs, an inhibitory effect on glial hemichannel activity, which result neuroprotective and open novel avenues for alternative treatments for AD.

Disclosures: J.A. Orellana: None. **K.F. Shoji:** None. **J.C. Sáez:** None. **C. Giaume:** None.

Poster

866. Neuroinflammation: Neurodegeneration

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:866.13/P16

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Quantitative autoradiography of neuroinflammation in Tg4510 mice and rats receiving kainic acid challenge using [^{3}H]PRB28

Authors: *J. XU[1], J. SUN[1], D. CAOUETTE[3], S. GRIMWOOD[3], Y. LU[3], K. BALES[3], D. HOLTZMAN[2], R. MACH[1]
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Abstract: Glia cells (astrocytes and microglia) play an important role in mediating immune processing in the central nervous system (CNS). Evidence indicates glial cell activation is directly linked to a variety of CNS diseases. Elucidating the impact of inflammatory processes on neurodegeneration has been a challenge; efforts have focused on biomarker identification. The peripheral benzodiazepine receptor (PBR) or translocator protein (TSPO) is a key biomarker used in the development of radiotracers for examining activated microglia, reactive gliosis, brain injury and other inflammatory processes. Here, quantitative autoradiography with [^{3}H]**PBR28** measured the change in TSPO density as an inflammatory response to neurofibrillary tangle pathology in Tg4510 mice and kainic acid (KA) lesioned rats. First, the dissociation constant (K_d) was determined using saturation binding and Scatchard analysis; K_d values were ~0.4-0.9 nM in frontal cortex, hippocampus, choroid plexus, thalamus and cerebellum of both rat and mouse. Tg4510 mice (3 and 6 months) and age-matched wild type (WT) cohorts were utilized for PBR autoradiography quantification of TSPO receptor expression. A high density of TSPO was detected in 3 month old Tg4510 vs. age-matched WT mice only in the hippocampus (fmol/mg tissue): 433 vs. 322 (p=0.004). Extensive regional increases in TSPO levels were found in 6 month old Tg4510 mice vs. age matched WT group in the frontal cortex 568 vs. 335 (p=0.011); pituitary gland 1506 vs. 1105 (p=0.034); thalamus 240 vs. 193 (p=0.034); and cerebellum 549 vs. 463 (p=0.029). These data were consistent with the reported temporal change of neurofibrillary tangles pathology in this transgenic model. As an additional model of neuroinflammation, adult male rats were given either intraperitoneal PBS or KA (12 mg/kg); KA-treated animals reached high seizure scores within 3 hours. 7 days post KA injection rats were euthanized and brains harvested for [^{3}H]**PBR28** autoradiography studies. A significant

increase in TSPO levels was found in rats receiving KA vs. controls in the frontal cortex (572 vs. 188, p=0.014), thalamus (1127 vs. 193, p=0.0003), hippocampus (1152 vs. 237, p=0.0002), and choroid plexus (3275 vs. 2565, p=0.011). Immunohistochemistry (IHC) staining on adjacent sections from Tg4510 mice and KA-treated rats was carried out using the microglia marker ionized calcium binding adaptor molecule 1 (Iba1) and the astrocyte marker glial fibrillary acidic protein (GFAP). IHC staining in the hippocampus, frontal cortex, thalamus, cerebellum and brain stem showed glial cell activation correlated with the [^{3}H]**PBR28** autoradiography binding.

Disclosures: J. Xu: None. **J. Sun:** Employment; Pfizer. **D. Caouette:** None. **S. Grimwood:** None. **Y. Lu:** None. **K. Bales:** None. **D. Holtzman:** None. **R. Mach:** None.

Poster

866. Neuroinflammation: Neurodegeneration

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIEHS R01ES014470 to KMZ

PMDF Grant to KMZ

T32NS041218-10 to DJ

Title: Fibrillar alpha-synuclein elicits complex morphofunctional activation states in microglia

Authors: *D. A. JOSEPH, L. R. FENG, L. CORTES-AVELLANEDA, K. A. MAGUIRE-ZEISS
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Abstract: Parkinson’s disease (PD), an age-related neurodegenerative disorder, is characterized by the loss of dopamine neurons in the substantia nigra, the accumulation of alpha-synuclein (SYN) in Lewy bodies and neurites, and neuroinflammation. While the exact etiology of sporadic PD remains unknown, a growing body of evidence suggests that misfolded SYN promotes inflammation and oxidative stress with subsequent neurodegeneration. Microglia are the resident immune cells of the brain capable of quickly reacting to danger/damage associated molecular patterns (DAMPs) through activation of pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs). When a proinflammatory pathway is initiated activated microglia release cytokines, chemokines and nitric oxide which if unchecked can adversely impact adjacent neurons. We have demonstrated that misfolded SYN containing SDS-resistant high

molecular weight and monomeric species promotes inflammation and alters the expression of TLRs1, 2, 3 and 7, suggesting that SYN acts as a DAMP. In order to determine the mechanism of activation we separated the misfolded SYN into HMW (~250 kDa) and LMW (~16 kDa) fractions. Using both primary microglia from C57 mouse cortices and BV-2 microglia we assessed morphofunctional activation using immunocytochemistry and quantification of proinflammatory cytokine gene expression, production, and release. Here we report upregulation of proinflammatory (TNF-α, IL-1β and MMP9) and alternative activation (Arg-1 and IL-10) profiles in HMW SYN-stimulated microglia. In contrast, LMW SYN does not promote microglial activation. We also show that conditioned media from SYN-activated microglia attenuates the neurite complexity of dopamine neurons indicating that these microglial secreted molecules are detrimental to neurons. Overall, these data suggest that SYN-directed microglial activation occurs through specific pathways that are dependent on the conformation of SYN. These TLR-mediated microglial responses to SYN open an avenue for novel PD therapies, where altering SYN structure would attenuate inflammation.

Disclosures: D.A. Joseph: None. **L.R. Feng:** None. **L. Cortes-Avellaneda:** None. **K.A. Maguire-Zeiss:** None.

Poster

866. Neuroinflammation: Neurodegeneration

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:866.15/P18

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: DOD Grant W81XWH

Title: Novel magnetic resonance imaging techniques to visualize inflammatory/degenerative mechanisms

Authors: *R. CLEMENTS, E. FREEMAN, J. BLANK, A. KHITRIN
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Abstract: Current therapies alter neuro-inflammatory/degenerative diseases (such as Multiple Sclerosis, MS), however, little is known about their impact on neuroprotection and repair. Further, the development of effective therapies for these important components of MS has been hampered by the lack of sensitive clinical response criteria. We propose new methods aimed at associating changes in magnetic resonance (MR) detectable white/gray matter disease activity with pathological outcomes enhancing our understanding of degenerative processes resulting in tissue atrophy. We present novel protocols for existing MR scanning systems providing the

ability to visualize structures that are impossible with current methods. Using techniques to concurrently stain and three-dimensionally analyze many cell types and structures related to auto-inflammatory pathology (glial cells, neurons, myelin, microvessels, infiltrates) and correlate these changes with existing and novel MR modalities will provide new ways of processing, acquiring, analyzing and understanding data from existing MRI hardware. Spinal cords from control mice and mice with induced experimental autoimmune encephalomyelitis (EAE) were subjected to MR imaging using standard protocols and newly developed MR imaging sequences. Novel imaging sequences were designed to resolve tissues with short T2 (invisible in conventional MRI) using new signal excitation methods. After MR acquisition, spinal cords were sectioned and immunofluorescently probed to concurrently label neurons, glial cells, microvessels, cellular infiltrates and myelin. 3D laser scanning microscopy and digital reconstruction were utilized to generate high resolution volumetric data. MR and multichannel microscopy data were co-registered and analyzed to identify novel cellular phenomena/structures related to autoimmune inflammatory diseases visible in the newly developed MR acquisition sequences that resolves tissue with a short T2.

Disclosures: R. Clements: None. **E. Freeman:** None. **J. Blank:** None. **A. Khitrin:** None.

Poster

866. Neuroinflammation: Neurodegeneration

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Program#/Poster#:866.16/P19

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CAPES

CNPq

FAPERJ

Title: Ablation of protocerebral tract in crustacean induces both migration of hemocytes and activation of glia cells.

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Abstract: Circulating hemocytes are crucial in injury processes by participating in recognition, phagocytosis, melanization and cytotoxicity (Jiravanichpaisal, 2006). Likewise, microglial cells - specialized macrophages of the central nervous system - act as primary responding cells by producing a large number of pro-inflammatory factors, including nitric oxide (Saijo and Glass, 2011). In a previous paper of our laboratory, the protocerebral tract (PCT) of the crab *Ucides cordatus* was analyzed after extirpation of the eyestalk (Corrêa et al., 2005). In this paper we showed that among axons with morphologic aspects of axoplasmic degeneration, granular cells resembling hemocytes (blood cells) were seen. Based on this observation, we classified these cells and their participation in an acute degenerative process, 24 hours after lesion (Chaves-da-Silva et al., 2010). In the present work, our objective was to study the morphological and biochemical relationships between glial cells and hemocytes 48 hours after lesion (subacute response), as well as to study possible molecules released during the degenerative process. Analyzing the distal stump of injured PCT by immunohistochemistry, histochemistry, light and electron microscopies, and western blot, we observed a large number of IB4-positive cells (macrophages/microglia) when compared with control animals. These cells showed granules in the cytoplasm and were labeled for iNOS (inducible nitric oxide) and histamine. In the same region we observed an increasing number of cells labeled for GFAP (glial cells). Some of the cells containing granules (hemocytes) migrated further into the damaged tract when compared with the acute response (24 hours) PCT (Chaves-da-Silva et al., 2010). Electron micrographs of the PCT exhibited alterations in the morphologic aspect of the cells which infiltrated into the injured tract, when compared with the cells that surrounded the tract close to the connective tissue. Our results suggest that the presence of iNOS and histamine in the lesion site may be considered an important factor involved in the activation of hemocytes and/or glial cells attracted to the injury. In addition, we propose that there are two functionally different types of glial cells: GFAP positive cells, which respond to injury by changing their nuclei morphology and another, which maintains its morphology, as seen in normal conditions, and is not capable to express GFAP. These results show that hemocytes and glial cells have a critical role during degenerative processes, being essential to remove cellular debris and to organize the microenvironment for neuroregeneration.

Disclosures: P.G. Chaves da Silva: None. **C. Corrêa:** None. **S. Allodi:** None.

Poster

866. Neuroinflammation: Neurodegeneration

Location: Hall F-J

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Program#/Poster#:866.17/P20

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Alzheimer's Association

Alexander von Humboldt Foundation

Title: Circulating pro-inflammatory monocytes rapidly infiltrate hippocampal tissue after kainic acid-induced neurodegeneration

Authors: *N. H. VARVEL[1,2], J. NEHER[1], I. CHARO[3], R. RANSOHOFF[4], M. JUCKER[1,5]
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Abstract: Cells of myeloid origin mediate immunological roles in the mature brain. Microglia, the resident innate immune cells, populate the central nervous system (CNS) early in development and continuously monitor parenchymal tissues. While monocytes do not infiltrate CNS tissues during normal conditions, in response to injury, circulating monocytes can, at least temporarily, engraft in the brain and contribute to neuroimmune responses. Identification and subsequent analysis of microglia and CNS-engrafted monocytes is difficult due to nearly overlapping protein expression patterns. Previous studies have utilized whole-body irradiation coupled with transfer of labeled bone marrow-derived hematopoietic progenitors to distinguish between brain resident and peripheral cells. However, this approach induces damage to the blood brain barrier and introduction of supraphysiological levels of uncommitted hematopoietic stem cells into the circulation, leading to exaggerated monocytic infiltration of CNS tissue. In the present study, we wanted to determine if pro-inflammatory, Ccr2-expressing, circulating monocytes engraft in CNS tissue in response to neuronal cell death. To accomplish this, we utilized novel animal models in which the open reading frame of Ccr2 was replaced by red fluorescent protein (RFP) cDNA, such that heterozygous mice have RFP-expressing Ccr2-positive cells and retain receptor function. Homozygous Ccr2rfp/rfp knockout mice, maintained on the C57BL/6 inbred background, were crossed to wild-type FVB animals to create F1 hybrid Ccr2 heterozygous (B6.FVB-Ccr2-RFP) mice. B6.FVB-Ccr2-RFP mice subjected to kainic acid (KA) experienced seizures and neuronal cell loss in the hippocampus. As expected, neuronal degeneration was accompanied by local microgliosis. However, KA-induced neuronal degeneration also induced robust infiltration of peripheral Ccr2-RFP-expressing monocytes. Strikingly, the infiltrating Ccr2-positive monocytes were encountered specifically in the hippocampus and were not encountered in other brain regions, suggesting that, like microgliosis, monocyte infiltrating was remarkably specific to injured brain sites. Our future studies will be designed to further characterize CNS-infiltrating monocytes during neuronal degenerative processes by examining the temporal sequence of neuronal cell loss, microgliosis and monocyte infiltration. In addition, the role of monocytes in the injured brain will be determined by inhibiting monocyte infiltration after KA exposure.

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Poster

866. Neuroinflammation: Neurodegeneration

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01 EY020496

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Career Development Award- Research to Prevent Blindness, Inc.

Unrestricted Departmental Grant from Research to Prevent Blindness, Inc.

Title: IL-6 trans-signaling and neuroprotection of retinal ganglion cells in glaucoma

Authors: *H. M. CATHCART[1], C. R. FORMICHELLA[2], R. M. SAPPINGTON[2]
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Abstract: The cytokine IL-6 initiates pro- and anti- inflammatory signaling cascades through two receptors: membrane bound IL-6Rα (classical signaling) and soluble IL-6R (sIL-6R; trans-signaling). Both receptors interact with membrane bound gp130, a common signal transducer, causing downstream activation of transcription factors with the potential to modulate neuroinflammatory responses. The purpose of this study was to evaluate the neurobiological relevance of trans-signaling in the onset and progression of retinal ganglion cell (RGC) pathology in chronic and acute murine models of glaucoma. Using a co-immunolabeling assay, the presence of sIL-6R and transmembrane IL-6Rα were assessed simultaneously in vertical paraffin sections of retina, whole mount retina and optic nerve head. For this assay, two antibodies against IL-6Rα were used; the first is specific to the cytoplasmic peptide sequence, while the second is specific to the extracellular sequence. IL-6Rα was identified by co-localization of both antibodies whereas sIL-6R was revealed by the presence of only the extracellular antibody. Furthermore, IL-6Rα, sIL-6R, gp130 and soluble gp130 (sgp130) were quantified from soluble and trans-membrane protein isolated from whole retina and both fractions were analyzed using the Milliplex Map mouse soluble cytokine receptor immunoassay. In retina whole mounts from young and old mice exposed to chronic (DBA/2) and acute

(microbead injected C57BL/6) increase in intraocular pressure, we found that both forms of IL-6R were associated with SMI-31 or DAPI in RGCs. However, localization of IL-6Rα was primarily associated with RGC and astrocyte somas in control or PBS injected C57BL/6 mice, while strong sIL-6R labeling was associated with RGC axons and retinal vasculature in DBA/2 and microbead injected mice. The cytokine receptor immunoassay suggests that IL-6Rα concentration increased in old DBA/2 (9.70 pg/ml) retinas when compared to retinas from old C57BL/6 (6.88 pg/ml) mice. The immunoassay also revealed that both sIL-6R and IL-6Rα decreased in young DBA/2 (1.74 and 0.35 pg/ml) retinas when compared with concentrations from retinas of young C57BL/6 (5.42 and 4.99 pg/ml) mice. Gp130 concentrations were increased in young DBA/2 retinas (33.73 pg/ml; C57BL/6 11.24 pg/ml), while sgp130 concentrations were below the level of assay detection. These results indicate that sIL-6R is consistently associated with RGC axons in the retina and optic nerve head. The location and potential variability of sIL-6R expression within glaucomatous retinas is crucial to defining the role of sIL-6R in augmenting glial and neuronal responses in this inflammatory microenvironment.

Disclosures: H.M. Cathcart: None. **C.R. Formichella:** None. **R.M. Sappington:** None.

Poster

866. Neuroinflammation: Neurodegeneration

Location: Hall F-J

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Program#/Poster#:866.19/Q2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: AA005965

AA013521-INIA

AA017168

Title: Evidence for neuroinflammation in the thiamine deficient rat

Authors: C. ALT[1], A. MANNING-BOG[2], E. V. SULLIVAN[3], *A. PFEFFERBAUM[4,3], N. M. ZAHR[3,2]
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Abstract: Wernicke's Encephalopathy (WE), a disorder induced by thiamine (vitamin B1) deficiency (TD), is a concomitant of alcoholism, anorexia, and bariatric surgery. WE is

associated with focal pathology in brain regions, including the thalamus, mammillary bodies, and inferior colliculus. Many biological processes, such as inflammation, have been proposed to explain the selective neuronal loss observed in WE. To provide support for a role for neuroinflammation in TD, wild-type Wistar rats were fed TD chow; animals were also given either pyrithiamine (PYR, n=5) to induce TD, or thiamine (CON, n=5) to ensure they were thiamine replete. Following TD, the PYR group was discernible from the CON group by weight loss, piloerection, ataxia, and slowed righting reflex. Animals were euthanized after 12 days of treatment and extracted brain hemisections were either quick frozen in liquid nitrogen for cytokine protein analysis or placed in 4% paraformaldehyde for immunohistochemical analysis of glial activation. The levels of the pro-inflammatory cytokines TNF-α, IL-6, and IL1-β and the chemokine MCP-1 (i.e., CCL2) were quantified in the thalamus and inferior colliculus, structures vulnerable to TD. MCP-1 levels were significantly higher in the inferior colliculus of the PYR than CON group (p=.0077) and showed an insignificant trend in the thalamus (p=.1229). Evaluation of histopathology (astroglial activation determined by GFAP and microglial activation determined by Iba-1 immunoreactivities) may provide morphological support for biochemical evidence of inflammation in the inferior colliculus. These data support a previous finding reporting a role for secreted chemokines (i.e., MCP-1) in the pathophysiology of TD.

Disclosures: C. Alt: None. **A. Manning-Bog:** None. **E.V. Sullivan:** None. **A. Pfefferbaum:** None. **N.M. Zahr:** None.

Poster

866. Neuroinflammation: Neurodegeneration

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSF GRFP

NIH NRSA

Title: Investigating the role of prostaglandin E2 mediated neuroinflammation in models of parkinsonism

Authors: *S. S. PRADHAN[1], J. JOHANSSON[1], Q. WANG[1], N. KO[2], A. MANNING-BOG[2], K. ANDREASSON[1]
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Abstract: Recent studies have identified non cell-autonomous mechanisms such as inflammation as major contributors to Parkinsonian disease (PD) pathology. Neuroinflammation in PD is primarily mediated by significant increases in reactive microglia, which secrete pro-inflammatory cytokines and chemokines and reactive oxygen and nitrogen species (ROS/RNS). The prostaglandin E2 (PGE2) receptors EP2 and EP4 can respectively amplify or dampen microglial inflammation in a disease setting. However the role of microglial EP2 and EP4 has not been studied in the context of PD. In this study we investigate the interaction between microglial PGE2 signaling and dopaminergic (DA) neuron degeneration in the MPTP neurotoxin model of Parkinsonism. We show that MPTP induced loss of DA neurons and striatal dopamine levels is correlated with an increase in activated microglia in the substantia nigra and striatum. Our data suggests that modulating EP2 and EP4 signaling in microglia influences dopaminergic neuron survival, loss of striatal DA neuron projections, and levels of striatal dopamine. Furthermore we show that PGE2 signaling regulates microglial oxidative stress and cytokine production in vitro. The data indicates that EP2 and EP4 receptors may interact with Parkinsonian mechanisms by regulating inflammatory pathways in microglia. Targeting PGE2 signaling may be a potential therapeutic avenue for PD and other neurodegenerative disorders with a neuroinflammatory component.

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Poster

866. Neuroinflammation: Neurodegeneration

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Program#/Poster#:866.21/Q4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Division of Intramural Research, NIDCR, NIH

Insertion of Researchers Program at the Academy, CONICYT, Chile

Title: Inhibition of tissue-type plasminogen activator expression does not ameliorate neuro-inflammation in forebrain-specific Cdk5 conditional knockout mice

Authors: *E. UTRERAS PURATICH[1,2], M. PROCHAZCOVA[2], A. TERSE[2], S. TAKAHASHI[3], A. B. KULKARNI[2]
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Abstract: Cyclin-dependent kinase 5 (Cdk5) is essential for brain development and its deregulation is involved in neurodegenerative diseases. We reported earlier that the forebrain-specific Cdk5 conditional knockout (Cdk5 cKO) mice displayed early lethality at 3 weeks of age associated with neuronal migration defects, transient tremors, and growth retardation. The expression levels of the neuronal tissue-type plasminogen activator (tPA), a serine protease known to mediate microglial activation, were significantly increased in Cdk5 cKO mice. To delineate the specific role of tPA in the Cdk5 cKO phenotype, we generated Cdk5 cKO; tPA double knockout (2cKO) mice. Interestingly, 2cKO mice displayed a partial rescue in body weight gain and astrogliosis but still died at around 3 weeks of age, suggesting that increased expression of tPA observed in Cdk5 cKO mice does not contribute significantly to the pathological process that leads to astrogliosis and neurodegeneration. Furthermore, as compared to Cdk5 cKO mice, 2cKO mice also displayed increased expression of pro-inflammatory cytokines such as TNF-alfa, IL-6, IL-1 beta, IFN-gamma, OSM, LIF, and TGF-beta 1 in the brain cortex. Moreover, the CD68 levels, which are markers of microgliosis, were increased in 2cKO mice as compared with Cdk5 cKO mice. In conclusion, these results suggest that inhibiting tPA expression in Cdk5 cKO has no benefits for preventing its lethal phenotype associated with increased neuro-inflammation.

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Poster

866. Neuroinflammation: Neurodegeneration

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Swedish Foundation for Strategic Research

Title: The effect of the transcription factor Nurr1 in the adult dopaminergic system upon toxin-induced neuroinflammation

Authors: *M. PAPATHANOU[1,2], T. PERLMANN[1,2]
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Abstract: Developmental transcription factors (TF) are involved in the specification and differentiation of neurones. *Nurr1* is one such TF that has been extensively investigated for its role in the development of midbrain dopaminergic (DA) neurones. *Nurr1* knockout in mice leads

to lack of DA neurones and perinatal lethality. Work in our lab demonstrated that *Nurr1* is also crucial for the maintenance of fully functional adult DA neurones, which is in turn supported by the association of *Nurr1* gene variants with rare cases of sporadic and familial Parkinson's disease. However, the function of *Nurr1* in response to toxin insult remains elusive. Studies using *Nurr1* heterozygous mice have suggested a potential neuroprotective role of *Nurr1* against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or lactacystin neurotoxicity. It nonetheless remains uncertain whether the effects are due to *Nurr1* deficiency in mature neurons or if they are a consequence of developmental abnormalities. Moreover, lentiviral short hairpin RNA knockdown of *Nurr1* has suggested a role in DA neuroprotection against lipopolysaccharide-induced neuroinflammation by an anti-inflammatory activity within reactive microglia. Yet, it is unclear if *Nurr1* expression within neurons is also important for protection against inflammation-induced neurodegeneration. The aim of this study was to thus investigate inflammation-induced neurotoxicity in *Nurr1* conditional knockout mice.
Nurr1 floxed (C57/Bl6) mice were crossed with the tamoxifen-inducible Cre recombinase transgenic line under the dopamine transporter promoter. Mice were treated with tamoxifen at 4 weeks, followed by intranigral injection of the polyinosine-polycytidylic acid or vehicle at 8-10 weeks of age. The brains were subsequently analysed by immunohistochemistry using dopaminergic, microglial, astrocytic, phagocytic and apoptotic markers.
Nurr1 ablation increased vulnerability in PolyI:C-induced neurotoxicity. There was a time-dependent reduction in DA striatal innervation and a dose-dependent axonal degeneration, accompanied by phagocytosis but without significant signs of apoptosis. Finally PolyI:C led to astrogliosis and microgliosis although the expression of the toll-like receptor was evident in neurones and microglia but not in astrocytes.
In conclusion these results suggest that *Nurr1* is continuously important for protection of adult DA neurons and also suggest that functions within neurons contribute to DA neuroprotection in response to inflammation. These studies may therefore be of relevance in the context of Parkinson's disease.

Disclosures: M. Papathanou: None. **T. Perlmann:** None.

Poster

867. Brain Imaging Alterations in Schizophrenia and Other Psychoses II

Location: Hall F-J

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Program#/Poster#:867.01/Q6

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH R01 MH083320

Title: The amygdala and bipolar disorder: A meta-analysis of volumetric change

Authors: P. RANE[1], C. HASELGROVE[1], S. HODGE[1], *J. F. BATES[2,3], J. A. FRAZIER[1], D. N. KENNEDY[1]
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Abstract: The amygdala's involvement in the processing and expression of various emotions has captured attention of researchers for many years. Many studies involving amygdala changes in Bipolar disorder (BPD), a mood disorder, are published every year with seemingly inconsistent reports of volumetric findings. The amygdala is reported to be larger in the BPD patients compared to normal controls in many publications while other reports point towards smaller amygdala in BPD group. Comparisons between studies are confounded by a number of factors. Various methods of image acquisition, and volumetric analysis are employed. The studies also vary significantly in the age ranges, illness severity, illness duration, treatment status and comorbidities. This study was an effort to perform a meta-analysis on currently published reports to get an insight into any abnormalities in amygdala development in BPD patients.
The Internet Brain Volume Database (IBVD) offers a repository of volumetric data of different brain structures. IBVD was queried for amygdala volume reports in BPD and normal subjects. Publications were limited to those that had reports for both the groups as well as age ranges. The BPD group amygdala volume was converted to a singular z-score per BPD group by comparing it to corresponding control group from the same publication. These z-scores were plotted against the age average of the two groups.
The resulting plot points towards an interesting correlation where the young BPD groups (age <25) have negative z-scores while the older BPD groups (age >35) have positive z-scores. A few outliers that do not follow this pattern either have co-morbidities such as diagnosis of psychosis or ADHD. We hypothesize that this could be an age effect, however, it can also point towards an effect of disease and/or treatment durations. This is supported by the fact that most of the drug naïve BPD groups have smaller z-scores than the treated BPD groups, irrespective of the treatment.
This meta-analysis points towards the presence of a substantial age effect in the nature of the volumetric differences seen in the amygdala. This is impacted directly by effects such as age of illness onset, illness duration, medication status and duration, etc. As it is clear that all these factors play in important role in the meaning of any specific observation of amygdala volume, it highlights the importance of report of more standardized and detailed population characteristics by the authors. Detailed understanding of these additional effects will be necessary to elucidate the intriguing age relationship and direction reversal of amygdala volumetric observations relative to healthy subjects.

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Poster

867. Brain Imaging Alterations in Schizophrenia and Other Psychoses II

Location: Hall F-J

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Program#/Poster#:867.02/Q7

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: 5R21MH086104

NIH T90

Title: Network-level differences in effective connectivity during affect labeling in bipolar disorder

Authors: *S. TORRISI, E. LAU, N. VIZUETA, C. A. PENFOLD, S. Y. BOOKHEIMER, L. L. ALTSHULER
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Abstract: Background:
Bipolar Disorder (BP) involves dysfunction of emotion regulation. Using Dynamic Causal Modeling (DCM) with fMRI, we examined differences in effective connectivity in a subset of regions comprising the (incidental) emotion regulation network, specifically evaluating the role of the posterior inferior frontal gyrus (Broca's Area, BA44/45) in the linguistic labeling of emotional faces.
Methods:
34 euthymic BP I subjects (average age: 41.6 ± 12 years, 19 male) and 42 age- and gender-matched control subjects (41.5 ± 12, 23 male) were tested with a well-validated affect labeling paradigm. Standard general linear model analysis of label affect in SPM8 revealed greater recruitment of right vlPFC (BA 47) in controls and greater recruitment of subgenual ACC in bipolar subjects. Regions demonstrating equivalent levels of activation in both groups, however, were chosen for DCM analysis. During labeling affect vs matching affect, all subjects together showed strong activations in anterior insula and Broca's area. Thus time series were extracted from each of five left hemisphere regions strongly activated by labeling emotional facial expressions (FFA, amygdala, anterior insula, vlPFC, and Broca's area). Our models tested within groups the causal involvement of Broca's area and its path of modulation to the amygdala during labeling. The model space was parsed into orthogonal families to test commonalities across model permutations, and Bayesian model averaging (BMA) was performed on winning families to determine parameter estimates and weights between regions.
Results:

Results of final model comparison revealed different winning models between groups, indicating that labeling negative emotional faces involves a different pattern of connections in BP than in controls. Family-level Bayesian Model Selection (BMS) and BMA also clarified the roles of cortical regions and their influence on the amygdala.

Conclusion:

These findings indicate that the network recruited during incidental emotion regulation differs between euthymic BP and controls, providing novel effective connectivity evidence for trait dysfunction of the disease.

Disclosures: S. Torrisi: None. **E. Lau:** None. **N. Vizueta:** None. **C.A. Penfold:** None. **S.Y. Bookheimer:** None. **L.L. Altshuler:** None.

Poster

867. Brain Imaging Alterations in Schizophrenia and Other Psychoses II

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Association between hippocampal grey matter volume and rates of associative learning rate in controls but not schizophrenia or bipolar patients

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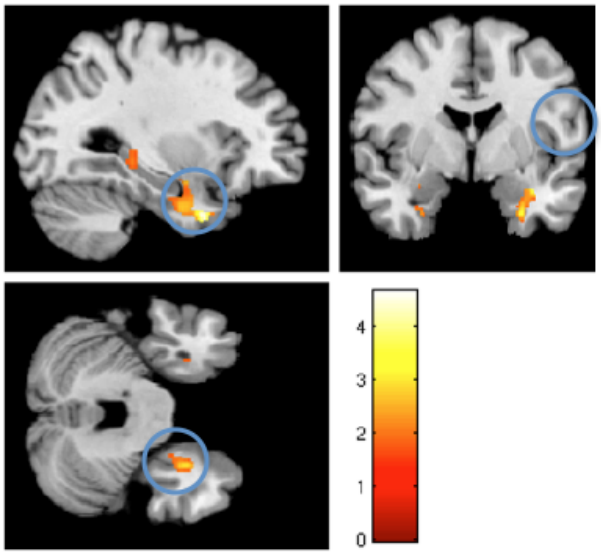
Abstract: Background: The hippocampus is central in memory formation and reported as central to the pathophysiology of schizophrenia (SCZ) and bipolar disorder (BP; Brambilla et al., 2011). Tasks of learning and episodic memory are particularly relevant to the study of hippocampal pathophysiology (Diwadkar et al., 2008). Hippocampal volume has been generally associated with integrity of episodic memory (Rodrigue & Raz, 2004) though these relationships have never been investigated in patients with SCZ or BP. Here we use Voxel Based Morphometry (VBM) to assess the relationship between hippocampal grey matter volume and learning rate during an associative learning paradigm (Brambilla et al., 2011) in a group of age- and gender-matched healthy controls (HC), SCZ and BP.

Methods: Subjects participated in an object-location paired associative learning task (Diwadkar

et al., 2008) administered the same day that structural MRI data were collected. Learning rates from the task were derived based on fitting negatively accelerated learning curves (performance=1-e$^{-rate*time}$) to individual subject data. Data were obtained from 17 SCZ (7 F, mean age=36 yrs, sd=8.1; learning rate=0.17, sd=.1), 12 BP(8 F,mean age:41, sd=9.6;learning rate=0.34, sd=.4) and 22 HC (8 F, mean age=33 yrs, sd=7; learning rate=1.24, sd=.4). Subjects wereassessed using the Structured Clinical Interview for Axis I DSM-IV Disorders (SCID-I). Data were analyzed using DARTEL (SPM8; Ashburner, 2007), and group differences in hippocampal volume and regressions with learning rate were investigated in 2nd level analyses corrected for cluster extent (p< .05).

Results: As seen in the figure, only HC evinced significant positive correlations between learning rate and hippocampal volume.

Discussion: HC were characterized by systematic positive relationships between hippocampal structure and learning related function, though these correlations were absent in the patient groups. These negative results imply that SCZ and BP (relative to HC) may be characterized by dysfunctional hippocampal structure-function relationships.



Hippocampal clusters (in HC) with a positive relationship to learning rate are depicted (insets) in sagittal, coronal and axial views.

Disclosures: B.B. Yanos: None. **R. White:** None. **M. Bellani:** None. **G. Rambaldelli:** None. **P. Brambilla:** None. **V. Diwadkar:** None.

Poster

867. Brain Imaging Alterations in Schizophrenia and Other Psychoses II

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Functional connectivity during facial-emotion recognition in euthymic bipolar disorder

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Abstract: Bipolar disorder is characterized by periods of mania, depression and euthymia; the exact cause of the disorder has proved elusive. Even during euthymia, a wide variety of brain regions show significant differences controls; dependent functional task. Differences in activation level and size have been reported in bilateral striatum [3, 2], Middle Frontal Gyrus (MFG) [4], Inferior Frontal Gyrus (IFG) [3, 4, 2] cingulate [4], thalamus, insula, lingual gyri, left amygdala , intrapietal sulcus, all three temporal gyri, right parahippocamopal gyrus, and intraparietal sulcus [1, 3, 2]. MPRAGE and a face/emotion task were carried out on a Siemens Trio 3T scanner, (TR 2s), and segmented using Freesurfer. 21 euthymic subjects (9F, 12M) and 18 control (10F, 8M) were scanned while performing three face/emotion tasks as in Altshuler et al. [1, 2]. We calculated full brain voxel-voxel connectivity using Mutual Information (MI). Data were motion corrected and signals from the ventricles, unlabeled voxels, and white matter regressed out. The upper 90th percentile of connectivity within each pair of regions was then taken for regional connectivities. MI lower than 0.2 bits were
treated as unconnected. To correct for multiple comparisons (168 × 168 regions), $1.77 \times 10{-}6$ was considered significant. During the face/emotion task, euthymic subjects showed higher connectivity than controls between: 1) right
caudate - ipsalateral temporal plane of the superior temporal gyrus, 2) right caudate - ipsalateral heschl's Gyrus, 3) right transverse temporal sulcus - left Frontal Marginal gyrus/sulcus 4) right heschl's gyrus - right transverse frontopolar gyrus/sulcus, 5) left amdala - right anterior occipital sulcus, 6) right medial orbital sulcus - ipsalateral occipital pole, 7) left orbital sulcus - right middle temporal gyrus (MTG), 8) left orbital sulcus - bilateral transverse collateral sulcus. The connections that showed increases in euthymic patients verse controls are necessary portions of facial emotion processing and were active in controls as well. Supranormal connectivity during emotion recognition suggests euthymic patients experience tighter neural integration, and less inhibition during emotion recognition. In particular higher amygdala-visual, MTG-prefrontal and prefrontal-transverse sulcus pairing indicates a lack modulatory influcence during face/emotion processing.
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[2] L. C. Foland-Ross et al. NeuroImage, 59(1):738-44, Jan. 2012.

[3] G. S. Malhi et al. Bipolar disorders, 7 Suppl 5:58-69, Jan. 2005.
[4] J. Townsend et al. Psych. Res, 182(1):22-9, Apr. 2010.

Disclosures: M.C. Chambers: None. **A. Irimia:** None. **L. Altshuler:** None. **J. Van Horn:** None.

Poster

867. Brain Imaging Alterations in Schizophrenia and Other Psychoses II

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: VA Merit CX000154 (KMS)

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Title: Auditory gamma oscillation and cortical volume deficits in schizophrenia

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Abstract: Objective:
Recent studies suggest that abnormalities in the oscillatory activity of neural circuits may have a critical role in the pathophysiology of schizophrenia. Forty Hz auditory steady state response (ASSR) impairments have been reported and hypothesized to reflect these neural network abnormalities in chronic schizophrenia patients (SZ) and first episode psychosis compared to healthy controls (HC). However, the relationship between gamma oscillation deficits and

structural abnormalities of the auditory cortex in schizophrenia is not clear. Here we examined whether 40 Hz ASSR deficits in SZ are associated with volume reductions of Heschl's gyrus (HG), particularly in the left hemisphere, where both abnormalities have been associated with auditory hallucinations.
Methods:
Electroencephalogram was recorded from 24 SZ (4 females) and 24 HC (4 females) matched for demographic data. Stimuli were 20-, 30-, and 40-Hz binaural click trains. Time-frequency analyses (PLF: phase locking factor, EP: evoked power) were performed for each ASSR. MRI volumes were calculated for gray matter of HG. Clinical symptoms were assessed by PANSS.
Results:
ASSR PLF at 40 Hz and HG volume were significantly reduced in SZ specifically in the left hemisphere compared to HC. There were no significant differences in EP. Left lateralized 40Hz ASSR PLF reduction was correlated with total positive symptoms and hallucination symptoms. For the ASSR-HG associations, 40 Hz ASSR PLF was positively correlated with left HG volume in SZ.
Conclusions:
These findings support the presence of functional neural oscillatory deficits, which are correlated with underlying structural abnormalities and positive symptoms. We propose that assessing ASSR-PLF in SZ may provide a sensitive biomarker for the integrity of functional and structural neural networks that are fundamentally altered in SZ.

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Poster

867. Brain Imaging Alterations in Schizophrenia and Other Psychoses II

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Program#/Poster#:867.06/Q11

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH R01MH073998

SNF PBGEP3_139835

NIDA RC2DA029475

Title: Resting state functional connectivity in pediatric bipolar disorder

Authors: *A. A. LISO NAVARRO, E. M. SIKOGLU, D. KENNEDY, J. A. KING, J. A. FRAZIER, C. M. MOORE
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Abstract: Bipolar disorder is a lifetime condition with a prevalence of 1%, and in the recent years it has been recognized that this disorder may present as early as childhood and adolescence. Children and adolescents with Pediatric Bipolar Disorder (PBD) may first exhibit cognitive impairments affecting domains such as attention and executive functions.
Non-invasive techniques such as functional Magnetic Resonance Imaging (fMRI) may allow us to further understand the mechanisms underling the pathophysiology of PBD and its associated cognitive deficits. Some of the brain regions linked to the pathophysiology of PBD include the Anterior Cingulate Cortex (ACC) and prefrontal cortex. In this work we studied resting state functional connectivity (rs-FC: a technique that allows one to assess spontaneous brain activity; using temporal correlations, one can examine the functional connections between a region of interest (ROI), namely seed region, and the rest of the brain) in a sample of children with PBD (n=7, mean age=12.14±2.67 years) and Typically Developing children (TD) (n=18, mean age=12.22±2.71 years).
Subjects were scanned on a 3T Philips System and an EPI sequence was performed (TE=30msec, TR=2500msec, slice thickness:3mm, 50 slices) Seed based rs-FC (seed at ACC MNI coordinates (0,44,10)). Analysis was carried using SPM-8 and DPARSFv2.0. The connectivity patterns for children with PBD and TD participants were compared through a two-sample t-test (corrected for False Discovery Rate, $p < 0.05$ considered significant).
There is more connectivity in the TD group than the PBD group, between the ACC base seed and left cortex orbitofrontal, right superior frontal gyrus and left medial temporal gyrus (FDR corrected $p<0.05$).
Importantly the orbitofrontal cortex, superior frontal gyrus (both part of the prefrontal cortex) and left medial temporal gyrus are implicated in different cognitive tasks, which are impaired in PBD. The decreased connectivity shown in PBD between the ACC and these areas could suggest a disruption in the brain “rewiring” in PBD, may be due to an asynchrony in brain maturation in PBD.
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Poster

867. Brain Imaging Alterations in Schizophrenia and Other Psychoses II

Location: Hall F-J

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Program#/Poster#:867.07/Q12

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: ARC risk predicts PFC function, hippocampal activation, and PFC-hippocampal connectivity in an encoding and retrieval memory task

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Abstract: Background: We have recently found evidence for an association between a single variant in ARC and risk for schizophrenia (Kirov et al., 2012). ARC is an activity-regulated immediate early gene thought to be important for activity-dependent neuronal specificity (Wang et al., 2006). Previously, we found risk at this variant was associated with primary deficits in PFC-hippocampal connectivity in an N-back working memory task (Zheutlin et al., 2012). Here, we investigated whether this variant of ARC predicts prefrontal and hippocampal activation, as well as altered coupling between these two regions, in a hippocampus-mediated task.
Methods: 187 healthy subjects completed a picture encoding and retrieval (PEAR) memory task and were scanned using GE EPI at 3T (Milwaukee, WI). ARC effects were assessed in SPM5 using ANCOVA with age, gender, and performance metrics included as nuisance variables. First level activation and PPI analyses included above were performed as described previously (Callicott et al., 1999; Rasetti et al., 2011). Connectivity was assessed with PPI analysis using a hippocampal seed.
Results: We found that genetic risk at this SNP was correlated with increased activation in the DLPFC and hippocampus in the PEAR encoding & retrieval episodic memory task. Additionally, increased risk was correlated with increased coupling between the prefrontal cortex and the hippocampus.
Conclusions: In healthy subjects, ARC risk predicted abnormal PFC function, hippocampal activation, and PFC-hippocampal connectivity in a hippocampus-mediated memory task.

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Poster

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Program#/Poster#:867.08/Q13

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Neuronal Coordination Research Focus Frankfurt (NeFF)

Title: Effects of ketamine on gamma-band oscillations in MEG-data: A comparison with chronic schizophrenia

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Abstract: There is evidence to suggest that the pathophysiology of schizophrenia involves hypofunctioning of the N-methyl-D-aspartate (NMDA) receptor. To test the validity of this model, we measured neural oscillations in the 30 -120 Hz range with Magnetoencephalography (MEG) during the administration of a sub-anesthetic dose of ketamine, a non-competitive antagonist at the NMDA-Receptor site in healthy controls. Furthermore, MEG-data was also obtained from patients with chronic schizophrenia and matched controls.
10 normal participants were administered ketamine (0.006 mg/Kg) or a placebo saline solution in a within-subject design. In addition, 16 controls and 16 chronic patients with schizophrenia were assessed. A moving sinusoidal grating (5° of visual angle) was presented and the task for participants was to detect the acceleration of the grating through a button press. MEG-activity was recorded with a 275-channel CTF MEG system. Structural brain images (MRIs) were collected and a beamforming technique was adopted to localize the source of oscillations.
In healthy controls, ketamine led to a reduction in induced gamma band oscillations (45-75 Hz) over occipital sensors, which was localized to early visual areas (V1/V2). This reduction in high-frequency oscillations during visual stimulation is comparable to the pattern of impairments in chronic patients with schizophrenia. Thus, our data support the view that gamma-band oscillations and associated cognitive deficits may involve the hypofunctioning of NMDA-receptors, suggesting a crucial role of aberrant glutamatergic neurotransmission in the pathophysiology of schizophrenia.

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Poster

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Program#/Poster#:867.09/Q14

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH Intramural Research Program

Title: Effective connectivity deficits in numerical set pattern evaluation and updating in schizophrenia

Authors: *C. M. KAPLAN[1], J. L. MOLINA[1], Q. CHEN[1], J. A. APUD[1], D. R. WEINBERGER[1,2], H. Y. TAN[1]
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Abstract: Introduction:
Inferring and abstracting logical patterns from information in the environment is impaired in psychotic disorders. It has been shown prefrontal cortex may be implicated in failure of these higher cognitive operations; striatal and hippocampal regions have also been implicated in failure to update and learn from information gathered. However, less is known about their integrated function in larger brain networks, such as how bilateral parietal, premotor, and sub-regions in prefrontal cortex interact with the subcortical regions in these operations, and which circuits are impaired or compensatory in psychosis.
Methods:
24 normal controls (NC) and 14 schizophrenia patients (SZ) performed an event-related fMRI task in a 3T-scanner. Participants were presented with numbers varying stochastically about an underlying base integer (number set). The sets of numbers also shifted stochastically up or down by one unit at the base integer. Subjects were to respond when they inferred the number set has shifted, and to update their representation of the new base integer. Imaging data was analyzed using SPM8. Dynamic Causal Models were estimated at subject level within ROI masks based on combined NC+SZ group-level peaks.
Results:
SZ performed with larger total prediction error ($p=0.0044$) and larger mean prediction error at response ($p=0.0075$). At set-updating, task modulated effective connectivity was reduced in SZ ($p<0.05$) at bilateral anterior (APFC), dorsolateral prefrontal (DLPFC), premotor (PMd), parietal (PAR), striatal (STR) and hippocampal (HIP) regions. Task-critical networks in NC that correlated with overall performance and were impaired in SZ included left to right connectivity at APFC and at DLPFC, and left prefrontal regions to STR and PAR connectivity. However, in

SZ there was negative correlation, leading to better prediction performance of number set shifting, from engagement of left hemisphere rostral to caudal effective connectivity (DLPFC to APFC and from APFC to STR, HIP, and PAR).
Discussion:
Effective numerical set evaluation and updating engaged bilateral rostral to caudal patterns of frontal-parietal-striatal-hippocampal effective connectivity, which was dysfunctional in SZ. However, increased left rostral to caudal effective connectivity, particularly in networks engaging APFC, was associated with improved performance in SZ and may be compensatory. The effects of medication in these findings remain to be explored.

Disclosures: C.M. Kaplan: None. **J.L. Molina:** None. **Q. Chen:** None. **J.A. Apud:** None. **D.R. Weinberger:** None. **H.Y. Tan:** None.

Poster

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Program#/Poster#:867.10/Q15

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Drug Abuse [R01DA026799-01]

Title: Resting state functional connectivity of brain reward circuitry in patients with schizophrenia and cannabis use disorder: Effects of cannabis and dronabinol

Authors: *A. S. FISCHER[1], S. WHITFIELD-GABRIELI[2], R. M. ROTH[1], A. I. GREEN[1]
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Abstract: Background:
Cannabis use disorder (CUD), present in over 40% of patients with schizophrenia (SCZ), worsens disease progression. Unfortunately, available treatments for SCZ are rarely successful in limiting cannabis use. Development of effective treatments for these patients will be facilitated by a more complete understanding of the basis of their cannabis use. We have proposed that patients with SCZ and CUD use cannabis to ameliorate abnormalities present within their brain reward circuit (BRC). In the present study, we used resting state functional connectivity (RSC) to assess the intrinsic functional architecture of the BRC. We then investigated the effects of cannabis and dronabinol (synthetic delta-9-tetrahydrocannabinol [THC]) on RSC of reward circuitry in these patients.
Methods:

Participants included 10 patients with SCZ and CUD and 6 healthy controls. Patients abstained from cannabis and other substance use for a minimum of seven days prior to scanning. Subjects completed an eight minute fMRI resting scan at baseline and again after either smoking a 3.6% THC cannabis cigarette and ingesting a placebo pill (n=5) or smoking a placebo cigarette and taking an active 15mg dronabinol pill (n=5). Connectivity was analyzed using a region of interest (ROI)-to-ROI analysis between bilateral nucleus accumbens (Nac) source ROIs (anatomically defined 4mm spheres surrounding peak Nac coordinates), 88 broadmanns regions, and 5 anatomically defined BRC ROIs. Between group connectivity differences were assessed using ANOVA. Group x Time interactions were determined with repeated measures ANOVA.

Results:

Measurements at baseline revealed decreased RSC between bilateral NAC ROIs and other key BRC regions (ventral tegmental area, amygdala, hippocampus, caudate, putamen, medial prefrontal, orbitofrontal, and anterior cingulate cortices) in patients with SCZ and CUD as compared to controls ($p<0.05$, uncorrected). Moreover, smoked cannabis and oral dronabinol each increased connectivity within the BRC ($p<0.05$, uncorrected).

Conclusion:

The data from this small study sample suggest that BRC connectivity is reduced in patients with SCZ and CUD, and that cannabis and dronabinol may move the connectivity toward that seen in normal subjects. Our findings support the use of RSC as a neuroimaging 'biomarker' to identify BRC abnormalities present in patients with SCZ and CUD and, potentially, to track the effect of pharmacologic agents on this circuitry. Moreover, if these findings are confirmed in a larger sample, they may suggest that dronabinol should be studied as an adjunctive treatment (with antipsychotic medications) to help reduce cannabis use in SCZ.

Disclosures: A.S. Fischer: None. **S. Whitfield-Gabrieli:** None. **R.M. Roth:** None. **A.I. Green:** Research Grant; National Institute on Drug Abuse [R01DA026799-01].

Poster

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Program#/Poster#:867.11/Q16

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Reduced n-acetylaspartate in the frontal cortex and ventral hippocampus following sub-chronic phencyclidine treatment in the female rat.

Authors: S. MARSH, S. L. MCLEAN, J. C. NEILL, *M. K. HARTE
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Abstract: Schizophrenia is a chronic psychiatric disorder characterised by positive, negative and cognitive symptoms. Although the exact cause remains elusive there is substantial evidence for neuronal dysfunction in the brains in patients with the disorder. N-acetylaspartate (NAA) is present in high concentrations in the CNS and is considered to be a general marker of neuronal loss and/or cellular dysfunction. Numerous magnetic resonance studies have shown reductions in levels of NAA in schizophrenia, with deficits correlated to disease severity. Studies have also demonstrated recovery of NAA levels following acute brain injury. Further studies have also reported increases in NAA following pharmacological therapy. Taken together these studies demonstrate its potential use as a biomarker for assessing restoration of neuronal function following successful treatments. We have consistently demonstrated that sub-chronic phencyclidine (PCP) treatment in the female rat produces robust cognitive deficits and pathological changes that mimic those reported in schizophrenia. The aim of this study was to investigate levels of NAA in the brain in the female rat following our sub-chronic PCP treatment regime.

Adult female (n=16) hooded lister rats received sub-chronic PCP (2 mg/kg bi-daily, i.p, n=8) or vehicle (saline 0.9% bi-daily, i.p, n=8) for 7 days followed by a 7 day washout period. Six weeks later the rats were killed and brains removed. Brains regions were dissected in relation to Bregma coordinates [Frontal Cortex, +4.7 to +3.2mm; Striatum, +1.7 to -0.2mm; Dorsal Hippocampus -3.3 to -4.8mm; Ventral Hippocampus -4.8 to -6.3mm; Temporal Cortex -3.3 to -4.8mm mm. Tissue was homogenised, samples prepared and extracted with ISOLUTE SAX anion exchange columns, followed by HPLC analysis using UV detection. Compared to vehicle, sub-chronic PCP treatment produced significant reductions in NAA, in the frontal cortex ($p<0.05$) and ventral hippocampus ($p<0.05$), with no significant changes in the striatum, dorsal hippocampus or temporal cortex. These preliminary findings demonstrate that sub-chronic PCP administration causes reductions in NAA in the frontal cortex and ventral hippocampus. This provides further evidence to suggest that sub-chronic PCP can mimic aspects of the neuronal pathology of schizophrenia and may provide a translational biomarker for assessing the effect of treatment on indicators of normal neuronal function. The relationship of the reductions in NAA in these regions to the cognitive deficits reported in the sub-chronic PCP model remains to be determined.

Disclosures: S. Marsh: None. **S.L. McLean:** None. **J.C. Neill:** None. **M.K. Harte:** None.

Poster

867. Brain Imaging Alterations in Schizophrenia and Other Psychoses II

Location: Hall F-J

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Program#/Poster#:867.12/Q17

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NARSAD Independent Investigator Award (AB)

Foundation of Hope Award (AB)

Title: Abnormal white matter dti connectivity in children and adolescents with a first degree relative for schizophrenia.

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Abstract: Background: Schizophrenia has most recently been characterized as a neurodevelopmental disorder associated with aberrant maturation of cortical connectivity. Whether aberrant connectivity precedes onset of psychosis and is present in at-risk children is unclear. The goal of the present study was to examine white matter connectivity in fronto-limbic and fronto-striate tracts and regions in familial high-risk children and adolescents.
Methods: DTI images were acquired on 23 children ages 9-18 with a first degree family member with schizophrenia (FHR) and 38 age-, gender, and ethnicity matched controls (CTLs). Acquisition consisted of two 15-direction (+ one baseline) 2mm isotropic full-brain EPI DWI acquisitions.Following DTI QA analysis and artifact removal, the diffusion tensors and diffusivity measures (FA, MD, GA, AD and RD) were calculated. Two analysis methods were implemented, one examining Regional Diffusion Statistics, and the second focusing on Fiber-Tract based statistics. For the ROI-based analysis, T1 and T2-weighted images for each subject were run through automatic tissue segmentation (itkEMS), and automatic cortical and sub-cortical parcellation tools. Regional diffusion statistics were calculated for each of the cortical and sub-cortical white-matter masked ROIs. For the tract-based analysis, subjects' FA maps were warped using a non-linear registration technique (AtlasWerks) to create an atlas for the study population. The resulting dataset of fiber tracts were run through FADTTS to measure the variability of diffusivity statistics along the fibers and their association with diagnosis and age as covariates.
Results: ROI-based analyses revealed significantly larger FA ($p<.02$) in prefrontal and cingulum regions in HR compared to CTL children. Preliminary fiber-tract analysis results revealed significant group and age effects on left and right cingulum pathways, consistent with the ROI analysis.
Summary: Our findings show that white matter organization along fronto-limbic regions is aberrant in children with familial risk for schizophrenia.

Disclosures: M. Weber: None. **S. Hart:** None. **C. Bellion:** None. **S. Ramsey:** None. **J. Bizzell:** None. **A. Belger:** None.

Poster

867. Brain Imaging Alterations in Schizophrenia and Other Psychoses II

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NARSAD Young Investigator Award

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Title: Structural and functional working memory deficits associated with adolescent-onset psychosis

Authors: *A. SCHVARCZ[1], K. H. KARLSGODT[2], P. BACHMAN[2,3], M. JALBRZIKOWSKI[1], T. G. VAN ERP[4], T. D. CANNON[1,2,3], C. E. BEARDEN[1,2,3]
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Abstract: Background: Adolescent-onset psychosis (AOP) is typically associated with more severe deficits in working memory and attention relative to the adult-onset form of illness. Given the relationship between cognitive abilities and the well-documented gray and white matter abnormalities that occur in schizophrenia, as well as the neuroanatomical changes that occur throughout adolescence, studies have just recently begun to investigate the correlation between structural changes and corresponding physiological and behavioral outputs in AOP. This study seeks to better understand how variability in structural brain connectivity constrains functional-physiological connectivity and capacity in individuals with AOP relative to healthy controls. Methods: Functional magnetic resonance imaging (fMRI) scans were obtained from AOP patients (N=16) and healthy comparison subjects (N=16) matched for age (range: 12-22), gender,

IQ, ethnicity, self and parent education, and handedness. Diffusion tensor imaging (DTI) scans were obtained from a subset of those participants (patient N=9, control N=13). While in the scanner, subjects performed a spatial capacity task manipulating working memory load in which 1, 3, 5, or 7 dots are shown per trial, with subjects asked to identify if a probe dot's location corresponded to a location of one of the target dots (12 trials per load). fMRI data were analyzed with FSL FEAT; DTI data were analyzed with Tract-Based Spatial Statistics. Threshold for cluster statistical significance was set at $Z>2.3$; $p<0.05$.
Results: fMRI analyses revealed a distinct pattern of age-associated changes in neural activity during task performance in AOP patients relative to controls in multiple brain regions. Under greater working memory demand, controls demonstrated greater neural activity as compared to patients in posterior brain regions. Of the working memory tracts examined in DTI analyses (inferior longitudinal fasciculus (ILF), superior longitudinal fasciculus (SLF), and cingulate gyrus), a significant positive association between age and fractional anisotropy (FA) was observed bilaterally in the ILF in controls, which was not observed in the AOP group. This suggests the possibility that patients fail to show expected age-associated increases in FA in working memory circuitry.
Discussion: This study provides additional clarity into brain structure-function relationships in AOP as they relate to working memory dysfunction. Future studies will employ more advanced joint modeling approaches such as joint independent component analysis to further assess these relationships and better elucidate endophenotypes of schizophrenia.

Disclosures: A. Schvarcz: None. **K.H. Karlsgodt:** None. **P. Bachman:** None. **M. Jalbrzikowski:** None. **T.G. van Erp:** None. **T.D. Cannon:** None. **C.E. Bearden:** None.

Poster

867. Brain Imaging Alterations in Schizophrenia and Other Psychoses II

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH Intramural Research Program

Title: Increased prefrontal-parietal effective connectivity at number processing in working memory in schizophrenia

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Abstract: Introduction:
Symbolic number processing engages parietal cortex, especially inferior parietal sulcus, and is a uniquely human ability. Working memory (WM) engages prefrontal-parietal cortices and is a limited capacity system that supports the brief storage and temporal integration of information. WM but not number processing is known to be dysfunctional in schizophrenia (SZ). However, less is known of how these brain processes may influence each other in SZ. Here, we examined, in healthy volunteers (HV) and SZ, effective connectivity of cortical networks during dissociable encoding of number symbols in WM, and subsequent processing of their relative numerosity.
Methods:
22 healthy volunteers (HV) without previous history of psychiatric or neurological disorders and 15 patients were processed in SPM8 and DCM10. Subjects were matched for performance, response time, age, and sex. Regions activated during WM conditions (Visual Cortex, VC; Dorsolateral Prefrontal Cortex, DLPFC; Ventrolateral Prefrontal Cortex, VLPFC; and Parietal Cortex, IPS) were selected for effective connectivity analyses.
Results:
During WM encoding, greater connectivity ($p<0.05$) was observed in forward VC→VL and backward VL→VC, DL→VC, VL→PC in SZ relative to HV. At numerosity comparison, there was greater connectivity from VC→PC in SZ. Moreover, SZ engaged sustained frontal-parietal network connectivity during numerosity comparison, whereas HVs performed equivalently with local parietal cortex processing and less cortical network connectivity ($p<0.05$).
Conclusion:
SZ patients engaged increased cortical network effective connectivity during WM encoding without performance advantage. The increased network coherence appeared sustained at numerosity comparison in SZ whereas HV engaged local parietal processing. The abnormal increased network coherence during WM encoding may reflect dysfunction in SZ that sets the stage for impairments in subsequent processing. Medication status, however, could be a confounder.

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Poster

868. Drugs of Abuse and Addiction: Translational Studies

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA 1 R21 DA21241-01A1

Title: Just not yet: Earlier age of marijuana use predicts poorer neurocognitive performance and alterations in brain structure

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Abstract: Marijuana (MJ) remains the most popular illicit drug in the US. While studies have reported that chronic MJ use is related to cognitive impairments, the perceived risk of MJ use has declined, which has been accompanied by an increase in rates of use, especially among younger adults. Diffusion tensor imaging (DTI) provides a quantitative measure of white matter microstructure, also reported to be altered in MJ smokers. In order to assess the impact of age of onset of MJ use on cognitive performance and white matter microstructure, we analyzed data from 46 chronic MJ-smokers and 31 non-MJ smoking control subjects who completed a neurocognitive battery and DTI scanning.

Overall, MJ smokers exhibited significantly worse performance on tasks of executive function, including the Wisconsin Card Sorting Test (WCST), Stroop Color Word Test, and the Trail Making Test (TMT), and significantly higher levels of impulsivity, measured by the Barratt Impulsiveness Scale (BIS) compared to control subjects. DTI analyses revealed MJ smokers also had significantly lower levels of fractional anisotropy (FA), a measure of directionality and coherence in white matter fibers, and higher levels of mean diffusivity (MD) in the left and right genu of the corpus callosum relative to controls. To clarify the impact of age of MJ onset, MJ smokers were separated into those who began smoking prior to age 16 (early onset; n=23) and those who began using MJ at age 16 or older (late onset; n=23). Interestingly, analyses revealed that the cognitive, impulsivity and white matter differences detected between the MJ smokers and control subjects were primarily driven by the early onset MJ group.

Within the MJ smokers, regression analyses revealed that earlier age of MJ onset was predictive of poorer performance and altered white matter. Earlier MJ onset predicted lower FA in the left and right genu (β= .344, p=.09; β= .358, p=.08) and higher MD in the right genu (β=-.345, p=.09), and predicted both longer time required to complete Part A (β= .304, p=.05) and increased errors on Part B (β=-.302, p=.05) of the TMT. Earlier MJ onset also predicted both higher frequency (β=-.346, p=.01) and magnitude of MJ use (β=-.375, p=.01), measured as smokes per week and grams per week. Taken together, data suggest that early MJ use results in poorer cognitive function, higher impulsivity and altered brain structure relative to non-MJ smokers. These findings underscore the importance of early identification and prevention of early, regular MJ use, as exposure to MJ during a period of developmental vulnerability appears to result in neurophysiological changes, which may have long-term implications.

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Poster

868. Drugs of Abuse and Addiction: Translational Studies

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH DA02019

Title: Differential consequences of repeated administration of a cannabinoid receptor agonist on limbic neuronal morphology in adolescent compared to adult rats.

Authors: *A. F. CARVALHO[1], B. A. S. REYES[3], N. SOUSA[2], E. E. J. VAN BOCKSTAELE[3]
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Abstract: Cannabinoid receptor agonists are known to alter mood, cognition and perception. Several lines of evidence indicate that the impact of cannabinoid-based drugs is different in adolescence compared to adulthood. For example, cannabinoid-induced cognitive impairment is more evident during adolescence when compared to adulthood. In addition, adolescents report fewer episodes of aversion to cannabinoid-based drugs in comparison to adults, a finding that could contribute to the higher incidence of cannabis use during adolescence. In the present study, we used three-dimensional analysis of Golgi-stained neurons in the nucleus accumbens (Acb) of adolescent and adult rats that had been chronically treated with a synthetic cannabinoid receptor agonist, WIN 55,212-2 or vehicle. Results showed that exposure to WIN 55,212-2 significantly decreased dendritic length, dendritic branching and spine density in adult animals compared to age-matched controls. Interestingly, WIN 55,212-2 treatment only affected spine density in adolescent-treated animals when compared to control. These findings indicate a differential consequence to the effects of cannabinoids on neuronal morphology during the period of adolescence in a critical brain region involved in motivated behaviors. These morphological differences may contribute to the differential effects of cannabinoid-based agents on behavior in adult compared to adolescent animals.

Disclosures: A.F. Carvalho: None. **B.A.S. Reyes:** None. **N. Sousa:** None. **E.E.J. Van Bockstaele:** None.

Poster

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Program#/Poster#:868.03/R2

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH

Title: Molecular targets of cannabis action in the developing nervous system

Authors: G. TORTORIELLO[1], C. MORRIS[2], C. H. BOTTING[3], Y. L. HURD[2], *T. HARKANY[1,4]
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Abstract: Cannabis abuse during pregnancy is a leading socioeconomic problem since it imposes life-long modifications to the behaviors and cognition of affected offspring. Recent studies suggest that Δ^9-tetrahydrocannabinol (THC), the major psychoactive component from cannabis, can disrupt the spatial and temporal organization and efficacy of endocannabinoid signalling at CB1 cannabinoid receptors (CB_1Rs) in developing cortical neurons. Axonal growth may be particularly sensitive to THC action since CB_1Rs and the enzymes metabolizing endocannabinoids preferentially accumulate in the growth cones of cortical neurons, affecting growth cone steering decisions. We hypothesized that cannabis smoking during pregnancy can permanently change the cytoarchitecture, synaptic connectivity and functions of fetal forebrain neurons by erroneous recruitment of signaling networks to CB_1Rs in immature axons. We find that maternal cannabis exposure during pregnancy introduces axon fasciculation errors leading to the mistargeting of excitatory synapses and permanently impaired endocannabinoid-dependent and -independent forms of synaptic plasticity in the hippocampus of adult offspring exposed to THC *in utero*. Pharmacological dissection revealed that THC acts as a partial CB_1R agonist in the fetal brain, and recapitulates the bulk of the molecular modifications associated with WIN55,212-induced impairments of cortical development. We used global protein profiling to identify a 37-member hierarchical protein network downstream from CB_1Rs that confers cannabis sensitivity to developing cortical neurons by decreasing the rate and randomizing the subcellular foci of microtubule polymerization. We find that maternal THC exposure impacts the expression of select members of this protein network in the fetal human forebrain at mid-gestation, reinforcing our notion that THC perturbs the developmental dynamics of a signalosome domain required to maintain ordered axonal growth and guidance during the formation of cortical connectivity maps.

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Poster

868. Drugs of Abuse and Addiction: Translational Studies

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Topic: C.17. Drugs of Abuse and Addiction

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SC Johnson Foundation

Title: High serum glutamate level is associated with positive response to acamprosate treatment.

Authors: *D.-S. CHOI[1], H. NAM[1], V. M. KARPYAK[3], X. LI[2], D. L. WALKER[1], D. J. HINTON[1], H. CHOI[1], O. ABULSEOUD[2], M. A. FRYE[2], D. A. MRAZEK[2]
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Abstract: Acamprosate treatment is most effective in the maintenance of abstinence from alcohol. However, the efficacy of acamprosate is not uniform in all patients. Therefore, serum biomarkers associate with acamprosate efficacy would be useful for the identification of potential responders and develop an individualized treatment approach for alcohol dependence. We used LC-MS/MS to quantify serum metabolites and acamprosate levels in 90 alcohol dependent subjects (DSM-IV-TR) completing a 3-month trial of acamprosate treatment. We compared acamprosate levels and metabolite profiles between 44 responders (no alcohol consumption) and 46 non-responders (any alcohol consumption). Glutamate levels were significantly higher at baseline in the responder group compared to the non-responder group. Following acamprosate treatment serum glutamate levels in the responder group decreased significantly compared to baseline. Consistently, levels of glutamate related metabolites in the urea cycle also showed similar changes and were negatively correlated with glutamine levels. There was no change in glutamate or other metabolite levels in the non-responder group when comparing baseline levels to that following acamprosate treatment. Our data demonstrated that responders to acamprosate treatment have elevated serum glutamate levels, which are decreased by acamprosate treatment.

Disclosures: D. Choi: None. **H. Nam:** None. **V.M. Karpyak:** None. **X. Li:** None. **D.L. Walker:** None. **D.J. Hinton:** None. **H. Choi:** None. **O. Abulseoud:** None. **M.A. Frye:**

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Poster

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Support: T32AA07468

R01AA011114

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The Wellcome Trust

VA

Title: Genetic models provide evidence for MPDZ involvement in sedative withdrawal and self-administration

Authors: *K. J. BUCK[1,2], L. C. MILNER[2], O. KOLOTUSHKINA[2], N. H. KOMIYAMA[3], D. ROSSI[2], S. GRANT[3]
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Abstract: Using robust behavioral models, positional cloning, expression and sequence analyses, we implicated *Mpdz* as a gene involved in ethanol and barbiturate withdrawal (*Nat Neurosci* 7:699, 2004), but *in vivo* evidence supporting its role in withdrawal behavior has been lacking. *Mpdz* codes for the multi-PDZ domain protein (MPDZ). Here, to assess its role *in vivo*, we report the development of transgenic mice with increased *Mpdz* expression (MPDZ-TG). Injection of an artificial bacterial artificial chromosome (BAC) construct (with *Mpdz* but no other full genes) into embryos resulted in transgenic founders from which we completed MPDZ-TG mice (DBA/2 background). We also developed an MPDZ knockout (heterozygote) model (MPDZ-KO; C57BL/6 background) using an embryonic stem cell line with an insertional

mutation in *Mpdz*, which we compared to wildtype (WT) littermates. Ethanol and pentobarbital (PB) withdrawal associated CNS hyperexcitability were assessed using the handling-induced convulsion. MPDZ-TG show less severe ethanol withdrawal than WT ($p<0.05$), with a trend for PB withdrawal ($p<0.1$). MPDZ-KO show more severe ethanol withdrawal than WT ($p<0.05$), and a trend for PB withdrawal ($p\sim0.1$). The strengths of the TG approach complement the limitations of the KO approach, and vice versa, so the finding that both support a role for MPDZ in sedative withdrawal is compelling. Additionally, self-administration analyses using a two-bottle choice paradigm indicate a main effect of genotype ($p<0.01$), with MPDZ-KO consuming more ethanol than WT. Ongoing analyses are comparing MPDZ-KO and WT to assess the potential impact of MPDZ expression on 5-HT_{2C} and $GABA_B$ receptor function. MPDZ physically associates with 5-HT_{2C} and $GABA_B$ receptors, and the substantia nigra pars reticulata (SNr) is implicated in mediating the effects of this QTL on withdrawal (*J Neurosci* 28:9840, 2008; *Behav Brain Res* 218:152, 2010) so we have initially examples voltage-clamped (V_h= -50 mV) SNr neurons. 5-HT (10 and 100 uM) induced inward currents in approximately 80 and 40% of MPDZ-KO and WT neurons, respectively; these currents are mediated by 5-HT_{2C} receptors in GABAergic neurons. MPDZ-KO also show a trend ($p<0.1$) for more severe convulsions in response to a 5-HT_{2C} receptor antagonist (SB242084, 10 mg/kg) compared to WT. The majority of SNr neurons had outward currents in response to a $GABA_B$ receptor agonist, with MPDZ-KO having greater response to 3 and 30 uM baclofen than WT ($p<0.001$). Our results suggest a relationship between reduced MPDZ expression and increased 5-HT_{2C} and $GABA_B$ receptor function.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant RO1 NS070715

NIH Grant T32 DA007027

Title: Pharmacological determinants of abuse-related morphine effects in an assay of intracranial self-stimulation in rats

Authors: *L. L. MILLER, S. S. NEGUS
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Abstract: Intracranial self-stimulation (ICSS) is a class of behavioral procedures that can be used to evaluate the abuse liability of drugs. Mu opioid agonist effects on ICSS reflect integration of both (a) rate-increasing effects that are associated with abuse liability, and (b) rate-decreasing effects that may limit abuse liability. This study investigated pharmacological determinants of the rate-increasing and rate-decreasing effects of morphine on ICSS. Male Sprague Dawley rats were equipped with electrodes targeting the medial forebrain bundle and trained to respond for stimulation under a fixed-ratio 1 schedule. Reinforcement rates were evaluated across a range of stimulation frequencies (56-158 Hz), and effects of morphine (cumulative doses of 0.32-10 mg/kg) on frequency-rate ICSS curves were determined. In drug naïve rats, significant but modest rate-increasing effects predominated at low morphine doses, but rate-decreasing effects emerged and predominated at higher morphine doses. Morphine effects were redetermined under two sets of conditions. The first experiment examined effects of morphine treatment history on morphine-induced changes in ICSS. Four groups of rats were exposed to protocols designed to establish graded histories of morphine exposure with or without the concomitant opportunity to engage in ICSS. In general, prior morphine exposure attenuated the rate-decreasing effects and enhanced the rate-increasing effects of morphine, and this effect of morphine exposure was greatest when morphine was administered daily prior to ICSS sessions. One neurobiological effect of morphine exposure is to decrease the density of functional mu opioid receptors in brain. Accordingly, the second experiment evaluated the degree to which effects of morphine exposure could be recapitulated by 24 hr pretreatment with the irreversible mu antagonist beta-funaltrexamine (ß-FNA; 0.32-3.2 mg/kg). The hypothesis predicted that opioid-induced rate-decreasing effects would be more sensitive than rate-increasing effects to pretreatment with ß-FNA; however, ß-FNA was not more potent to block morphine rate-decreasing effects. These results are consistent with earlier data to suggest that rate-increasing and rate-decreasing effects are mediated by anatomically distinct populations of mu receptors with similar sensitivities to ß-FNA but different sensitivities to morphine exposure. These data also suggest that a history of opioid agonist exposure may produce pharmacological and behavioral tolerance to morphine effects that limit abuse potential and enhance expression of morphine effects associated with abuse.

Disclosures: L.L. Miller: None. **S.S. Negus:** None.

Poster

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Merck, Sharp, & Dohme Ltd

Title: Self-administration of compounds lacking intrinsic efficacy at α1 subunit-containing $GABA_A$ receptors

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Abstract: Benzodiazepines are widely prescribed but their utility is limited by unwanted side effects, such as abuse potential. The mechanisms underlying the abuse-related effects of benzodiazepines are not well understood, although α1-containing $GABA_A$ receptors are thought to play a critical role. Furthermore, previous drug history has been shown to be a major determinant of the abuse-related effects of benzodiazepine-type drugs. In the present study, we examined the reinforcing effects of several α1-sparing compounds that vary with respect to intrinsic efficacy (determined by patch-clamp electrophysiology in cloned receptors) at α2, α3, and α5 subunit-containing $GABA_A$ receptors: MRK-623 (functional selectivity for α2/α3-containing receptors), TPA023B (functional selectivity for α2/α3/α5-containing receptors), and TP003 (functional selectivity for α3-containing receptors). The reinforcing effects of these α1-sparing compounds were compared with those of the non-selective partial agonist MRK-696, and non-selective full agonists, midazolam and lorazepam. Comparison of relative reinforcing effectiveness was determined in rhesus monkeys trained to self-administer either a benzodiazepine (midazolam) or a stimulant (cocaine), under a progressive ratio schedule of i.v. drug injection. Lacking efficacy at α1-containing receptors resulted in lower break points than the reference benzodiazepines. Moreover, the α1-sparing compounds were self-administered in midazolam-trained, but not cocaine-trained monkeys. Notably, the α3-selective compound TP003 had significant reinforcing effects, albeit with less reinforcing effectiveness relative to reference benzodiazepines. These results suggest α3-containing $GABA_A$ receptors may be important for the reinforcing effects of benzodiazepines in midazolam-trained monkeys, whereas α1-containing $GABA_A$ receptors may be crucial for the reinforcing effects of benzodiazepine-type compounds in animals with a history of stimulant self-administration.

Disclosures: **N.M. Shinday:** Other Research Support; Merck Research Laboratories. **E.K. Sawyer:** None. **B.D. Fischer:** None. **D.M. Platt:** None. **S.C. Licata:** None. **J.R. Atack:** None. **G.R. Dawson:** None. **D.S. Reynolds:** None. **J.K. Rowlett:** None.

Poster

868. Drugs of Abuse and Addiction: Translational Studies

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:868.08/R7

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH K01DA027756 (CAH)

Title: Differentiating neural networks with interleaved TMS-BOLD imaging: Insight into addiction

Authors: *C. A. HANLON[1], M. CANTERBERRY[2], J. J. TAYLOR[2], W. DEVRIES[2], M. S. GEORGE[2,3]
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Abstract: Introduction: Interleaved transcranial magnetic stimulation (TMS) and BOLD imaging in the MR environment provides us with a unique opportunity to probe neural circuitry. The purpose of the current study was to determine if, through the use of an optimized interleaved TMS-BOLD sequence in two cortical targets, we could differentially activate lateral and medial prefrontal cortex neural circuits.
Methods: Interleaved TMS/BOLD imaging data was acquired for 15 healthy individuals and 10 cocaine users who received TMS in 2 runs with the coil positioned over the: 1) dorsolateral prefrontal cortex (DLPFC, EEG: F3), and 2) orbitofrontal/medial prefrontal cortex (OFC/MPFC, EEG: FP1)(Magstim MR-compatible coil). The TMS pulse started 100ms before the onset of the next TR (9 second interpulse interval, 100% motor threshold). BOLD data was analyzed using standard parametric techniques. Additionally 5 participants were scanned twice to evaluate test-retest reliability.
Results: Among healthy controls, DLPFC TMS was associated with a significant elevation of BOLD signal in multiple dorsal cortical areas (middle frontal gyrus, middle temporal gyrus, superior frontal gyrus, brainstem, inferior parietal/angular gyrus, precuneus)**.** In contrast, OFC/MPFC TMS was associated with a significant elevation of BOLD signal in multiple medial and limbic subcortical areas (cingulate cortex, medial frontal gyrus, cuneus, caudate, middle temporal cortex, cerebellum ($p<0.05$, $k = 50$, corrected clusters). The cocaine users demonstrated

a similar pattern of activity, but has selective dysregulation in the OFC/MPFC network. **Conclusions:** These novel data demonstrate that it is possible to differentially activate known cortical-subcortical networks through an optimized TMS/BOLD sequence over the DLPFC and the OFC/MPFC. Test-retest reliability is high in healthy controls and among cocaine users there is a selective deficit in OFC/MPFC circuit function. These data have important implications for both basic neuroscience research and in patient populations that may have pathology differentially affecting mesolimbic versus mesocortical circuitry.

Disclosures: C.A. Hanlon: None. **M. Canterberry:** None. **J.J. Taylor:** None. **W. DeVries:** None. **M.S. George:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Topic: C.17. Drugs of Abuse and Addiction

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NIH Grant AA16849

Title: The relationship between excessive ethanol drinking, level of intoxication, and plasma corticosterone across a bitonic function of fixed time intervals in ethanol-preferring and non-preferring mouse strains.

Authors: *M. M. FORD, A. D. MCCRAKEN, A. M. STEELE, K. A. GRANT
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Abstract: Scheduled-induced polydipsia (SIP) employs intermittent schedules of food reinforcement to generate excessive drinking, and accumulating evidence suggests that drinking typographies established during induction in cynomolgus monkeys are predictive of future chronic heavy drinking. The current work sought to further explore the relationship between the amount/pattern of 5% v/v ethanol (5E) or water consumed, the level of intoxication achieved (via blood ethanol concentration; BEC) and the response of the hypothalamic-pituitary-adrenal axis (via plasma corticosterone; CORT) across a range of fixed time (FT) intervals of food delivery. Male mice (C57BL/6 and DBA/2; n = 6/strain/fluid) were food restricted to 90% free-feeding body weight and then serially exposed to FT schedules (0, 0.5, 1, 2, 3, 5, 7, 10, 20 and 60 min) during daily 60 min sessions. Both strains exhibited a bitonic function (ascending and descending limb) of 5E intake as the FT interval was progressively lengthened, with peak

intoxication occurring at FT-5 (2.1±0.2 mg/ml in C57BL/6 versus 1.0±0.4 mg/ml in DBA/2 mice). Plasma CORT levels were positively correlated with BEC during the ascending limb in C57BL/6 ($r = 0.87$; $P < 0.001$) and DBA/2 ($r = 0.48$; $P < 0.01$) mice, but not during the descending limb. This suggested that the CORT response was driven primarily by the induction schedule and not by ethanol consumption, as CORT levels remained elevated as 5E intakes declined during the descending limb and similar CORT concentrations were observed across FT intervals when water was the available solution. Clear strain differences in 5E lick distribution across the inter-pellet intervals (IPIs) and bout patterns were also apparent. C57BL/6 mice consumed 5E throughout the entire IPI whereas DBA/2 mice concentrated their licking exclusively within the initial 30-sec following pellet deliveries, regardless of FT interval length. Peak intoxication at FT-5 was associated with a 3-fold increase in first bout size, a doubling of % total intake consumed within the first bout, and a 2-fold escalation in the rate of drinking onset (licks/min) in C57BL/6 mice only. In contrast, the 5E drinking typography of DBA/2 mice remained relatively fixed across FT intervals of the ascending limb, resulting in a lower peak BEC in this non-preferring strain. Although the peak interval of SIP (FT-5) culminates in elevated ethanol intake and intoxication in both C57BL/6 and DBA/2 strains, significant HPA axis activation and expression of binge-like drinking typographies are observed only in the ethanol-preferring C57BL/6 mice.
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Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Title: Ethanol intake under social circumstances or alone: Impact of age, sex, social activity and social anxiety.

Authors: *E. M. TRUXELL, L. P. SPEAR, E. I. VARLINSKAYA
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Abstract: Alcohol use is normative among American adolescents, with drinking typically occurring in social situations. Recent studies in laboratory rodents suggest that responsiveness to certain desired effects of ethanol may be influenced by social context, with these influences being more pronounced during adolescence than in adulthood. Therefore, the present study was designed to assess the impact of drinking alone or in a social group on ethanol intake in group-housed adolescent and adult male and female rats. Another aim was to investigate whether individual differences in baseline levels of social activity and anxiety influence subsequent ethanol intake. On postnatal day (P) 30 or P70, levels of social activity and anxiety were assessed using a modified social interaction test. Animals were then given a 30-min drinking session every other day (six sessions) from P36 to P46 for adolescents and P76-86 for adults. During each session, animals were given access to 10% ethanol in “supersac” (3% sucrose + 0.125% saccharin) solution in a novel cage. On alternating days for each animal, ethanol access occurred either alone or in a group of five littermates, with the drinking context on the first day counterbalanced for each age/sex group. Animals were not food or water deprived prior to these limited access sessions. All experimental sessions were videotaped for later scoring, with individual intakes in group sessions indexed as proportional time spent drinking per animal x total g EtOH consumed/body weight. Trunk blood samples were collected immediately after the last session, and BECs determined. In general, adolescents drank more than adults, and females demonstrated higher intake than males regardless of age. Adolescent males, adult males and adult females
consumed more ethanol under social than alone circumstances. In contrast, adolescent females showed notable increases in ethanol intake under alone test circumstances. Highly socially active adolescent males demonstrated elevated levels of ethanol intake relative to their low and medium socially active counterparts when drinking in groups, but not when drinking alone. In adolescent females, level of social anxiety was associated with their intake under social circumstances, with highly socially anxious females consuming more ethanol than less socially anxious animals – an effect not seen under alone drinking conditions. In adults, neither social activity nor social anxiety predicted drinking behavior, suggesting that responsiveness to social stimuli exerts a substantial impact on “social drinking” during adolescence, but not in adulthood.

Disclosures: E.M. Truxell: None. **L.P. Spear:** None. **E.I. Varlinskaya:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Title: Kappa-opioid receptors in neurons expressing the dopamine transporter mediate ethanol preference but not escalation of ethanol intake or binge drinking in mice.

Authors: *K. L. SMITH, A. VAN'T VEER, C. S. JOHN, E. I. SYPEK, W. A. CARLEZON JR., B. M. COHEN, A. J. BECHTHOLT-GOMPF
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Abstract: An important role for endogenous opioids in regulating the rewarding value of ethanol (EtOH) has been established both by findings using opioid antagonists in preclinical studies and by the effective clinical use of non-selective opioid antagonists in the treatment of alcoholism. The critical involvement of mu- and delta-opioid receptors is reported; however, the role of kappa-opioid receptors (KORs) requires further clarification, as changes in KOR functionality can both contribute to and diminish EtOH reward. Here, using cell type specific KOR deletion, we investigated the role of KORs in the expression of three types of ethanol consumption: 1) preference for EtOH, using a 2 bottle choice paradigm, 2) acquisition of moderate to excessive drinking (escalation drinking) and 3) binge drinking, using the drinking in the dark (DID) paradigm.
Methods: For selective ablation of KORs in dopamine transporter (DAT) containing neurons, we crossed our floxed KOR mice to DAT-Cre mice (mice expressing Cre recombinase under the control of the DAT promoter). Separate cohorts of female mice were used for each experiment. Two bottle choice: mice were given access to tap water through two sipper tubes for 4 days (acclimation). During the choice phase of the experiment, one of the two water bottles was replaced with a bottle containing EtOH in increasing concentrations (3%, 6% and 10%, 15% v/v). Escalation drinking: mice were acclimated to tap water through two sipper tubes for 4 days, before intermittent EtOH (15% v/v) access; mice were subjected to 24-hour cycles of ethanol access and deprivation for 14 days. DID: Water bottles were replaced with a 20% (v/v) EtOH solution sipper tube daily for 2h, presented 3h after lights out. After 6 days (acquisition), mice were given ethanol access for 4 h.
Results: Floxed KOR DAT-Cre+ (DAT neuron KOR knock-out) mice demonstrate a lower preference for EtOH compared to their littermates that lacked Cre in the two-bottle choice paradigm. No difference was detected between genotypes in escalation of EtOH intake or in

binge EtOH drinking (DID).
Conclusions: These data indicate that KOR activation contributes to the rewarding value of EtOH and that EtOH preference is mediated, at least in part, by regions expressing DAT, which would include the VTA. Although KOR deletion decreases preference for EtOH, this effect appears to be overcome when EtOH intake increases from low levels to moderate/excessive intake. Taken together these findings suggest that KORs in regions expressing DAT may mediate vulnerability to low and moderate intake, but play a negligible role in the regulation of excessive EtOH intake.

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Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Title: During early ontogeny age and Picrotoxin determine ethanol intake and reinforcement, and whether ethanol is aversive or appetitive

Authors: *S. K. SANDERS, D. WATERS, A. GOLDBERG, N. ARANA, T. VILLALUNA, D. MENDOLA, F. LIRIANO, N. E. SPEAR
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Abstract: Ethanol (EtOH) intake is high in the infant rat. Blood EtOH concentrations (BECs) of 300 mg% have been found after 30 min of drinking. EtOH intake does not directly reflect EtOH reinforcement; they often have been dissociated empirically. EtOH can condition aversions, but EtOH intake of doses that can condition aversions has been recorded. EtOH acts as a GABA agonist, and GABAergic drugs have been found to affect EtOH-related behavior. GABA function changes throughout early development, which could explain ontogenetic changes in EtOH intake. To assess the relationship between EtOH intake and reinforcement during infancy and the possible role of GABA, infant rats were given multiple EtOH exposures on postnatal day

6 (P6)-P10 or P12-P16. These ages differ in intake when EtOH is given by the technique of " consume off the floor" (COF), a self-administration paradigm. In the present experiment pups in the younger and older groups were given EtOH or water (H20). Intake sessions occurred every 24 hrs and were 20 min long. 48 hrs after the last intake session all pups were given an injection of Picrotoxin (PTX), a GABAA antagonist, prior to a test of H20 ingestion. In the younger group, pups previously given EtOH consumed more H20 at test than pups previously given H20. In the older group pups previously given EtOH ingested less H20 at test than pups previously given H20. In the younger group, PTX decreased intake of H20 regardless of what they were given previously, and his was more evident with previous EtOH. In the older group (P12-P16), PTX did not affect intake of H20 in pups previously given EtOH, but it did decrease H20 intake on P18 in pups given H20 on P12-P16. The younger infants appeared to condition acceptance of EtOH and the older infants appeared to condition aversion to EtOH. Since PTX decreased H20 intake on P18 and slightly on P12, additional P12 and P18 pups were given an injection of PTX prior to their first exposure to H20. PTX did not affect intake of H20 at P12 or P18 in pups given neither EtOH nor H20 previously. PTX apparently altered the effects of prior testing experience in general. Since PTX did not increase H20 intake in P18 pups given EtOH previously, it apparently did not affect aversive conditioning. Overall, the younger group ingested more EtOH and greater EtOH reinforcement was seen in the younger rats on P12, whereas the older group had lower EtOH intake on P12-P16 and they showed an EtOH aversion on P18. Taken together this suggests that EtOH is appositively reinforcing in the younger infants, but aversive in the older infants.

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Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Program#/Poster#:869.05/R12

Topic: C.17. Drugs of Abuse and Addiction

Title: Continuous intravenous caffeine infusions dose-dependently increase the motivation to obtain alcohol in rats.

Authors: *S. JONES, E. GARCIA, B. L. RINDT, M. I. PALMATIER
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Abstract: Caffeine (CAFF) and alcohol (ETOH) are self-administered simultaneously in human subjects. In pre-clinical models the bitter taste of CAFF reduces oral self-administration of ETOH, however bolus CAFF injections increase ETOH intake as well as motivation to obtain ETOH in rats. The present study investigated the effects of continuous intravenous (IV) experimenter-administered CAFF infusions on ETOH self-administration in order to more closely approximate the time course of co-administration in humans. ETOH drinking was initially established via intermittent access drinks of ETOH (20% v/v) in the home cage, with water freely available throughout this initiation phase. Once stable intake levels were reached, rats were briefly water restricted and shaped to respond for water in operant conditioning chambers that were fitted with two levers and two liquid dippers. Water was available in each dipper for pressing the lever located directly above that dipper on a fixed-ratio 1 (FR1) reinforcement schedule. Rats were then implanted with IV jugular catheters and allowed to recover for 7-10 days. During recovery, rats were randomly assigned to one of 2 groups [CAFF (1.36 mg/kg/session) or saline (SAL, 0.9%)] with the preference for the left and right levers balanced across groups. For each rat, one lever/dipper was assigned ETOH and the other water, with the constraint that preference for each dipper did not differ in either group. In subsequent tests, ETOH and water were available under an FR1 schedule before being shifted to an FR2 schedule. Rats received IV infusions of either CAFF or SAL every minute of a 60 minute session. CAFF significantly increased responding for ETOH under the FR2 schedule, but did not alter responding for water. Rats were then shifted to a progressive ratio schedule and CAFF concentrations were manipulated in order to determine the relationship between IV CAFF dose and the motivation to obtain ETOH. CAFF dose-dependently increased motivation to obtain ETOH, and preliminary findings suggest that the peak CAFF dose in this paradigm is approximately 5 mg/kg/h. These findings suggest that IV CAFF increases ETOH self-administration and motivation to obtain ETOH when CAFF is administered in conjunction with access to ETOH. Future studies will investigate whether CAFF will increase the motivation to obtain ETOH when oral ETOH and IV CAFF are both contingent upon operant behavior.

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Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Topic: C.17. Drugs of Abuse and Addiction

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Title: Gaboxadol infusion in the nucleus accumbens shell decreases ethanol self-administration in mice

Authors: *M. RAMAKER[1], M. N. STRONG[1], M. M. FORD[1], D. A. FINN[1,2]
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Abstract: Recent in vitro studies suggest that recombinantly expressed GABAA receptors containing the δ subunit may show enhanced sensitivity to low doses of ethanol (i.e., 3 - 30 mM), when compared to receptors without the δ subunit. The δ subunit-containing GABAA receptors are thought to be located exclusively in the extrasynaptic space and contribute to tonic inhibition. Previous research in our lab has found that systemic injections of gaboxadol (THIP), a GABAA receptor agonist selective for the δ subunit, dose-dependently reduced intake of 10% (v/v) ethanol (10E) in a 2-bottle choice procedure (both 24 hr access and 2 hr access). In order to elucidate which brain regions may be underlying this effect, the present study aimed to examine the effect of a nucleus accumbens (NAc) shell infusion of THIP on 2 hr ethanol intake in an identical experimental setup. The NAc shell was chosen based on evidence in rats in which a conditional knockdown of the δ-subunit in the NAc shell reduced ethanol self-administration. In the current study, C57BL/6 (B6) male mice had 2-hour access to 10E and water beginning two hours into their dark cycle. Following approximately 4 weeks of stable ethanol intake, mice were surgically implanted with bilateral cannulae aimed at the nucleus accumbens shell. Following recovery from surgery, stable ethanol intake was re-established (2.7 g/kg/2 hr). Mice received a bilateral infusion of 0 or 500 ng (in 200 nl acsf) THIP per side into the NAc shell (n=11, within-subjects design), with a week between infusions. Infusion of THIP led to a 51% decrease in ethanol self-administration ($p = 0.008$). Importantly, these results indicate that activation of GABAA receptors in the NAc shell mimicked the effect seen following systemic THIP administration and that the δ subunit-containing GABAA receptors in the NAc shell may be an important mediator of ethanol reinforcement.

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Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Program#/Poster#:869.07/R14

Topic: C.17. Drugs of Abuse and Addiction

Title: The role of midbrain dopamine neurons in mediating alcohol drinking behaviors

Authors: *B. JUAREZ[1,2], A. K. FRIEDMAN[3], D. CHAUDHURY[3], J. J. WALSH[2], M. CRUMILLER[2], M.-H. HAN[3]
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Abstract: In contrast to most drugs of abuse, such as cocaine, human alcohol dependence research has shown that there are multitudes of factors that predispose an individual to alcoholism or alcohol abuse. This suggests that there may be a "susceptibility" to alcohol preference that could ultimately lead to alcohol dependence. To understand the physiological properties that underlie this susceptibility to alcohol preference, we used C57BL/6J male mice, a strain that has a well-established alcohol drinking preference behavior. Previous studies have shown that this strain of mice displays a bi-model distribution of low and high preference alcohol drinking phenotypes. Using a two-bottle choice (2-BC) paradigm with increasing concentrations of alcohol (24 hr access), we were able to clearly define the low preference drinkers and the high preference drinkers by their statistically different consumptions of alcohol. We then performed *in vivo* electrophysiological recordings from dopamine neurons in the ventral tegmental area (VTA), a key component of the mesolimbic reward circuit, between the two groups. Surprisingly, we found that low preference drinkers have a significantly higher firing rate, higher bursting frequency and percent time bursting in VTA dopamine neurons as compared to the high preference drinkers. To further investigate this phenomenon, we used optogenetic techniques to manipulate firing of VTA cells *in vivo* to mimic the firing changes we observed in low preference drinkers. Using a burst/phasic stimulation protocol, we show that activation of channel-rhodopsin (ChR2)-infected VTA neurons causes a significant decrease in alcohol drinking preference and consumption during a four-day binge drinking paradigm. This occurs in both one-bottle choice (1-BC) and 2-BC in alcohol consumption and preference, when compared to control mice and mice that received a tonic stimulation pattern. We are currently using halorhodopsin to decrease the firing of these cells in order to observe changes in alcohol drinking behavior. These studies, and continuing work, will provide highly useful information about cell type-specific functional roles of the brain's reward circuit in mediating alcohol drinking behaviors.

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Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Support: NIAAA Grant R01 AA016848

The State of California for medical research on alcohol and substance abuse through the University of California, San Francisco

Title: MetBDNF polymorphism promotes persistent alcohol drinking behavior in mice.

Authors: *V. WARNAULT, E. DARCQ, D. RON
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Abstract: The brain-derived neurotrophic factor (BDNF) plays an important role in neurogenesis and neurodevelopment, as well as in learning and memory (1). A single nucleotide polymorphism (G196A) in the BDNF gene results in an amino acid substitution of Valine (Val) for Methionine (Met) in the pro-region of BDNF causing a deficit in neuronal activity-dependent release of the neurotrophic factor (2). An average of 23% of the world population carries the MetBDNF allele (3) and this polymorphism has been associated with the severity level of several psychiatric disorders (1-2).

We generated data to suggest that BDNF is part of a homeostatic pathway that gates the transition from moderate to excessive alcohol drinking (4-7). Therefore, we hypothesized that the protective role of the growth factor against the development of adverse phenotypes such as excessive alcohol intake will be diminished in carriers of the MetBDNF allele. To test this hypothesis, we generated homozygous knock-in mice carrying the MetBDNF allele on a C57Bl/6J background (MetBDNF), and tested whether this polymorphism promotes excessive alcohol drinking in adult male mice.

Using a paradigm of intermittent access to 20% alcohol (v/v, 2 bottle choice), we found that the MetBDNF mice drink a higher level of alcohol than wild type (WT) mice. Interestingly, the MetBDNF but not WT mice continued to consume alcohol even when the solution was adulterated by quinine (0.1-0.3g/l). Importantly, this difference was not due to a change in quinine palatability. These data suggest that carrying the MetBDNF allele results in a persistent consumption of high levels of alcohol despite negative consequences (e.g., ingestion of an unpalatable solution). We also found that the higher level of alcohol intake in MetBDNF mice was not due to faster alcohol metabolism. Importantly, the two genotypes did not differ in general compulsivity-like behavior as measured by the T-Maze. Furthermore, MetBDNF and WT mice consumed the same amount of a saccharin solution (0.03%w/v) even after adulteration by quinine (0.1-0.3g/l), indicating that the persistent alcohol intake by the MetBDNF mice is specific to alcohol and is not due to a general increase in motivation to drink a rewarding solution. Together, our results suggest that the MetBDNF allele increases the risk for developing alcoholism by promoting compulsive alcohol intake.

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5-McGough et al 2004 J. Neurosci.
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7-Logrip et al 2008 FASEB J.

Disclosures: V. Warnault: None. **E. Darcq:** None. **D. Ron:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Program#/Poster#:869.09/R16

Topic: C.17. Drugs of Abuse and Addiction

Title: Effect of chronic alcohol use on steady state plasma levels of stavudine, lamivudine and nevirapine in HIV/AIDS patients in Uganda using chronic alcohol-use biomarkers

Authors: *G. BBOSA
Dept Pharmacol & Therapeut., Makerere Univ. Med. Sch., Kampala, Uganda

Abstract: Chronic alcohol consumption by some HIV/AIDS patients on ARVS may affect their therapeutic outcome due to the interaction of ethanol and the drugs. The study determined the effect of chronic alcohol use on the steady state plasma drug concentrations (therapeutic window) of d4T, 3TC and NVP in the HIV/AIDS patients. A longitudinal cohort using repeated measures design with serial measurements model was used. The alcohol-use bioamrkers were used to screen the patients and also to standardise the gender differences in alcohol use. A total of 41 patients (26 alcohol group and 15 control group) were followed up for 9 months with sampling done at 3 month intervals. Plasma was obtained by centrifuging. The Shimadzu Class-VPTM HPLC Chromatography data system version 6.1 was used to measue the plasma drug levels of d4T, 3TC and NVP. Data was analysed using the SAS 2003 version 9.1 statistical package with the repeated measures fixed model and the means were compared using the student t-test. Generally the mean steady state plasma drug levels of d4T and 3TC were higher than the reference ranges in both the control and alcohol-use groups but the plasma drug levels in the alcohol group were lower than in control group during the 9 months period of follow-up though the difference was not statistically significant ($p>0.05$). The mean steady state plasma drug levels of NVP in both groups were within the reference ranges but though the plasma drug levels were higher in the alcohol group as compared to the control group and there was no significant

difference ($p<0.05$) in the groups. Chronic alcohol use by the HIV/AIDS patients may reduce the steady state plasma
drug levels of d4T and 3TC but may increase the NVP drug levels in the patients.

Disclosures: G. Bbosa: None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:869.10/R17

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA020676

Title: Ethanol intake is insensitive to dihydromyricetin in adult rats with a long history of established alcohol self-administration.

Authors: *D. J. WOODWARD, A. V. AZAROV
Neurosci. Res. Inst. of North Carolina, WINSTON-SALEM, NC

Abstract: The aim of this work was to study the influence of dihydromyricetin (DHM) on established ethanol intake in adult rats. A flavonoid component of anti-hangover herbal medicines and a relatively new compound for US, DHM (~6.7 mg/kg/day in drinking fluids) was recently shown to suppress ethanol intake by ~80% in young rats with initial weight of 200-250 g, after 6 weeks (PND ~49-56 to ~91-98) of ethanol vs. water drinking under alternate-day access in “Wise-Simms procedure” (Shen et al., 2012, J. Neurosci., 32, 390-401). Upon drinking in the same access conditions, ethanol self-administration was sensitive after 1.5-months, but resistant after 3-4 months, to pairing with normally aversive influence of quinine (Hopf et al., 2010, Alcohol. Clin. Exp. Res., 34, 1565-73). In current study, Sprague Dawley rats weighing 400-450 g were cage and spout trained for 1 week and then allowed to drink 20% v/v EtOH (20E) in choice with water on Mon-Wed-Fri, with access to only water on remaining days, for 16 weeks, in 15-hr sessions. On week 16, EtOH intake was 3.3 g/kg and preference ~ 50%, total fluid intake was ~ 38 ml/kg and very close on both alcohol and water days. Then for 2 weeks, DHM (0.15 mg/ml) was added on alcohol days to both 20E and water. Total fluid intake under DHM was similar to pretreatment weeks, and between alcohol and water days, with the respective dose of self-administered DHM at ~5.7 mg/kg/day. EtOH intake was 2.8 and 3.4 g/kg, and preference - 47 and 60%, on DHM weeks 1 and 2. Our finding is that in adult rats with a long experience of alcohol self-administration, ethanol intake under DHM showed only slight downward trend in the 1st week of treatment followed by a recovery during 2nd week, indicating

an overall resistance to DHM suppression. These data, together with those already published suggest that therapeutic potential of dihydromyricetine for alcohol use disorders may depend on the length of a drinking history and / or the age of an individual. (AA020676 to DJW)

Disclosures: D.J. Woodward: None. **A.V. Azarov:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:869.11/R18

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01AA019458

Title: Activation of glutamate transporter 1 attenuates relapse to alcohol- seeking behavior in alcohol-preferring rats

Authors: A. M. QRUNFLEH, A. ALAZIZI, S. N. SREEMANTULA, *Y. SARI
Univ. of Toledo, Col. of Pharm., Toledo, OH

Abstract: Relapse to alcohol-seeking behavior depends on increased glutamate transmission in key regions of the mesocorticolimbic motive circuit, including prefrontal cortex (PFC) and nucleus accumbens (NAc). Extracellular glutamate is regulated by a number of glutamate transporters in various brain regions. Of these transporters, glutamate transporter 1 (GLT1, also termed excitatory amino acid transporter 2, EAAT2), is responsible for the uptake of the majority of extracellular glutamate. We have recently reported that ceftriaxone (i.p.), a b-lactam antibiotic known to elevate GTL1 expression, reduces alcohol intake in alcohol-preferring (P) rats (Sari et al., 2011). We investigated here whether ceftriaxone-induced GLT1 upregulation or activation attenuates relapse to alcohol-seeking behavior. We found that P rats that have been exposed to free choice of 15% and 30% alcohol for 5 weeks and treated with ceftriaxone (50 and 100 mg/kg, i.p.) during the last 5 days of the two-week deprivation period showed a reduction in alcohol intake upon relapse that persisted for at least 10 days. Moreover, ceftriaxone-mediated attenuation in relapse to alcohol drinking was associated with upregulation of GLT1 level in PFC and NAc core. The upregulation of GLT1 level was found only at the higher dose of ceftriaxone, which suggests that the lower dose may have other pharmacological effects that cause reduction in alcohol intake. In addition, ceftriaxone has no effect on relapse to sucrose-seeking behavior. Given previous work indicating that up-regulation of GLT1 attenuates cue-induced reinstatement of cocaine-seeking behavior in rats (Knackstedt *et al.,* 2010; Sari *et al.,* 2009) and reduces

alcohol consumption in P rats, GLT1 is considered now as potential therapeutic target for treatment of alcohol dependence.

Disclosures: A.M. Qrunfleh: None. **A. Alazizi:** None. **S.N. Sreemantula:** None. **Y. Sari:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Program#/Poster#:869.12/R19

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01AA019458

NIH Grant AA013522

Title: Effects of ceftriaxone on the acquisition and maintenance of ethanol drinking in peri-adolescent and adult female alcohol-preferring (P) rats

Authors: *P. RAO[1], J. E. TOALSTON[2], A. ALAZIZI[1], Y. SARI[1], R. L. BELL[2]
[1]PHARMACOLOGY, UNIVERSITY OF TOLEDO, TOLEDO, OH; [2]Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Increased glutamatergic neurotransmission has been demonstrated to mediate the reinforcing properties of drugs of abuse, including ethanol (EtOH). We have recently shown that administration of ceftriaxone, a β-lactam antibiotic known to upregulate glutamate transporter 1 (GLT1) levels/activity, decreased the maintenance of EtOH intake in male adult alcohol-preferring (P) rats (Sari et al 2011). In the present study, we tested whether ceftriaxone administration would reduce the acquisition and maintenance of EtOH drinking in adolescent and adult female P rats. Adolescent (~35 days of age) and adult (~75 days of age) EtOH-naïve female P rats were used in this study. Animals were treated with 200 mg/kg ceftriaxone for 7 days prior to acquisition to and during the maintenance of EtOH intake in both adolescent and adult female P rats. The results show that pretreatment with ceftriaxone reduced the acquisition of EtOH intake compared with controls in adolescent, but not adult, rats. However, there was increased water intake after ceftriaxone treatment in both age groups as well. Ceftriaxone treatment significantly reduced the maintenance of EtOH intake compared with controls in both animals that started as adolescents and adults. As before, water intake was increased following ceftriaxone treatment in both groups. Reductions in EtOH intake by ceftriaxone were inversely associated with the upregulation of GLT1 protein levels in both the nucleus accumbens (~25% in

rats that started at both ages) and prefrontal cortex (~65% in rats that started as peri-adolescents and ~50% in those that started as adults). These findings provide further support for continued research examining GLT1-associated mechanisms for the treatment of alcohol abuse and dependence.

Disclosures: P. Rao: None. **J.E. Toalston:** None. **A. Alazizi:** None. **Y. Sari:** None. **R.L. Bell:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:869.13/R20

Topic: C.17. Drugs of Abuse and Addiction

Support: MNiSW N N405 143238

POIG DEMETER 1.1

Title: Novelty- seeking behaviors and the escalation of alcohol drinking after abstinence are controlled by mGluR5 receptors on dopaminoceptive neurons.

Authors: *J. RODRIGUEZ PARKITNA, M. SIKORA, S. GOLDA, R. PRZEWLOCKI
Inst. of Pharmacol. of the Polish Acad. of Sci., Krakow, Poland

Abstract: Novel experiences activate the brain's reward system in a manner similar to drugs of abuse, and high levels of novelty- and sensation-seeking behavior have been associated with increased susceptibility to alcohol and drug abuse. Here we investigate how glutamatergic modulation of dopaminoceptive neurons affects novelty seeking and alcohol drinking in a mouse model with selective knock-down of the metabotropic glutamate receptor 5 (mGluR5) in neurons expressing D1 dopamine receptors. Mice harboring a transgene expressing microRNA hairpins against mGluR5 mRNA under the control of the D1 dopamine receptor gene promoter ($mGluR5^{KD\text{-}D1}$) showed no behavioral deficits and exhibited normal anxiety levels and learning abilities. However, $mGluR5^{KD\text{-}D1}$ animals showed reduced locomotor activity when placed in a novel environment and exhibited decreased interaction with a novel object. Moreover, unlike control animals, mutant mice did not perform instrumental responses under the operant sensation-seeking paradigm, although they learned to respond for food normally. When $mGluR5^{KD\text{-}D1}$ mice were provided access to alcohol, they showed similar patterns of consumption as wild-type animals. However, mutant mice did not escalate their alcohol consumption after a period of forced abstinence, but control mice almost doubled their intake.

These data identify mGluR5 receptors on D1-expressing neurons as a common molecular substrate of novelty-seeking behaviors and behaviors associated with alcohol abuse.

Disclosures: J. Rodriguez Parkitna: None. **M. Sikora:** None. **S. Golda:** None. **R. Przewlocki:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:869.14/S1

Topic: C.17. Drugs of Abuse and Addiction

Support: 1 F32 AA 20143-01

Title: Clozapine inhibition of alcohol intake in Syrian golden hamsters: selectivity, motivation and reward

Authors: *D. GULICK[1], L. BONVINI[1], E. TEMPLETON[1], A. SONSTEGARD[1], D. BUCCI[2], A. GREEN[1]
[1]Psychiatry Dept., Dartmouth Med. Sch., LEBANON, NH; [2]Psychological and Brain Sci., Dartmouth Col., Hanover, NH

Abstract: Introduction: Although the atypical antipsychotic drug clozapine (CLOZ), unlike other antipsychotics, effectively reduces alcohol intake in patients with schizophrenia (SCZ), it is prescribed infrequently because of its severe side effects. CLOZ also decreases free access alcohol drinking in the Syrian golden hamster, an animal model of alcohol drinking, while haloperidol (HAL), an antipsychotic that does not limit alcohol drinking in SCZ, has no effect on alcohol drinking in the hamster. In order to develop novel therapeutics based on CLOZ's action, we sought to elucidate the basis of CLOZ's effect on alcohol intake in the hamster. We first tested whether CLOZ would reduce sucrose or saccharin intake, as well as alcohol intake, to determine if it has a selective effect on drug rewards vs. natural rewards. Next, to determine whether CLOZ reduces alcohol intake by interfering with the motivation to consume alcohol or by reducing alcohol-associated reward, we tested the effects of CLOZ vs. HAL on operant responding and conditioned place preference (CPP) for alcohol or sucrose. Methods: In Experiment 1a, animals were given free access to food and two bottles - one water bottle and the other containing either a solution of sucrose (15.5 g/L) or saccharin (0.732 g/L). In Experiment 1b, animals were given free access to food and three bottles - one water bottle, one 15% v/v alcohol bottle, and the other containing either a high concentration solution of sucrose (210 g/L) or saccharin (0.732 g/L). Drug treatment consisted of 14 daily injections of 8 mg/kg CLOZ or

vehicle after fluid consumption levels steadied. In Experiment 2, we measured the effects of CLOZ or HAL on operant lever pressing for water, sucrose, or alcohol rewards. Finally, in Experiment 3, we tested the effects of CLOZ or HAL on CPP for alcohol or sucrose. Results: CLOZ was more effective at reducing alcohol intake than either sucrose or saccharin intake. HAL, but not CLOZ, reduced lever pressing for alcohol and sucrose. HAL also completely blocked CPP, whereas CLOZ produced only a modest, non-significant attenuation of CPP. Discussion: CLOZ selectively reduces alcohol intake without significantly impacting the intake of natural rewards. Further, the decreased alcohol intake in response to CLOZ treatment occurs without a change in alcohol-seeking behaviors or alcohol-associated reward, supporting the hypothesis that CLOZ, unlike HAL, may decrease alcohol drinking by ameliorating dysfunction of the brain reward circuit without blunting the ability of the reward circuit to respond to rewarding stimuli.

Disclosures: **D. Gulick:** Research Grant; 1 F32 AA 20143-01. **L. Bonvini:** None. **E. Templeton:** None. **A. Sonstegard:** None. **D. Bucci:** None. **A. Green:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:869.15/S2

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant P20GM103499

Title: Acute administration of SB-277011A, a selective dopamine D3 receptor antagonist, decreases binge-like consumption of ethanol in mice as measured by the “drinking-in-the-dark” paradigm

Authors: *O. RICE, Sr.[1], D. SCHONHAR[1], T. RUNYAN[1], J. GAAL[2], E. L. GARDNER[3], C. R. ASHBY, Jr[4]
[1]Furman Univ., GREENVILLE, SC; [2]MegaPharma Pharmaceuticals, Budapest, Hungary; [3]Intramural Res. Program, Natl. Inst. on Drug Abuse, Baltimore, MD; [4]Pharmacol., St. John's Univ., Queens, NY

Abstract: Selective and acute blockade of the dopamine (DA) D3 receptor by SB-277011A attenuates ethanol (EtOH) preference, intake, and lick responses in EtOH-preferring rats. However, alcohol consumption that leads to abuse is often marked by binge drinking, characterized as bringing blood EtOH levels to ≥ 80mg/dl within two hours of the initial drink. It is unclear if brain mechanisms implicated in EtOH reward are equally implicated in EtOH binge

consumption and abuse. Therefore, the present study utilized the "Drinking-in-the-Dark" paradigm to examine the acute effects of SB-277011A, on binge consumption of EtOH in mice. Male C57BL/J6 mice were given access to 2% EtOH (diluted in near beer) 3hrs into their dark cycle and were allowed to drink EtOH for 2hrs. The total EtOH consumed during this time was measured for 7consecutive days to provide a baseline. On test day, the mice were given i.p. injections of either vehicle (10% 2-hydroxypropyl-β-cyclodextrin in 0.9% saline) or SB-277011A (15 or 30 mg/kg), thirty minutes prior to their drinking session. The results demonstrated that mice voluntarily brought their blood alcohol levels to ≥ 80mg/dl. In addition, a single i.p. injection of 15 mg/kg i.p. of SB-277011A produced a significant decrease in EtOH consumption. These data show that the acute administration of SB-277011A decreases voluntary binge consumption of EtOH and provide evidence that the DA D3 receptor plays an integral role in binge drinking-in-the-dark in mice.

Disclosures: O. Rice: None. **D. Schonhar:** None. **T. Runyan:** None. **J. Gaal:** None. **E.L. Gardner:** None. **C.R. Ashby:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:869.16/S3

Topic: C.17. Drugs of Abuse and Addiction

Support: Finnish Foundation for Alcohol Studies

Title: Subchronic anabolic steroid administration affects voluntary alcohol drinking in alcohol preferring AA rats

Authors: S. KAILANTO, *K. KIIANMAA, T. SEPPÄLÄ
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Abstract: Introduction: Alcohol abuse and dependence with the resulting medical and social harm remain among the greatest substance abuse problems worldwide. Furthermore, a large number of young adults abuse anabolic androgenic steroids (AASs). Whether the abuse of AAS constitutes a risk factor for abuse of other intoxicants and drugs, or vice versa, is still unknown. This question is, however, of importance, since several surveys conducted young adults and adults not connected to sports, have revealed that AAS use may be concomitant with use of alcohol or drugs. This type of mixed-use probably involves more than a desire to enhance the users' appearance or sports performance. Several studies have shown that chronic AAS use may affect behaviour by influencing neuronal activity and plasticity. The specific aim of the present

study was to assess if nandrolone decanoate, the AAS, exposure modulates the voluntary ethanol intake by alcohol preferring AA (Alko Alcohol) rats.
Methods: Three months old AA rats were placed into single wire mesh cages, where food and water (n=24), or food and a free choice between 10% (v/v) ethanol and water (n=24) were continuously available. After four weeks of ethanol intake, nandrolone decanoate (20 mg/kg i.m.) or vehicle oil was administered to the rats every other day, five times in total. Ethanol and water consumption measurements were continued for four weeks after the last steroid injection.
Results: The results showed that all ethanol drinking animals achieved stable consumption levels of ethanol (4.9 g/kg/day) during the first four weeks. The ten day nandrolone treatment decreased ethanol intake (3.0 g/kg/day) compared to the vehicle oil treated animals (4.4 g/kg/day). Intake of ethanol stayed on a lower level also during the four weeks following administration of the drug, although the difference between the groups narrowed during the last week.
Discussion: Suppression of ethanol intake after administration of nandrolone decanoate suggests that AAS treatment may modify the rewarding effects of ethanol in alcohol preferring rats. Nandrolone treatment may therefore be used in certain conditions to reduce intake of ethanol. Since ethanol consumption remained on a reduced level after discontinuation of the nandrolone decanoate treatment, the anabolic steroid may induce long lasting changes in the functioning of the brain.

Disclosures: S. Kailanto: None. **K. Kiianmaa:** None. **T. Seppälä:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Topic: C.17. Drugs of Abuse and Addiction

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NIH grant AA10760

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Grant from Dept. of Veterans Affairs

Acheivement Rewards for College Scientists Foundation

Title: Microstructure of binge-like ethanol drinking in a high drinking selected mouse line

Authors: *A. M. BARKLEY-LEVENSON[1,2], J. C. CRABBE[1,2]
[1]Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Portland Alcohol Res. Ctr., VA Med. Ctr., Portland, OR

Abstract: The High Drinking in the Dark (HDID) mouse line was selected for high blood ethanol concentration (BEC) following limited access to an ethanol solution. These mice voluntarily drink to intoxicating BECs in a short period of time, and consequently represent a genetic animal model of binge-like drinking. Recently, we have shown that these mice have a larger average bout size than their control line, without differing in average bout duration, when tested in the BioDAQ Episodic Intake Monitoring system on a 4 day Drinking in the Dark (DID) procedure. This suggested a possible genetic difference in ethanol drinking efficiency (e.g. lick rate or lick volume), and the present study sought to address this possibility. Male HDID-1 and HS/Npt (HS; heterogeneous control line) were given a 4 day limited-access DID test while housed in lickometer chambers that record each contact with the drinking tube (licks). During the 4 hours of access on the final test day, HDID-1 mice drank significantly more g/kg ethanol, had a greater total number of licks, and reached higher BECs than the HS mice. The lines did not differ significantly in overall lick rate or lick volume. Drinking bout structure was examined, with a bout defined as at least 20 licks with no more than one minute between licks. HDID-1 mice had a significantly larger average bout size (licks/bout) than HS mice. This was due to a significantly larger bout size during the first 2 hours, but not during the second 2 hours. These results differ from those obtained using the BioDAQ system, wherein genotypic differences in bout size were primarily observed during the latter half of the drinking session. We also observed a trend towards the first bout in the session being larger on average in HDID-1 mice than HS mice, suggesting that HDID-1 mice might be more initially receptive to ethanol than HS mice. HDID-1 and HS mice did not differ significantly in number of bouts or average bout duration. Overall, these results replicate the previously reported larger bout size of the HDID-1 mice than the HS mice, and suggest that the greater ethanol consumption by the HDID-1 mice than the HS mice does not seem to be due to consistent differences in lick rate or lick volume. Additionally, the temporal pattern of genotypic differences in bout size may vary depending on the continuous consumption recording system used and subsequent total intake. Supported by AA13519, AA10760, and a grant from the US Department of Veterans Affairs. AMB-L is supported by AA007468, and the Achievement Rewards for College Scientists Foundation.

Disclosures: A.M. Barkley-Levenson: None. **J.C. Crabbe:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:869.18/S5

Topic: C.17. Drugs of Abuse and Addiction

Support: AA07611

AA13522-INIA

Title: Effects of ivermectin on free-choice maintenance and relapse ethanol drinking in two rat lines selectively bred for high alcohol preference

Authors: *K. M. FRANKLIN[1,2], R. L. BELL[2], W. J. MCBRIDE[2,3]
[1]Indiana Univ. Sch. of Med., INDIANAPOLIS, IN; [2]Psychiatry, [3]Biochem., IU Sch. of Med., Indianapolis, IN

Abstract: On-going studies in our, and other, labs have implicated purinergic P2X4 receptors in ethanol (EtOH) drinking. The objective of the current study was to assess the ability of systemic (i.p.) and central (intracerebroventricular [i.c.v.]) exposure to the P2X4 receptor positive allosteric modulator ivermectin (IVM) to reduce the maintenance of and relapse to EtOH drinking in two rat lines selectively bred for high EtOH consumption. Methods: Experiment 1: Adult, male high-alcohol drinking replicate (HAD1/HAD2) rats were provided 24-hr free access to 15% EtOH, water, and food. Following stable EtOH drinking levels, rats (n=8/dose) were injected (i.p.) for 5 consecutive days with 1.5-7.5 mg/kg IVM or a vehicle (10% propylene glycol into sterile saline) 30 min prior to the dark cycle. EtOH intake (g/kg) was assessed 1, 4, and 24 hrs following IVM treatment. Following the 5 test sessions, rats were maintained on the 24-hr EtOH access protocol without IVM treatment to serve as a “washout period” and assess EtOH drinking recovery. Rats then were EtOH-deprived for a 2-week period. These methods were repeated to assess IVM’s effects on relapse to EtOH drinking. Rats received the same IVM dose in both test-phases. Experiment 2: Adult, female HAD2 rats were provided 2-hr scheduled access to 15% EtOH, with free food and water access. Following stereotaxic cannula implantation into the lateral ventricle and stable EtOH drinking levels, rats (n = 5-7/dose) were infused i.c.v. for 5 consecutive days with 0.5-2.0 ug/ul IVM or a vehicle (10% propylene glycol into artificial cerebrospinal fluid) 30 min prior to the dark cycle. EtOH intake (g/kg) was assessed 2 hrs following IVM treatment. Results: Experiment 1: Systemic IVM treatment reduced on-going and relapse EtOH drinking in HAD1 and HAD2 rats (up to ~50% of vehicle-values) with the greatest effects occurring at 5.0 and 7.5 mg/kg IVM doses. However, these reductions were variable, due to several testing conditions, including rat line and test-phase. Also, the effects were time-dependent, with the greatest reductions relative to controls occurring 4 hrs after IVM treatment. Experiment 2: Central infusion with 2 ug/ul IVM revealed a trend toward reducing 2-hr EtOH drinking over the 5-day test period, up to ~30% of vehicle-values ($p = 0.06$). Conclusions: The current findings support several avenues of research implicating P2X4 receptors in EtOH-drinking behavior. Our results indicate that systemic and central IVM administration reduce EtOH intake in two rat lines genetically selected for high alcohol

preference. Overall, these findings suggest that an IVM-like compound could prove useful in treating alcohol abuse and dependence.

Disclosures: K.M. Franklin: None. **R.L. Bell:** None. **W.J. McBride:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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NIH Grant UO1AA016647

NIH Grant P60AA010760

Title: Urocortin-1: Selective involvement in long-term alcohol consumption

Authors: *W. J. GIARDINO, M. M. FORD, A. E. RYABININ
Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Converging lines of evidence support a role for the neuropeptide urocortin-1 (Ucn1) in alcohol preference and reward. Earlier studies showed that, relative to wild-type littermates, genetic deletion of Ucn1 in mice on a C57BL/6J background dampened intake of 6% and 10% alcohol in a continuous access “two-bottle choice” (2BC) drinking procedure. However, we observed no effect of Ucn1 deletion in a limited access “drinking-in-the-dark” (DID) model, in which mice had access to a single bottle of 20% alcohol for 2-4 hours, beginning 3 hours into the circadian dark period. Because these procedures differed in several aspects, we investigated the relative importance of each variable in mediating Ucn1’s effects on alcohol drinking.
Experiment A used a continuous access 2BC procedure with 10-40% alcohol (four sessions at each concentration). Experiment B used the standard DID procedure (four sessions total), except access to alcohol was accompanied by access to water. Experiment C used an intermittent access 2BC procedure in which mice were offered 3-20% alcohol every other day for five weeks. Consumption in Experiment C was tracked using a lickometer apparatus, allowing for analysis of changes in the drinking microstructure across time.
In Experiment A, genetic deletion of Ucn1 dampened intake and preference of 20-40% alcohol, relative to wild-type littermates. In Experiment B, deletion of Ucn1 had only minor and sex-

specific effects on alcohol preference. In Experiment C, deletion of Ucn1 decreased 20% alcohol consumption during the first four hours of the session, relative to wild-type littermates. Importantly, this effect emerged only after several alcohol drinking sessions.
In conclusion, it appears that Ucn1 involvement in alcohol consumption depends on the extent of alcohol history, rather than alcohol concentration, concurrent water availability, or schedule of access. These findings lend further support for Ucn1's importance in the development of alcohol use disorders resulting from prolonged drinking.
Additional studies are currently aimed at evaluating whether Ucn1 plays a role in sensitivity to alcohol-induced sedation, and whether Ucn1's contribution to alcohol consumption can be explained by generalized effects on fluid intake, caloric intake, or taste reactivity.

Disclosures: W.J. Giardino: None. **M.M. Ford:** None. **A.E. Ryabinin:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R24 grant AA015512-02 from NIAAA

NIH grant P50 DA027840 from NIDA

Title: Caffeine reduces alcohol intake but increases nicotine self-administration in rats

Authors: *A. H. REZVANI[1], H. G. SEXTON[1], J. JOHNSON[1], C. WELLS[1], K. GORDON[1], L. LUMENG[2], J. ROSE[1], E. D. LEVIN[1]
[1]Dept. of Psychiatry, Duke Univ., Durham, NC; [2]Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Caffeine, alcohol and nicotine are three of the most widely used psychoactive drugs in the world. They are often taken together. However, the interaction of caffeine, either promoting or inhibiting alcohol or nicotine self-administration, has not been well characterized. After the establishment of a stable alcohol intake in alcohol preferring (P) rats, the acute (0, 5, 10 and 20 mg/kg) and chronic (0, 20 mg kg for 10 days) effects of caffeine given subcutaneously (SC) or orally on alcohol intake and preference were determined. In addition, the acute effects of caffeine (0, 20 mg/kg given orally) on alcohol intake and preference after 4-day alcohol deprivation were evaluated. The acute effects of oral administration of caffeine (0, 20 mg/kg given) on saccharin (0.2%) preference was also assessed. Furthermore, we explored the effects of acute oral administration of caffeine on nicotine self-administration in Sprague Dawley rats. Acute SC

administration of caffeine (20 and 10 mg/kg) significantly reduced alcohol intake and preference at 24 hr. as well as saccharin intake in P rats. Caffeine also reduced alcohol intake following a 4-day alcohol deprivation. Chronic oral treatment with caffeine for 10 days reduced alcohol intake and preference with some signs of tolerance over that period. In contrast to its effects in reducing alcohol consumption, acute oral treatment with caffeine significantly increased rates of nicotine self-administration in Sprague Dawley rats. Caffeine and other adenosine receptor antagonists may hold promise for the treatment of excessive alcohol drinking. However, caffeine may be contraindicated with people who are trying to quit smoking given that it promoted nicotine intake in this preclinical study.
This research was supported by the R24 grant AA015512-02 from NIAAA and P50 grant DA DA027840- from NIDA.

Disclosures: A.H. Rezvani: None. **H.G. Sexton:** None. **J. Johnson:** None. **C. Wells:** None. **K. Gordon:** None. **L. Lumeng:** None. **J. Rose:** None. **E.D. Levin:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:869.21/S8

Topic: C.17. Drugs of Abuse and Addiction

Title: Sprague-Dawley rats self-administer alcohol and show alcohol preference using the two-bottle choice intermittent access drinking model

Authors: *R. K. VASUDEVA[1], S. WARD[1], L. G. KIRBY[2]
[1]Pharmacol., [2]Anat. & Cell Biol., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Alcoholism afflicts 1 in every 13 US adults, and comorbidity with depression is common. Levels of serotonin (5HT) metabolites in both alcoholic humans and alcohol-preferring rat strains are lower compared to non-alcohol dependent counterparts. While strains of rats are bred for alcohol preference (P, sP, AA) and others (Wistar, Long-Evans) are more commonly used in alcohol studies, we wanted to demonstrate that the standard out-bred Sprague-Dawley (SD) rat can also develop a preference for alcohol. This strain of rat was selected to coincide with several studies examining models of depression and anxiety in our laboratory and others. Two groups (n = 8) of SD male rats were housed singly and placed on the intermittent-access (IA) two-bottle choice drinking paradigm. Within each group, an average of 5 rats demonstrated a natural preference for ethanol (EtOH) by week 5, with an average of 3 rats not consuming significant amounts of EtOH for the duration of the study. Rats that demonstrated a preference

for ETOH consumed up to 4g ETOH/kg/day, while those who did not show a preference consistently drank less than 0.5g ETOH/kg/day. After 8 weeks on the IA paradigm, Group 1 was transferred to an operant conditioning task for EtOH self-administration. For comparison, a third group of SD rats (n = 6) was trained to self-administer EtOH using a sucrose fading (SF) paradigm. Under a fixed ratio 1 (FR1) schedule, the EtOH concentration-effect curve for IA rats was shifted downward and rightward compared to the SF rats, indicating that experience with IA led to an increased schedule-controlled intake of EtOH at higher doses. Responding for EtOH was more similar between the IA and SF groups under a progressive ratio (PR) schedule, indicating that motivation to consume EtOH did not differ following the different training paradigms. Additionally, IA and SF rats could be divided into high and low responders based on their EtOH self-administration behavior. Future work will investigate drinking behavior and EtOH consumption following depletion of 5HT. We predict that levels of EtOH consumption will increase significantly above baseline levels following cerebral 5HT depletion, thus mimicking alcoholic-like behaviors. Overall, we found that SD rats demonstrate a natural preference for EtOH in the IA paradigm. We also showed that SD rats acquire EtOH self-administration in a similar manner to more commonly used rat strains using two established operant training paradigms. Interestingly, these results also revealed the presence of distinct high and low self-administering SD rats, representing the opportunity to further investigate neurobiological correlates to these two phenotypes.

Disclosures: R.K. Vasudeva: Employment; Temple University School of Medicine. Research Grant; 5 T32 DA 7237-22. **S. Ward:** None. **L.G. Kirby:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:869.22/S9

Topic: C.17. Drugs of Abuse and Addiction

Support: Capes

Title: Social deprivation and social instability stress during adolescence modify anxiety-like behavior and plasma corticosterone, but not alcohol self-administration.

Authors: *P. E. OLIVEIRA, L. C. CASULA, P. C. BIANCHI, R. M. LEÃO, F. C. CRUZ, C. S. PLANETA
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Abstract: Adolescence is a critical period of ontogeny which individuals of several species presents typical characteristics such as increasing in novelty-seeking, risk-taking and peer interaction behaviors. These characteristics could facilitate initial drug-seeking, enhancing the vulnerability to addiction in later stages of life. Epidemiological studies have shown links between early life stressors and future alcohol problems. Pre-clinical researches corroborate these evidences demonstrating that stress during adolescence enhances the behavioral effects of drugs of abuse. In this study we evaluated the influence of early life social stressors on alcohol self-administration, anxiety-like behavior and plasma corticosterone. Adolescent male Wistar rats were submitted to 15 days of social deprivation or social instability. During this period the control group was left undisturbed. Blood samples were collected twenty four hours after the last stress session and five minutes after elevated plus maze test (EPM test) from tail vein. EPM test were applied forty eight hours after the end of the stress protocol. Anxiety-like indices in the EPM (% of frequency of entries and time spent in the open arms of maze) were registered during five minutes. Then animals of three groups were subjected to ethanol (6%) or saccharin (0.05%) self-administration protocol. Progressive ratio schedule and 24h-unlimited access parameters of alcohol (6%) or saccharin (0.05%) reinforcement was analyzed. Rats submitted to chronic social stress have shown increased percentage of open-arms entries and time spent in open-arms compared to control group. Basal plasma corticosterone was not altered by stress, however, single reared animals showed a pronounced increase of corticosterone secretion in response to a novelty. Furthermore, alcohol and saccharin self-administration behavior were not modified by previous stress. In conclusion, social deprivation and social instability during adolescence rats promote anxiolytic-like behavior, alteration of HPA axis when animals are submitted to an unknown environment, but were not able to alter alcohol- and saccharin-seeking behavior.

Disclosures: P.E. Oliveira: None. **L.C. Casula:** None. **P.C. Bianchi:** None. **R.M. Leão:** None. **F.C. Cruz:** None. **C.S. Planeta:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Topic: C.17. Drugs of Abuse and Addiction

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NIH Grant UL1-DE019586

NIH Grant PL1-DA024859

Title: Expression of MicroRNA10a and target genes is up-regulated by psychological stress, and correlates with drinking behavior in heavy, but not moderate drinkers.

Authors: *R. D. BEECH[1], J. J. LEFFERT[1], A. LIN[2], K. A. HONG[1], J. HANSEN[1], S. UMLAUF[3], S. MANE[3], H. ZHAO[4], R. SINHA[1]
[1]Psychiatry, [2]W.M. Keck Fndn. Biostatistics Resource, [3]Ctr. for Genome Analysis, [4]Epidemiology and Publ. Hlth., Yale Univ. Sch. Med., New Haven, CT

Abstract: Background: Alterations in stress-related gene-expression may play a role in stress-related drinking and the risk for alcohol dependence.
Methods: Microarrays were used to measure changes in gene-expression in peripheral blood in subjects exposed to three types of personalized imagery: neutral, stressful (but not alcohol-related), and alcohol-related. Gene-expression was measured at baseline, immediately after, and 1 hour after stimulus presentation. Subjects were allowed to drink up to 750cc of beer in a taste-test during the hour following stimulus presentation in each imagery condition, and the amount of beer consumed was recorded. Gene-expression levels were compared in 2 groups of subjects categorized into heavy drinkers (HD, defined as regular alcohol use over the past year of at least 8 standard drinks/week for women and at least 15 standard drinks/week for men), and moderate drinkers (MD, defined as up to 7 standard drinks/week for women and 14 standard drinks/week for men). Expression of microRNA10 (miR-10a) was assessed by quantitative real-time PCR (qRT-PCR).
Results: After correction for multiple testing (FDR<0.05), 79 genes were identified that changed by > 1.3 fold in the HD group, but not the MD group, following exposure to stress. No changes were observed for any of these genes in either group following exposure to neutral or alcohol-related imagery. Pathway analysis suggested that many of these genes were positively regulated by miR-10a. MiR-10a expression was up-regulated following psychological stress in HD, but not MD subjects, and miR-10a levels were correlated (R^2= 0.59) with amount drunk in HD, but not MD subjects.
Conclusions: These results demonstrate that expression of miR-10a and several of its target genes can be regulated by acute psychological stress, and is correlated with stress-induced drinking in a laboratory setting. Alterations in miRNA expression may be one mechanism linking psychological stress with changes in both gene-expression and behavior.

Disclosures: R.D. Beech: None. **J.J. Leffert:** None. **A. Lin:** None. **K.A. Hong:** None. **J. Hansen:** None. **S. Umlauf:** None. **S. Mane:** None. **H. Zhao:** None. **R. Sinha:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:869.24/S11

Topic: C.17. Drugs of Abuse and Addiction

Title: How does early life trauma contribute to changes in behavior after a normative adult stressor? Alcohol consumption in male Long Evans rats exposed to postnatal maternal separation and adult social defeat stress.

Authors: *B. E. CALDWELL, M. FAIN, K. L. HOCKENBERRY
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Abstract: The relationship between stress and risk for developing alcohol (ETOH) use disorders has been studied extensively in human and nonhuman animal models. Some rodent models show that stress exposure leads to an increase in preference for ETOH, but this finding is not consistently reported across experimental protocols. The relationship between stress and ETOH drinking is likely modulated by many factors. The nature of the stressor (type, duration, and controllability/predictability), rearing environment, and individual differences in reactivity to ETOH may be important factors in determining whether an organism will develop a heightened preference for ETOH after a stressful event. The present study examined the effects of maternal separation stress during the postnatal period, followed by social defeat stress in adulthood, on ETOH drinking in male Long Evans rats. Newborn litters of Long Evans rats were culled to 6 pups per litter the day of parturition. Litters were randomly assigned to either maternal separation (MS) or animal facility rearing (AFR). The day after parturition (PND1), MS litters were separated from the dam for 180 minutes per day for 14 days. All pups were weaned on PND21. Once mature (at least 60D old), rats were randomly assigned to either defeat stress (DS) or non-stress (NS) control groups. DS animals were exposed to five daily 5-minute defeat sessions. Finally, rats were tested for ETOH preference using a three-bottle choice protocol (10% ETOH/.5% saccharin; 5% ETOH/.5% saccharin consumption; .5% saccharin only). Preliminary results show animals exposed to both MS and DS preferred the 10% ETOH solution over the 5% and drank more (measured in g/kg) ETOH on average than AFR/NS animals. Pilot data obtained to date do not suggest a difference between NS rats and those exposed to DS only, and show a marginal difference between MS/DS and MS/NS animals. In addition to the alcohol drinking protocol, individual differences in sensitivity to ETOH were determined by a single administration of 1.5 g/kg ETOH, injected IP, followed by a test for motor coordination/ataxia using an automated “rotarod” (Med Associates) and observation in an open field to measure locomotor activity. Future analyses are planned for examining the correlation between initial response/sensitivity to ETOH (ataxia and locomotor behavior) and the amount of ETOH consumed following defeat stress. Finally, pharmacological targeting of dorsal raphe CRF is

under way in order to examine whether stress-related alcohol preference is determined by HPA axis activity surrounding the defeat experience.

Disclosures: B.E. Caldwell: None. **M. Fain:** None. **K.L. Hockenberry:** None.

Poster

869. Alcohol Intake and Preference: Neural Mechanisms and Biomarkers

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Program#/Poster#:869.25/S12

Topic: C.17. Drugs of Abuse and Addiction

Support: USPHS Grant AA-12882

E.H. Lane Foundation

Title: Differential effects of d1 and d2 receptors in the perifornical lateral hypothalamus in controlling ethanol drinking: possible interaction with orexin neurons

Authors: *Y.-W. CHEN[1,2], I. MORGANSTERN[2], J. R. BARSON[2], B. G. HOEBEL[1], S. F. LEIBOWITZ[2]
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Abstract: The neurotransmitter, dopamine (DA), is known to promote motivated behaviors, such as ethanol drinking, by acting in various mesolimbic brain regions. Indirect evidence from feeding and electrophysiological studies suggests, however, that the DA system in the perifornical lateral hypothalamus (PFLH), where both DA1 (D1) and DA2 (D2) receptor subtypes exist, has different functions in controlling ethanol consumption. In addition, these functions may be a consequence of DA acting on local neurons expressing the orexigenic peptides, orexin/hypocretin (OX) or melanin-concentrating hormone (MCH). The present study therefore tested the specific hypothesis that activation of the D1 receptor in the PFLH increases the consumption of ethanol, possibly by stimulating local peptide neurons, whereas activation of the D2 receptor in this region produces the opposite behavioral and neurochemical effects. In Experiments 1-2, adult, male Sprague-Dawley rats, trained to drink 7% ethanol, were microinjected in the PFLH with a D1 receptor agonist (SKF81297; 5.4, 10.8 nmol) or antagonist (SCH23390; 8.7, 17.4 nmol) or a D2 receptor agonist (quinelorane; 3.1, 6.2 nmol) or antagonist (sulpiride; 11.7, 23.4 nmol), with measurements of ethanol, chow, and water intake taken for 4 hours after injection. In Experiments 3-4, the effect of PFLH injection of the receptor agonists, SKF81297 and quinelorane, on local expression of OX and MCH was measured using both

radiolabeled *in situ* hybridization (ISH) and digoxigenin-labeled ISH. Results showed that PFLH injection of SKF81297 significantly stimulated ethanol intake but had no effect on chow or water intake. This is in contrast to the D2 agonist quinelorane, which caused a specific suppression of ethanol consumption. Injections of their antagonists into the PFLH produced opposite effects from the agonists, suggesting that both D1 and D2 receptors are tonically active in this brain area in controlling ethanol intake. In addition to their opposite effects on ethanol drinking, PFLH injection of SKF81297 and quinelorane had differential effects on OX mRNA expression. The former compound increased OX expression, while the latter reduced it, with both effects occurring specifically in the LH subregion. In contrast to OX, the expression of MCH was largely unaltered by these D1 and D2 agonists. These results show that DA in the PFLH, by acting on distinct receptor subtypes, may differentially control ethanol consumption by interacting locally specifically with OX neurons.

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Poster

870. Alcohol: Chronic Effects

Location: Hall F-J

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Program#/Poster#:870.01/S13

Topic: C.17. Drugs of Abuse and Addiction

Support: William Webster Fund

Title: Strain and sex dependent effects on the severity of ethanol-induced brain damage

Authors: S. LATTIMER[1], A. J. ELBERGER[1], L. LU[1], *K. M. HAMRE[2]
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Abstract: Chronic ethanol exposure has been shown to cause significant damage to the CNS. Recent evidence has suggested that the genetic background and/ or sex of the individual may impact the level of damage observed. However, little is known about whether these variables are critical or whether there is an interaction between the two variables that alters the level of CNS

damage following chronic ethanol exposure. To begin to address whether sex, strain or their interaction impacts ethanol-induced cell damage, male and female mice from four different BXD strains and the C57/BL6J (B6) parental strain were examined. Cell damage was assessed by evaluating changes in expression levels of 3 markers: 1) the astrocytic marker GFAP to assess whether there was an astroglial response, 2) the dendritic marker MAP2 to assess changes in neuronal processes, and 3) the inflammatory marker TLR-4 to assess inflammatory changes. Mice were treated through gavage for ten days with 5 g/kg of ethanol or isovolumetric saline following the protocol of Qin et al. (J. Neuroinflammation 5:10, 2008). Messenger RNA was isolated from the forebrain of each mouse. Expression levels in ethanol-treated or saline-treated control mice were determined through qRT-PCR. Control values were computed for each strain and each sex within a strain, and changes from controls were determined for each ethanol-exposed animal in each strain and sex. The analyses demonstrated that each marker showed different sensitivities among the strains. These experiments revealed significant effects of strain in the analysis of MAP2 and significant effects of sex within the B6 strain in the analysis of GFAP expression. Moreover, even with this limited strain analysis, we have identified one strain, BXD 73, that showed large effects on all measures, suggesting it to be highly sensitive to ethanol-induced brain damage. These data suggest that genetics and sex are critical variables in determining the level of damage observed in the CNS following chronic ethanol exposure. Additional studies are needed to further test the role of these variables and to identify the genetic networks that underlie these differences.

Disclosures: S. Lattimer: None. **A.J. Elberger:** None. **L. Lu:** None. **K.M. Hamre:** None.

Poster

870. Alcohol: Chronic Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA Grant R01 AA016848 (DR)

the State of California for medical research on alcohol and substance abuse through the University of California, San Francisco (DR)

Title: Excessive ethanol consumption disrupts the protective function of BDNF signaling within the dorsolateral striatum.

Authors: *E. DARCQ, V. WARNAULT, J. JEANBLANC, D. RON
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Abstract: The brain-derived neurotrophic factor (BDNF) plays an important role in neuronal proliferation, differentiation and survival, as well as synaptic plasticity [1]. BDNF binds and activates the high affinity TrkB receptor as well as the low affinity p75 neurotrophin receptor (p75-NTR) which can negatively modulate TrkB signaling [2]. Previously, we reported that the expression of BDNF in the dorsolateral striatum (DLS) of rats is induced in response to moderate self-administration of ethanol [3]. We further found that the activation of the BDNF receptor TrkB in the DLS of rats attenuates operant self-administration of moderate levels of ethanol, whereas siRNA-mediated knockdown of BDNF expression in the DLS increases self-administration of moderate levels of ethanol [3]. Together, these results suggest that the BDNF/TrkB-mediated signaling pathway in the DLS is part of a homeostatic pathway that gates the level of moderate ethanol intake [3-4]. Next, we hypothesized that excessive ethanol intake results in the breakdown of the protective actions of BDNF. To test this hypothesis, rats with a history of high levels of ethanol intake (20%v/v, 2-bottle choice) were trained to self-administer 20% ethanol in an operant self-administration paradigm. We then tested whether manipulations of BDNF levels still affect ethanol self-administration. We found that under these conditions, knockdown of BDNF levels or activation of the BDNF pathway in the DLS ceases to alter ethanol intake, suggesting that the transition from moderate to excessive ethanol drinking results in a breakdown of the BDNF pathway. Activation of p75-NTR produces responses that are opposite to those of TrkB [2]. We hypothesized that a history of excessive drinking alters p75-NTR expression and/or neuronal localization. We found that the synaptic localization of p75-NTR is increased only in the DLS of rats with a history of excessive ethanol drinking after 24 hrs of withdrawal, but not after moderate ethanol intake. Together, our results suggest that excessive ethanol drinking disrupts the protective BDNF signaling by changing p75-NTR localization.
1 Chao Nat Rev Neurosci 2003
2 Teng Dev Neurobiol 2010
3 Jeanblanc J Neurosci 2009
4 McGough J Neurosci 2004

Disclosures: E. Darcq: None. **V. Warnault:** None. **J. Jeanblanc:** None. **D. Ron:** None.

Poster

870. Alcohol: Chronic Effects

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:870.03/S15

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH to RG

Title: Diving deeper into zebrafish shoaling data: A focus on embryonic alcohol exposure effects

Authors: *C. BUSKE[1], R. T. GERLAI[2]
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Abstract: The mechanisms and development of vertebrate social behavior are not fully understood. Numerous human clinical conditions exist in which abnormal social behavior is part of the symptoms, among these are alcoholism and fetal alcohol syndrome. Animal models may facilitate the understanding of the mechanisms of social behavior and the mechanisms underlying abnormal social behavior.

We investigated how single zebrafish move in an open field and quantified swim speed and thigmotaxis. We also investigated zebrafish shoaling (group forming) in the open field by quantifying shoal cohesion in groups of ten fish. In the latter experiment, we studied how embryonic alcohol exposure affected the ontogenesis of shoaling. Although a large body of literature exists on the effects of fetal alcohol exposure, many studies have been completed using unrealistically high alcohol concentrations. To make our investigation more easily extrapolated to clinical circumstances, we employed low doses of alcohol and a short exposure time (2 hours) at 24 hours post-fertilization. A previous study has demonstrated this dosing regiment to be sufficient to cause significant reduction in social preference when measured in adult fish (Fernandes & Gerlai, 2009).

Preliminary results have suggested that embryonic alcohol exposure decreases shoal cohesion in maturing (>40dpf aged) and adult (90dpf) zebrafish. The current study expands on these findings by analyzing shoal cohesion for a larger sample size (n=10), and at smaller time intervals (15 different age points during development). Previous studies from our research group have also shown an oscillating pattern in shoal cohesion of zebrafish (Miller & Gerlai, 2008), here we study whether these oscillating patterns are affected by embryonic alcohol exposure. Miller & Gerlai also reported a change in shoaling oscillations when a stimulus like a predator or food was presented. Additional work published previously has shown that embryonic alcohol exposure lowers Dopamine, DOPAC, Serotonin, and 5-HIAA levels in the brain, throughout development and in the adult (Buske & Gerlai, 2011). These neurochemicals are implicated in various behaviours, with Dopamine also playing a role in social behaviour. Measuring changes in shoaling behaviour following the same treatment that lead to neurochemical changes in previous work serves as a starting point of improving our understanding of the underlying mechanisms affected by embryonic alcohol exposure.

Disclosures: C. Buske: None. **R.T. Gerlai:** None.

Poster

870. Alcohol: Chronic Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA Training Grant T32 AA007474

Charleston Alcohol Research Center Grant P50 AA010761

NIAAA Post Doctoral Fellowship Grant 1F32AA021321-01

Title: Effect of chronic intermittent ethanol exposure on extracellular glutamate levels and uptake in nucleus accumbens in C57BL/6J mice

Authors: *V. S. RAMACHANDRA, L. A. KNACKSTEDT, W. C. GRIFFIN, 3rd, C. L. HAZELBAKER, H. L. HAUN, L. SNYDER, H. BECKER
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Abstract: Previous studies using a mouse model of ethanol dependence and conventional microdialysis procedures have demonstrated that repeated cycles of chronic intermittent ethanol (CIE) exposure increased baseline extracellular glutamate levels in the nucleus accumbens (NAc). Elevated glutamate tone in the NAc was observed beyond acute withdrawal (1-2 weeks following CIE exposure) and was associated with increased ethanol drinking. However, the source of elevated glutamate levels in NAc is currently unknown. This study tested the hypothesis that CIE exposure produces elevated basal glutamate levels by decreasing glutamate re-uptake via excitatory amino acid transporters (EAATs) and/or increasing glutamate release via system xc-. Adult male C57BL/6J mice received repeated cycles of CIE vapor exposure (16 hr/day, 4 days/week to achieve BECs 175 - 225 mg/dL) every other week. Using quantitative microdialysis procedures, we confirmed that CIE-exposed mice showed significant elevation (nearly 2-fold increase) in basal accumbal glutamate levels compared to air-exposed controls. In separate groups of mice, an ex-vivo glutamate uptake assay was used to measure both glutamate uptake (via EAATs) and export (via system xc-) in the NAc at 72-hr following the 4th cycle of CIE exposure. [3]H-glutamate was measured in tissue samples in the presence and absence of sodium to assess sodium-dependent (EAATs) and sodium-independent (system xc-) transporter function, respectively. Results indicated no significant differences in NAc glutamate uptake or export between CIE-exposed and control mice. Studies are currently underway to examine other potential sources of glutamate release, such as neuronal hyperexcitability as possibly contributing to the increased accumbal extracellular glutamate levels observed in ethanol dependent (CIE-exposed) mice.

Disclosures: V.S. Ramachandra: None. **L.A. Knackstedt:** None. **W.C. Griffin:** None. **C.L. Hazelbaker:** None. **H.L. Haun:** None. **L. Snyder:** None. **H. Becker:** None.

Poster

870. Alcohol: Chronic Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA020103

NIH Grant RR017701

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Title: Ethanol increases KLF11 (TIEG2) promoter activity in SH-SY5Y cells

Authors: *S. L. SWILLEY[1], S. JOHNSON[1], C. UDEMGBA[1], P. R. ALBERT[2], J. LUO[3], *D. SITTMAN[1], *X.-M. OU[1]
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Abstract: Alcohol abuse results in brain damage and one possible mechanism is through its activation of the enzyme monoamine oxidase B (MAO B). The catalytic activity of MAO B generates reactive oxygen species and oxidative stress and ethanol-induced increases in MAO B activity likely contribute to ethanol's neurotoxicity. Although the molecular mechanisms involved in ethanol-induced MAO B activation have not been fully elucidated, our laboratory has shown that the TGF-β inducible early gene transcription factor, transforming growth factor beta-inducible early gene 2 (TIEG2, also known as Kruppel-like factor 11, KLF11) is involved in the process. KLF11 (TIEG2) has been found to inhibit cell growth, induce apoptosis, and increase the expression of MAO B. Moreover, our studies have shown that ethanol exposure increases the gene expression of KLF11, with KLF11 leading to further increases in ethanol-induced MAO B expression. The human KLF11 promoter contains DNA elements previously reported to be responsive to ethanol and/or oxidative stress. Therefore, the purpose of the present study was to determine whether the KLF11 promoter could be activated upon ethanol exposure.
The KLF11 1.5 kb promoter was generated and fused in frame to the pGL4 luciferase reporter gene vector (Promega). Human neuroblastoma SH-SY5Y cells were co-transfected with the KLF11 promoter-luciferase gene construct and the pRL-TK plasmid (renilla luciferase expression vector used as an internal control) using the superfect transfection reagent (Qiagen). The transfected cells were subsequently treated with ethanol (75 mM, 24 hr) in a closed chamber system. Following the exposure period, the cells were harvested and luciferase activity was determined.

There was a significant increase in the KLF11 1.5 kb promoter activity of the transfected cells treated with ethanol compared to untreated controls (Controls: 9.58±0.412 vs Ethanol-treated: 12.29±1.95, $p < 0.05$), suggesting that ethanol activates the KLF11 promoter. Because ethanol may activate the promoter through other transcription factor(s), future studies will determine the proteins and DNA interacting elements that are responsible for the effects of ethanol exposure. Our present and future studies will expand our understanding of the molecular mechanisms involved in ethanol-induced KLF11 (TIEG2) activation as well as provide further insight in to the mechanisms by which ethanol leads to brain cell damage.

Disclosures: S.L. Swilley: None. **S. Johnson:** None. **C. Udemgba:** None. **P.R. Albert:** None. **J. Luo:** None. **D. Sittman:** None. **X. Ou:** None.

Poster

870. Alcohol: Chronic Effects

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Program#/Poster#:870.06/S18

Topic: C.17. Drugs of Abuse and Addiction

Support: AA020103

RR17701

Title: Chronic ethanol intake increases immunohistochemical expression of Kruppel-like factor 11, a transcriptional activator of monoamine oxidase B, in the prefrontal cortex of rats

Authors: J. W. DUNCAN[1], C. A. STOCKMEIER[1], J. WANG[1], J. LUO[2], S. JOHNSON[1], *W. L. WOOLVERTON[3], X.-M. OU[1]
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Abstract: Alcoholism is a substance dependence disorder that affects daily responsibilities, physical health, memory, and neuropsychological fitness and may represent an acquired insult to the brain. Neuronal atrophy occurs in subjects with alcohol dependence, as seen with reduced prefrontal cortex (PFC) volume and decreased densities of neuronal and glial cells in human postmortem studies. One cause of cell death may be the Kruppel-like factor (KLF) 11 (also referred to as transforming growth factor-beta-inducible early gene 2, TIEG2)-mediated signaling pathway that is activated during ethanol exposure. Previous reports indicate that glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a multifunctional protein also involved in transcriptional regulation, can translocate to the nucleus in the presence of ethanol, augment

KLF11 and increase monoamine oxidase (MAO) B transcription *in vitro*. An increase in MAO B-mediated oxidative stress results in neuronal atrophy due to the formation of hydrogen peroxide, a byproduct of MAO B metabolism. Therefore, the GAPDH-KLF11-MAO B cell death cascade may be a novel mechanism promoting ethanol-induced neuronal death in alcoholism. We examined whether immunohistochemical expression of KLF11 protein would be increased in the PFC of rats chronically exposed to ethanol (N=10), as compared to ethanol-preferring controls (N=10). The dose of ethanol was physiologically comparable to that in humans. We examined the medial prefrontal cortex (mPFC) and the pre-motor frontal cortex. Rats were fed an isocaloric quantity of ethanol or glucose liquid control diet for 28 days, after which brains were removed and sectioned coronally. Unbiased 3-dimensional cell counting revealed a significant increase in the density of KLF11-immunoreactive cells in both the mPFC and frontal cortex by 36% and 28%, respectively, when compared to ethanol-preferring controls. These results indicate that chronic ethanol exposure in rats increases the recruitment of KLF11 which may enhance downstream expression of MAO B, resulting in excessive neurotransmitter metabolism and consequently, increased oxidative cellular stress. Enhanced KLF11 (TIEG2) expression in prefrontal cortex may critically affect learning, memory and autonomic responses regulated in this region.

Disclosures: J.W. Duncan: None. **C.A. Stockmeier:** None. **J. Wang:** None. **J. Luo:** None. **S. Johnson:** None. **W.L. Woolverton:** None. **X. Ou:** None.

Poster

870. Alcohol: Chronic Effects

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:870.07/S19

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA015741

Title: Effects of insular cortex inactivation on chemosensory responsiveness to ethanol

Authors: N. CASTRO, E. DRIVER, J. DUDLEY, J. GODFREY, B. FERETIC, C. QUINTANILLA, *S. M. BRASSER
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Abstract: Recent findings indicate that the insular cortex (i.e., limbic sensory cortex) is critically involved in addictive behavior to multiple drugs of abuse by regulating an organism's responsiveness to drug-associated sensory cues that trigger craving or "urge to use" (Naqvi & Bechara, 2009). Damage sustained to the insula in addicted smokers results in a disruption of

nicotine addiction (Naqvi et al., 2007) and pharmacological inactivation of the insula in rodents disrupts conditioned preference for environments previously paired with amphetamine or nicotine (Contreras et al., 2007; Scott & Hiroi, 2011) and prevents or suppresses cue-induced reinstatement of nicotine- and cocaine-seeking (Forget et al., 2010; DiPietro et al., 2006). Human imaging studies have demonstrated activation in the insula during the experience of drug-related craving and exposure to drug-associated cues (Bonson et al., 2002; Brody et al., 2002; Wang et al., 1999, 2007), including the taste of alcohol in heavy drinkers (Filbey et al., 2008). The present study measured chemosensory responsiveness to ethanol in chronically ethanol-exposed or naive rats under conditions of pharmacological "silencing" of the caudal granular/dysgranular insular cortex to directly examine the role of this brain region in mediating responses to ethanol-associated sensory cues. Outbred Wistar rats were initially exposed for five weeks to either a 20% ethanol intermittent access paradigm or were given access only to water. Following implantation of intracranial cannulae, rats from each exposure condition were subsequently tested over five consecutive days for short-term lick responses to ethanol (3-40%) and a water control after receiving bilateral insula infusions of saline or muscimol. Alcohol-experienced rats displayed a concentration-dependent increase in chemosensory avidity for ethanol compared to alcohol-naive rats, evidenced by elevated lick responses and trial sampling frequency particularly at higher ethanol concentrations (15-40%). Inactivation of the insula eliminated this concentration-dependent response in chronically exposed animals, with total licks to ethanol and water not differing, which was driven primarily by a decrease in number of ethanol trials sampled. Insula inactivation did not modify orosensory responses to ethanol in alcohol-naive animals. These data support insular cortex involvement in mediating responsiveness to conditioned alcohol chemosensory cues following chronic association of ethanol's taste and post-absorptive effects.

Disclosures: N. Castro: None. **E. Driver:** None. **J. Dudley:** None. **J. Godfrey:** None. **B. Feretic:** None. **C. Quintanilla:** None. **S.M. Brasser:** None.

Poster

870. Alcohol: Chronic Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant K99AA020537

NIH Grant R01AA010983

NIH Grant U01AA0119967

Title: Pharmacological enhancement of extinction learning attenuates alcohol-seeking behavior and alters neuronal plasticity within the addiction neurocircuitry.

Authors: *J. T. GASS, A. S. KASSAB, K. B. LOGOTHETIS, L. J. CHANDLER
Med. Univ. South Carolina, CHARLESTON, SC

Abstract: Extinction-based cue exposure therapies have proven ineffective in the treatment of alcoholism. Our lab has shown that positive allosteric modulation of mGluR5 with CDPPB can enhance the extinction learning of alcohol cues in rats. The current study was designed to assess changes in neuronal plasticity in specific brain regions that correlate with facilitated extinction learning and examine the impact of CDPPB treatment on relapse-like behavior. Male Wistar rats were trained to self-administer a 10% alcohol solution. Active lever presses were accompanied by a light/tone complex stimulus. Rats were then exposed to an extinction training or forced abstinence procedure and were given either an injection of CDPPB (30 mg/kg s.c.) or vehicle (10% Tween 80) 20 minutes prior to each session. Lever pressing behavior was monitored until extinction criteria were met. A diolistic-labeling technique combined with 3D image analysis was used to assess changes in dendritic spines in areas of the prelimbic (PrL) and infralimbic (IfL) subregions of the prefrontal cortex (PFC) and nucleus accumbens (NAc) after extinction learning. To investigate the impact of CDPPB treatment on relapse-like behavior, a separate group of rats was treated with CDPPB during extinction training and subsequently exposed to a cue-induced or contextual-reinstatement session. Treatment with CDPPB during extinction training facilitated extinction learning and resulted in a significant attenuation of cue-induced alcohol-seeking behavior, suggesting that this compound can reduce the impact of alcohol-associated cues on drug-seeking behavior. Additionally, extinction learning was associated with a significant increase in total spine density in the IfL-PFC. Importantly, this effect was promoted in animals that displayed facilitated extinction learning. Further analysis according to spine type revealed that this increase in spine density was primarily associated with an increase in mature “mushroom” shaped spines. These data are consistent with studies that implicate the IfL-PFC in extinction learning and suggest that positive allosteric modulation of mGluR5 may facilitate the formation of extinction memory traces in this region to increase the efficacy of cue exposure therapy.

Disclosures: J.T. Gass: None. **A.S. Kassab:** None. **K.B. Logothetis:** None. **L.J. Chandler:** None.

Poster

870. Alcohol: Chronic Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant P50 AA10761

NIH grant UO1 AA019967

NIH grant R01 AA010983

Title: Chronic alcohol exposure disrupts cognitive function and D2/D4 receptor modulation of prefrontal cortical function.

Authors: *H. TRANTHAM-DAVIDSON, S. W. CENTANNI, L. J. CHANDLER
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Abstract: The medial prefrontal cortex (mPFC) plays a critical role in decision-making and cognitive control of behavior, and these functions are highly influenced by dopaminergic (DA) activity in the mPFC. Previous studies in humans have reported disruption of cognitive processes following repeated episodes of excessive alcohol consumption and withdrawal, suggesting a possible link to altered mPFC function and DA activity in the mPFC. Recurrent network activity between excitatory pyramidal neurons is shaped by recruitment of inhibitory GABAergic neurons, specifically those that are characterized as fast-spiking interneurons (FSIN's). Dopaminergic inputs to mPFC exert powerful effects on network activity via D1/D2 receptor modulation of the balance between excitatory and inhibitory tone. Behavioral assessment of cognitive function revealed delay-dependent impairment of working memory performance in CIE rats compared to controls. These deficits were also associated with a reduction in cognitively modulated increase in phase association of the activity of mPFC neurons to the hippocampal theta rhythm during the epoch of the task that required the rat to use working memory to guide decision-making. In acute slices obtained from both control and CIE animals, D1 stimulation (SKF38393; 5 uM) increased the firing rate of both FS-interneurons and pyramidal neurons indicating that CIE did not alter D1 receptor modulation of cortical activity. However, in slices from CIE animals, the effects of both D2 and D4 stimulation were lost in both cell types. While the cellular mechanisms that underlie the loss of D2/D4 modulation of cortical activity following CIE are unclear and are currently under investigation, our preliminary analysis of spike characteristics and synaptic responses suggests that CIE primarily disrupts D2 signaling in pyramidal neurons and preferentially affects D4 signaling in FSIN's. These changes in D2/D4 receptor function will likely significantly impact DA modulation of PFC activity and may contribute to the cognitive dysfunction associated with CIE exposure. This is consistent with observations in detoxified alcoholics of altered D2 receptor signaling. We suggest that loss of D2/D4 receptor modulation following CIE exposure may shift the cortical network into a D1-

receptor dominated state that may contribute to cognitive deficits observed in human alcoholics. Preliminary experiments suggest that mGluR1 stimulation of FSIN's could be used as a novel therapeutic strategy that will restore the excitatory-inhibitory balance required for network synchrony that supports cognitive function.

Disclosures: H. Trantham-Davidson: None. **S.W. Centanni:** None. **L.J. Chandler:** None.

Poster

870. Alcohol: Chronic Effects

Location: Hall F-J

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Program#/Poster#:870.10/T2

Topic: C.17. Drugs of Abuse and Addiction

Support: Hope for Depression Research Foundation Grant RGA 11-014

NIAAA Grant R01AA020394-01

Title: Kappa-opioid receptor agonist-induced enhancement of 22-kHz ultrasonic vocalizations is indicative of a negative affective state

Authors: *A. WILLIAMS, B. M. WALKER
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Abstract: Alcohol Use Disorders, comprised of alcohol abuse and alcohol dependence, are a severe problem in the United States. High levels of comorbidity exist between alcohol dependence and negative affective disorders such as anxiety and depression during withdrawal. Alcohol-dependent individuals "self-medicate" by consuming alcohol to reduce the negative affective symptoms of withdrawal, which promotes excessive alcohol consumption. Given that there are no FDA-approved treatments for comorbid alcohol dependence and negative affect, it is important to understand the neurobiological mechanisms of dependence and withdrawal. Dynorphin (DYN), the endogenous ligand for kappa-opioid receptors (KORs), is increased in motivational and affective areas of the rat brain by alcohol dependence and has been shown to regulate excessive alcohol self-administration during acute withdrawal in dependent rats. Measurement of 22-kHz ultrasonic vocalizations (USVs) is an ethologically valid strategy that can be used to assess negative affective-like states in rats. During acute withdrawal, alcohol-dependent rats with escalated patterns of alcohol self-administration also display an increased number and duration of 22-kHz USVs when compared to non-dependent rats. The goal of this study was to mimic an alcohol withdrawal-like state in rats via intracerebroventricular (ICV) infusions of the KOR-agonist U50,488 and assess negative affective-like behavior by measuring

induced 22-kHz USVs. Male Wistar rats received an ICV infusion of one of four doses of U50,488 (0.0, 0.25, 2.5 or 25 µg) 15-min prior to the measurement of induced 22-kHz USVs. U50,488 dose-dependently increased 22-kHz USVs. To assess whether this effect was KOR-dependent, a separate group of rats were treated with the KOR-antagonist nor-BNI 24-hours prior to being infused with U50,488 (25 µg only), and USVs were assessed under the same conditions. Nor-BNI reversed the U50,488-induced increases in USVs. These results demonstrate that activation of KORs is sufficient to induce 22-kHz USVs and support the concept that DYN / KOR systems play an important role in the pathogenesis of alcohol withdrawal-induced negative affective-like states. Collectively, these results add to the field's understanding of the neurobiological mechanisms underlying alcohol dependence and provide an alternative strategy for the characterization of negative affective-like states during withdrawal.

Disclosures: A. Williams: None. **B.M. Walker:** None.

Poster

870. Alcohol: Chronic Effects

Location: Hall F-J

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Program#/Poster#:870.11/T3

Topic: C.17. Drugs of Abuse and Addiction

Support: University of Houston, Department of Psychology

University of Houston Division of Research Small Grants Program

Title: Exercise neurorestoration after repeated binge alcohol exposure

Authors: *M. E. MAYNARD[1], T. J. RASHAUD[1], A. FONSECA[1], J. L. LEASURE[1,2]
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Abstract: This study assessed long-term neural consequences of repeated binge alcohol exposure and potential neurorestorative effects of exercise. Rodent models show that binge alcohol exposure damages the brain, particularly the hippocampus, where it kills cells and inhibits neurogenesis. We have previously shown that exercise protects vulnerable brain regions from subsequent binge alcohol exposure. The present study investigated whether exercise could help restore the brain after binge-induced damage has already occurred. Adult female Long-Evans rats underwent 0, 1 or 2 binge episodes. Rats in the 1 or 2 binge groups were gavaged with ethanol (25% w/v) in nutritionally complete diet every 8 hours for 4 days. Control animals (the 0 binge groups) received isocaloric control diet. Recent evidence from a rodent model suggests that the hippocampus is undergoing self-repair during the first week following a single binge, so

we timed the second binge to begin on day 6 of abstinence, in order to disrupt ongoing neurorestoration. Animals in the exercise groups had access to running wheels 5.5 hours per day, for 28 days, beginning 7 days after their final binge. Rats were sacrificed 35 days after the end of the final binge. Compared to rats that underwent 1 binge, 2-binge animals took longer to reach peak intoxication levels, and withdrawal symptoms were more severe. For all binge groups, there was a positive correlation between behavioral intoxication and severity of withdrawal symptoms. Prior binge alcohol exposure had no effect on distance run. Stereological analysis of dentate gyrus cells positive for doublecortin (DCX, an early neuronal marker) revealed no effect of a single binge, but a second binge significantly reduced the number of DCX+ cells. As has been previously shown, exercise increased the number of DCX+ cells in control animals. However, there was no increase in DCX+ cells in exercising single binge animals, indicating a binge-induced suppression of the neurogenic effect of exercise. The effect of exercise after repeated binge episodes on cell proliferation and granule cell survival after 35 days, and the effect on DCX+ positive cells after a second binge, are currently being investigated. We conclude that repeated binge alcohol exposure causes a lasting suppression of neuronal differentiation in the hippocampus, and that exercise, which promotes neurogenesis in the alcohol-naïve brain, may have limited ability to do so in the binge-exposed brain.

Disclosures: M.E. Maynard: None. **T.J. Rashaud:** None. **A. Fonseca:** None. **J.L. Leasure:** None.

Poster

870. Alcohol: Chronic Effects

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Program#/Poster#:870.12/T4

Topic: C.17. Drugs of Abuse and Addiction

Support: Faculty of Medicine, University of Toronto: Dean's Fund

Title: Autobiographical memory and social cognition in youth with Fetal Alcohol Spectrum Disorder.

Authors: *S. AGNIHOTRI[1], J. ROVET[3], G. KOREN[3], C. RASMUSSEN[4], D. CAMERON[2], J. RYAN[2], M. KEIGHTLEY[2]
[1]Grad. Dept of Rehabil. Sci., [2]Univ. of Toronto, Toronto, ON, Canada; [3]Hosp. for Sick Children, Toronto, ON, Canada; [4]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Background: Adolescents with Fetal Alcohol Spectrum Disorder (FASD) often demonstrate deficits in social cognition and emotion processing, which negatively impact the

development of appropriate social skills. Consequently, they can experience social and community withdrawal and display an increased likelihood of remaining socially isolated in adulthood. Autobiographical memory (AM) has been found to be critical for the development of appropriate social behaviors; however, it has not been determined whether AM is impaired in children and adolescents with FASD and if this contributes to inadequate social skill development and social withdrawal found with these youth.
Specific Objectives: The specific objectives of the study are to: (1) compare AM recall performance between adolescents living with a diagnosis of FASD and a group of control participants without an FASD-diagnosis and; 2) determine whether AM recall can predict performance on tasks designed to measure social cognition (i.e. theory of mind and emotion processing).
Methods: Participants were 45 adolescents with FASD and 45 control participants aged 13-17 years. The groups were compared on the number of internal and external AM details that were recalled using the Children's Autobiographical Memory Interview, as well as scores on the Everyday Memory Questionnaire. Social cognition was compared using clinical and experimental measures of emotion processing and theory of mind. Regression analyses were used to understand whether AM can predict social cognitive performance.
Results: Marked deficits in AM performance, poor performance on tests of theory of mind and emotion processing and deficits in AM performance predicting social cognitive impairments is suggested.
Significance: This study is the first to our knowledge that has investigated AM in youth with FASD and lays the groundwork for future neuroimaging research to better clarify the negative impacts of prenatal alcohol exposure on AM development. A better understanding of the function of AM throughout adolescence in these youth will also increase our knowledge of the underlying mechanisms that may be related to the development of mental health problems faced by these youth.

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Poster

870. Alcohol: Chronic Effects

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Program#/Poster#:870.13/T5

Topic: C.17. Drugs of Abuse and Addiction

Support: SDSURF-JRF grant

Title: Neuronal nicotinic receptor ligands modulate chronic ethanol-induced ΔFosB up-regulation in mouse striatum

Authors: *S. RAHMAN, R. K. SAJJA
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Abstract: Chronic exposure to drugs of abuse including alcohol (ethanol) induces persistent accumulation of ΔFosB, a unique transcription factor that regulates long lasting neuronal adaptations, in the brain reward system. Recently, we have demonstrated that neuronal nicotinic acetylcholine receptor (nAChR) ligands such as cytisine attenuate binge-like ethanol drinking and preference in mice. Here, we examined the effects of cytisine (partial agonist at α4β2* nAChR subtype) and mecamylamine (non-selective nAChR antagonist), on chronic ethanol-induced striatal expression of ΔFosB in male C57BL/6J mice. Following exposure to free-choice 24-h ethanol (15% v/v) and water drinking for 25 days, animals (n=5/group) received intraperitoneal injections of saline, cytisine (0.5 mg/kg) or mecamylamine (3 mg/kg) on six consecutive days (once-a-day prior to fluid access). After 18-24 h from the termination of ethanol access, mice were sacrificed and ΔFosB expression in dorsal and ventral striatum was determined by western blot analysis. Cytisine or mecamylamine significantly suppressed 24-h average ethanol intake (56 ± 4.5% or 63 ± 6.7% of saline, respectively), following the third day of treatment. Consistent with the behavioral data, cytisine significantly reduced (116 ± 11% or 114 ± 13% of water control) chronic ethanol-induced expression of ΔFosB (194 ± 14% or 203 ± 24% of water control) in dorsal and ventral striatum respectively, while the reducing effects of mecamylamine were modest (146 ± 23% and 143 ± 18% of water control). Overall, these results indicate that nAChR ligands modulate the up-regulation of striatal ΔFosB following chronic voluntary ethanol consumption in mice. Thus, this study provides further evidence for the role of nAChRs in neurobiology of alcohol addiction. (*Supported in part by SDSURF-JRF grant)*

Disclosures: S. Rahman: Employment; South Dakota State University. **R.K. Sajja:** None.

Poster

870. Alcohol: Chronic Effects

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:870.14/T6

Topic: C.17. Drugs of Abuse and Addiction

Title: Putative NMDA receptor hypofunction contributes to impaired contextual memory consolidation in a rodent model of fetal alcohol syndrome

Authors: *M. J. GOODFELLOW, D. H. LINDQUIST
Behavioral Neurosci., The Ohio State Univ., Columbus, OH

Abstract: Fetal alcohol spectrum disorders (FASD) encompass a constellation of physical and mental abnormalities that may arise from prenatal alcohol exposure. In the present study, a rat model of fetal alcohol syndrome (FAS) is employed to investigate the effects of postnatal day (PD) 4-9 ethanol exposure, a period equivalent to the human third trimester. Perinatal exposure induces neuronal loss in the hippocampus (Gil-Mohapel et al., 2010; Livy et al., 2003), as well as deficiencies in hippocampal-dependent contextual fear conditioning (Murawski & Stanton, 2010). Such exposure is also reported to alter normal developmental changes in forebrain NMDA receptor (NMDAR) subunit composition and expression (Samudio-Ruiz et al., 2010). We hypothesize that ethanol exposure early in development dysregulates NMDAR function, which may contribute to impaired contextual fear learning, acquisition of which relies on NMDAR-dependent synaptic plasticity within the dorsal hippocampus (Matus-Amat et al., 2007). Indeed, 5 minutes of simple context exposure dramatically increases expression of activity-regulated cytoskeleton-associated protein (Arc/Arg3.1), a proposed biomarker of NMDAR activity involved in the maintenance of LTP (Ramirez-Amaya et al., 2005). The current study aims to relate NMDAR activity in FAS rats, as revealed by Arc protein expression, and hippocampal-dependent learning, based on the context pre-exposure facilitation effect (CPFE). In this task, pre-exposure to a specific context in phase 1 facilitates its subsequent association with an immediate footshock in phase 2, resulting in enhanced freezing rates during phase 3 retention testing (Fanselow, 2000; Rudy & O'Reilly 2001). In an attempt to identify the stage of learning in which FAS rats are compromised (encoding, consolidation, or retrieval), two different intervals separating the context pre-exposure and immediate shock are employed- 2 or 24 h. The former relies on a short-term memory of the contextual representation which can be associated with the shock independent of consolidation, whereas the standard 24 h interval requires memory consolidation for successful learning. Preliminary data indicates adult FAS rats are impaired with the 24 h, but not the 2 h, exposure-to-shock interval. Separately, Western blot protein analyses prepared from the dorsal hippocampus 1 h after context exposure (phase 1) provisionally suggest that Arc expression may be reduced in FAS rats. Taken together, we propose that FAS rats are capable of encoding the contextual representation but may be specifically impaired in its consolidation, possibly due to ethanol-induced NMDAR hypofunction.

Disclosures: M.J. Goodfellow: None. **D.H. Lindquist:** None.

Poster

870. Alcohol: Chronic Effects

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:870.15/T7

Topic: C.17. Drugs of Abuse and Addiction

Title: Long-term changes in cortical excitability following a single binge ethanol exposure.

Authors: *J. ZIBURKUS[1], F. GU[1], M. MAYNARD[2], A. AULAKH[1], J. L. LEASURE[2]
[1]Dept Biol & Biochem, [2]Dept of Psychology, Univ. Houston, HOUSTON, TX

Abstract: Recent evidence suggests that a single binge exposure may have long-lasting effects on brain areas vulnerable to ethanol, including the hippocampus and frontal cortex. In the present study, we investigated neural network changes in these regions 35 days after a single 4-day binge ethanol exposure. Adult female Long-Evans rats were gavaged with ethanol (25% w/v) in nutritionally complete diet every 8 hours for 4 days. Control animals received isocaloric control diet. Five weeks later, network activity was studied using electrophysiology and fast voltage-sensitive dye imaging (VSDI) in vitro. Studies were performed in the hippocampus areas CA1 and dentate gyrus and in the prefrontal cortex, layers II-III. The strongest effect of binge drinking on neural network activity was observed in the neocortical slices. Intracortical activity was probed using single pulse, paired-pulse and train stimulations in the gamma range frequency (40Hz). In the binged animals, stimulations of layer IV evoked abnormally large field potential responses in the superficial layers II-III. Measurements of evoked responses during the ten pulse train stimulation revealed subtle changes in the intracortical synaptic short-term plasticity (STP). On average, in binged animals evoked neocortical field potential responses were depressing, while they were facilitating in the control animals. VSDI performed concurrently with extracellular electrophysiology showed that layer IV stimulation evoked spatially wider activity maps in the binged animals, suggesting reduced specificity in the spatial neocortical network activation and aberrant excitability. Taken together, our results suggest that five weeks following a single binge ethanol exposure, the neocortical network activity and synaptic STP are altered. Further ongoing investigations are characterizing if the subtle changes in neural circuit activity seen at five weeks following single binge ethanol exposure are time-dependent and more pronounced at earlier times after the binge.

Disclosures: J. Ziburkus: None. **F. Gu:** None. **M. Maynard:** None. **A. Aulakh:** None. **J.L. Leasure:** None.

Poster

870. Alcohol: Chronic Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R01 AA018037

Title: CrebA and acquired drug tolerance in *Drosophila*

Authors: *B. TROUTWINE[1], N. ATKINSON[2]
[2]iCMB, [1]Univ. of Texas, Austin, TX

Abstract: We study the mechanisms that underlie the acquisition of tolerance to drugs of abuse using Drosophila as a model. Drug tolerance is defined as to a reduced response to a drug caused by previous drug exposure, and is a core component of addiction. We are attempting to understand how the nervous system changes after drug exposure in order to reduce the effect of a drug upon subsequent exposure. Our lab has previously shown that one such mechanism is the induction of the BK channel gene *slowpoke* after drug exposure. This leads to an increased capacity for repetitive firing, which counters the sedative effect of the drug. Our lab has also shown that the Drosophila CREB homolog *Creb2* plays a role in the regulation of *slowpoke* following sedation and that *CrebA* was induced after sedation, hinting that it may be involved. In this study we seek to establish *CrebA*'s role in the acquisition of tolerance to ethanol.
In this study we use a two day behavioral assay to test for the ability to acquire tolerance to the sedative effects of ethanol. Animals are divided into two groups, and on the first day one group is sedated with ethanol vapor while the other group receives a mock treatment. 24 hours later both groups are exposed to ethanol vapor while movement and climbing are recorded. Wild type flies receiving their second dose of ethanol take longer to sedate than naïve flies.
We show that three alleles that disrupt the *CrebA* locus interfere with the acquisition of tolerance to ethanol. Additionally, induction of a *CrebA* transgene mimics the tolerant state in drug-naïve animals. We are currently investigating whether *CrebA* regulates *slowpoke* following sedation in order to induce the tolerant state.

Disclosures: B. Troutwine: None. **N. Atkinson:** None.

Poster

870. Alcohol: Chronic Effects

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Program#/Poster#:870.17/T9

Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA T32 AA007468

Department of Veterans Affairs

Title: Sex and age related differences in the impact of binge alcohol drinking on hippocampal mGluR1/5 levels

Authors: *D. K. COZZOLI[1,2], M. N. STRONG[1], M. R. FORQUER-ANDREW[2], K. M. WIREN[1,2], D. A. FINN[1,2,2]
[1]Dept. of Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR; [2]VA Med. Res., Portland, OR

Abstract: Binge alcohol drinking is a growing health concern, and a history of binge alcohol use is a strong predictor of subsequent alcohol dependence. Previous studies in male rodents indicate that systemic inhibition of metabotropic glutamate receptor 5 (mGluR5) decreases alcohol consumption, including binge drinking, while systemic inhibition of metabotropic glutamate receptor 1 (mGluR1) fails to consistently alter alcohol consumption. Therefore, the present study sought to examine whether binge drinking differentially impacts hippocampal mGluR1 and mGluR5 levels in a sex- and age-dependent manner. C57BL/6J mice (males and females, 8 week old adults and 4 week old adolescents) were single-housed and introduced to the Scheduled High Alcohol Consumption paradigm. In this paradigm the animals receive intermittent (every 3rd day) access to 5% alcohol for 30 minutes, which produces blood alcohol concentrations that average 150mg% and exceed the NIAAA criteria for a binge (80mg%). Following 24-hour withdrawal from the seventh binge session, the hippocampus was dissected, homogenized, and protein levels were analyzed using western blotting. The levels of mGluR1 within the hippocampus were not affected by binge alcohol consumption, and there was no difference between males and females or adults and adolescents. On the other hand, hippocampal mGluR5 levels were altered in a sex-, age-, and treatment-dependent manner. In females, basal mGluR5 levels differed between adults and adolescents, with adolescents having a 40% increase in mGluR5 when compared with adults. However, binge alcohol consumption did not impact mGluR5 levels in either age group. In males, there was an age- and treatment-dependent effect of alcohol consumption, with binge alcohol consumption increasing mGluR5 levels by 42% in adults but decreasing the levels by 15% in adolescents. As mGluR5 lies up-stream of the PI3 kinase signaling cascade, these results suggest that binge alcohol consumption may not only impact the levels of mGluR5 but may also influence the levels and/or phosphorylation of PI3K, PDK1, mTOR, 4EBP1, and p70s6k in a sex- and age-specific manner. This line of investigation is ongoing in the laboratory. Together these data indicate that binge alcohol consumption produces sex- and age-specific alterations in hippocampal mGluR5 levels and suggest that further investigation may elucidate how males and females as well as adults and adolescents may benefit from unique pharmacotherapeutics.
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Poster

870. Alcohol: Chronic Effects

Location: Hall F-J

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Program#/Poster#:870.18/T10

Topic: C.17. Drugs of Abuse and Addiction

Support: WSU start up funds

NIAAA AA0169670 (TAM)

NIGMS R25GM05890513 (BDN)

Title: The influence of alcohol on striatal dopamine dynamics in BDNF-deficient mice

Authors: *B. NEWMAN, K. BOSSE, A. APAWU, T. MATHEWS
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Abstract: Understanding how the brain reacts and adapts to moderate alcohol intake is a critical step in figuring out what makes some individuals more vulnerable to alcoholism, despite the many detriments associated with the disease. The molecular mechanism that turns a 'casual' drinker into a 'compulsive' drinker is unknown, but it is thought that persistent alcohol consumption induces long-term changes in the reward pathways of the brain at both the cellular and molecular levels. Clinical studies have shown connections between SNPs in the gene-encoding brain-derived neurotrophic factor (BDNF) and vulnerability and relapse in alcoholism. Furthermore alcohol consumption has been shown to be altered through animal studies in which BDNF-signaling is manipulated. The evidence of BDNF's role in alcohol consumption and dependence is substantial, but recently BDNF has also been shown to be a potent modulator of dopamine (DA) neurotransmission. Using knockout mice with a 50% reduction in BDNF protein ($BDNF^{+/-}$) the objective of this research is to understand how genetic manipulations and alcohol consumption influence DA dynamics in the striatum. In this study the drinking-in-the-dark (DID) paradigm was used for 7 days of voluntary ethanol exposure to both wildtype (WT) and $BDNF^{+/-}$ mice. In the drinking behavior alone, significant differences were observed between genotypes, with the WT mice consuming a consistent amount (2.82 g/kg) over 7 days while the $BDNF^{+/-}$ mice showed a two-fold increase in ethanol consumed from day 1 to day 7. Ethanol induced changes in DA levels following 7 days of DID were determined using microdialysis. In naïve animals, ethanol challenge elicited an increase in DA levels and this increase was significantly blunted following DID exposure in both genotypes. Using slice fast scan cyclic voltammetry (FSCV), $BDNF^{+/-}$ mice showed alterations in electrically-stimulated DA release and uptake, whereas the WT mice illustrated no change. Overall the DA dynamics of the $BDNF^{+/-}$ mice seem

to be more heavily affected by 7 days of DID compared to their WT counterparts. In the future, other alterations will be explored further using pharmacological manipulations, as well as further characterization of the DA system such as autoreceptor functionality.

Disclosures: B. Newman: None. **K. Bosse:** None. **A. Apawu:** None. **T. Mathews:** None.

Poster

870. Alcohol: Chronic Effects

Location: Hall F-J

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Program#/Poster#:870.19/T11

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA020445

NIH Grant AA020930

NIH Grant AA010983

NIH Grant AA019967

Title: Chronic intermittent alcohol alters dendritic spine morphology and plasticity-related proteins in the mouse prefrontal cortex

Authors: *N. N. NEW[1], S. KROENER[2], J. T. GASS[1], P. J. MULHOLLAND[1], H. C. BECKER[1], L. J. CHANDLER[1]
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Abstract: The medial prefrontal cortex (mPFC) is implicated in complex cognitive operations including impulse control, behavioral plasticity, and motivation. The mPFC is of particular interest in the development and maintenance of addiction due to its role in top-down (e.g., experience-driven) processing. Long-term alcohol consumption is associated with structural abnormalities and neurological deficits attributed to dysfunction of the PFC. A recent study from our group demonstrated PFC-dependent behavioral impairments and aberrant spike-timing dependent synaptic plasticity in PFC pyramidal neurons following chronic intermittent ethanol (CIE) vapor inhalation. Thus, it is possible that chronic alcohol exposure may induce neuroadaptations in the mPFC that are associated with maladaptive decision-making that is a hallmark of alcohol use disorders. These experiments address CIE exposure-induced structural and biochemical plasticity of glutamatergic synapses in the prelimbic (PL) mPFC. We determined the effect of CIE exposure on dendritic spine morphology and subunit composition

of glutamate receptors in the PL mPFC immediately following CIE exposure and 1 week post-withdrawal. Dendritic spine analysis indicates that CIE exposure produces a significant and persistent increase in mature mushroom spines without altering overall dendritic spine density or spine length. The morphological neuroadaptations were associated with an increase in both GluN1 and GluN2B subunits of the NMDA receptor in a PSD-enriched fraction immediately after CIE exposure. However, these changes were no longer present when examined 1 week post-exposure. CIE exposure did not alter the expression levels of the GluR1 subunit of AMPA receptors at either time point. Taken together, the findings from these studies suggest that CIE exposure induces changes in dendritic spine morphology and glutamatergic subunit composition in the PL mPFC, suggesting that these neuroadaptations may contribute to the cognitive dysfunctions that characterize addictive processes.

Disclosures: N.N. New: None. **S. Kroener:** None. **J.T. Gass:** None. **P.J. Mulholland:** None. **H.C. Becker:** None. **L.J. Chandler:** None.

Poster

870. Alcohol: Chronic Effects

Location: Hall F-J

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Program#/Poster#:870.20/T12

Topic: C.17. Drugs of Abuse and Addiction

Support: NADIA of the NIAAA

Title: Danger signals HMGB1, TLRs, and RAGE are increased following adolescent intermittent ethanol treatment in adult rats and in human alcoholic post-mortem prefrontal cortex

Authors: *R. P. VETRENO[1], L. QIN[2], F. T. CREWS[2]
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Abstract: Adolescence is a critical period of prefrontal refinement, neuroplasticity, and increased risk-taking and sensation seeking that promotes the acquisition of skills necessary for adult independence. This period is also a time of considerable alcohol experimentation and abuse that prompted the hypothesis that adolescent binge drinking might exert long-term effects on adult brain and behavior. Our laboratory recently linked alcohol-induced neuroinflammation to neurodegeneration through the induction of innate immune danger signaling (i.e., high-mobility group box 1 [HMGB1]-Toll-like receptor [TLR]-receptor for advanced glycation end-products [RAGE] signaling). To determine whether adolescent binge drinking increased innate immune danger signaling in the adult brain, male Wistar rats received adolescent intermittent ethanol

(AIE [5.0 g/kg, i.g., 2-day on/2-day off schedule]) treatment from P25 to P55. During abstinence, reversal learning was measured, and 25 days later (P80) tissue was collected for danger signal expression assessment. Adult rats exposed to AIE had increased levels of TLR4 mRNA (>400% increase) and TLR3 mRNA (>300% increase) relative to controls as well as increased TLR4, TLR3, and RAGE immunohistochemical (IHC) protein expression, which was approximately double control levels in the adult prefrontal cortex. This persistently upregulated receptor expression was accompanied by increased expression of the danger signal receptor agonist, high-mobility group box 1(mRNA: 160% increase; IHC protein: 150% increase). Behavioral assessments indicated that AIE exposure did not alter Barnes maze spatial learning, but did induce long-term reversal learning deficits while increasing perseveration. Danger signal receptor expression correlated with reversal learning deficits. Human post-mortem brain assessment found increased danger signal expression in alcoholic orbitofrontal cortex, which correlated with lifetime alcohol consumption and age of drinking onset. Together, these findings provide evidence that adolescent binge drinking leads to long-term activation of innate immune danger signaling that contributes to adult neurocognitive dysfunction.

Disclosures: R.P. Vetreno: None. **L. Qin:** None. **F.T. Crews:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

Location: Hall F-J

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Program#/Poster#:871.01/T13

Topic: C.17. Drugs of Abuse and Addiction

Support: OTKA K 73219

Title: Embryonic and post-embryonic effects of the designer drug mephedrone on early neurogenesis and learning

Authors: A. ADAM[1], C. ZSEDENYI[1], *A. L. CSILLAG[2]
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Abstract: The designer stimulant
4-methylmethcathinone (mephedrone) is a new and popular drug of abuse widely
available on the Internet and still legal in some parts of the world. Given the
fact that pregnant females may also be exposed, gestational damage
to the brain of the developing embryo is likely to occur. Earlier work on
neuroactive drugs has indicated impairment of neurogenesis following developmental exposure
of embryonic brain regions such as the hippocampus or in cell cultures relevant to adult

neurogenesis. Here we present
data on the effect of a cathinone derivative mephedrone, administered in ovo, on the development of specific regions of chicken striatum as well as on early adaptive learning of young post-hatch chicks. In the first group of experiments, mephedrone (2 mg/kg egg weight) was injected into incubated chicken eggs in critical stages
(days 6-10) of striatal development. This was followed by quantitative analysis of post-embryonic proliferating neurons in the subventricular zone of striatum, based on incorporation of the labeled nucleoside BrdU, visualized
by immunohistochemistry, and quantified by stereological method. A decrease in the density of BrdU-labeled cells
in the subventricular zone of subpallium including the medial striatum, nucleus accumbens and striopallidal organ was observed primarily in those birds injected in ovo in the later phase of striatal neurogenesis (day 10 of incubation). To investigate the effect of direct
post-hatch drug exposure on learning ability, one-day old chicks injected i.p. with mephedrone (10 mg/kg b.w.) were tested for the passive avoidance learning task. No impairment of memory retention was evident when the drug was administered 30 min prior to training. The drug-injected birds showed hyperactivity without apparent distress at the time of recall testing (6 h).

Disclosures: A. Adam: None. **C. Zsedenyi:** None. **A.L. Csillag:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

Location: Hall F-J

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Topic: C.17. Drugs of Abuse and Addiction

Support: INSERM

Fundação para a Ciência e para a Tecnologia

Title: Caffeine exposure during pregnancy disrupts GABAergic circuits in offspring

Authors: C. G. SILVA[1,2,3], C. MÉTIN[4,5], N. J. MACHADO[3], S. DARMOPIL[1,2], P. LAUNAY[4,5], A. GHESTEM[1,2], M.-P. NESA[1,2], Y. BAQI[6], C. E. MULLER[6], A. IVANOV[1,2], Y. ZILBERTER[1,2], R. A. CUNHA[3], C. BERNARD[1,2], *M. T. ESCLAPEZ[1,2]
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INSERM UMR-S 839, Paris, France; [5]Univ. Pierre et Marie Curie, Paris, France; [6]PharmaCenter, Univ. of Bonn, Bonn, Germany

Abstract: Psychoactive drug consumption during pregnancy interferes with fetal brain development, with psychiatric, neurologic and cognitive deleterious long-term consequences. The effects of caffeine, the most widely consumed psychoactive substance, on brain development, are largely unknown. Since caffeine is a naturally-occurring antagonist of adenosine receptors, which regulate numerous intracellular processes, we investigated the consequences of exposing dams to caffeine, throughout gestation until weaning, on their offspring. Here we report that caffeine, or a type 2A adenosine (A2A) receptor antagonist, delayed the migration of GABAergic neurons and their insertion into the hippocampal circuitry, and increased network excitability during the first postnatal week. Early life caffeine exposure had long-term consequences in adult offspring, with a loss of hippocampal GABA neurons and robust cognitive deficits. These results demonstrate the deleterious effects of maternal caffeine consumption during development, through its action on A2A receptors, compromising the structural and functional properties of neuronal networks in offspring.

Disclosures: C.G. Silva: None. **C. Métin:** None. **N.J. Machado:** None. **S. Darmopil:** None. **P. Launay:** None. **A. Ghestem:** None. **M. Nesa:** None. **Y. Baqi:** None. **C.E. Muller:** None. **A. Ivanov:** None. **Y. Zilberter:** None. **R.A. Cunha:** None. **C. Bernard:** None. **M.T. Esclapez:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: R01DA020796

P30NS045776

R01DA022339

Title: Changes in frontal cortical BDNF and Glutamate receptor expression produced by prenatal cocaine exposure are heritable via epigenetic mechanisms

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Abstract: Prenatal cocaine exposure impairs fetal brain development and produces long-term alterations in cognitive function. Animal models of prenatal cocaine exposure confirm changes in multiple events associated with brain development, including neurogenesis and neuronal migration. There is also evidence that alterations in gene expression influence behavior that can be inherited across multiple generations via epigenetic modifications. The effects of exposure to environmental factors such as cocaine appear to become incorporated into the germ line through the sustained expression of associated epigenetic marks. We used a mouse model to test the hypothesis that prenatal cocaine exposure produces epigenetic alterations that are manifested as biochemical and behavioral changes and transmitted across multiple generations. We exposed timed-pregnant Swiss Webster mice to cocaine from the 8th day of pregnancy until parturition. The offspring (F1 generation) upon reaching maturity showed decreased cocaine sensitization and increased expression of BDNF and glutamate receptor mRNA in the frontal cortex. The increased BDNF mRNA expression was associated with increased histone H3 acetylation. We crossed the F1 females (prenatally exposed to cocaine) with drug naïve males to produce the F2 generation. Similarly, the F2 females were crossed with drug naïve males to produce the F3 generation. Remarkably, the F2 and F3 progeny also showed the frontal cortical gene expression changes observed in the F1 generation, even though neither the F2 nor the F3 mice were directly exposed to cocaine. Our results indicate that changes in frontal cortical gene expression produced by the prenatal cocaine exposure may be heritable via epigenetic mechanisms

Disclosures: D.M. Mc Carthy: None. **J. Zhu:** None. **S.B. Darnell:** None. **G.R. Sangrey:** None. **P.G. Bhide:** None. **G. Sadri-Vakili:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: The Department of Veterans Affairs

NIH NIDA P50DA018165

T32DA07262

Title: Effects of prenatal methamphetamine exposure on adult cognitive performance

Authors: *J. H. HARKNESS[1,2], E. EASTWOOD[1,2], J. M. LOFTIS[6,2,3], J. RABER[1,2,4,5], T. J. PHILLIPS[1,2,6]
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Abstract: Prenatal exposure to methamphetamine (MA) has been suggested to cause developmental and cognitive deficits in adulthood. However, due to polydrug abuse, pure MA effects are difficult to study in humans. Methods have not yet been described in the literature for a prenatal MA exposure model in mice that can be used to examine behavioral consequences associated purely with MA. The present experiments used a twice daily 8 mg/kg MA treatment regimen to explore effects of prenatal MA exposure on adult (~85 day old) spatial navigation learning using an 8-day Morris Water Maze task. In addition, brain tyrosine hydroxylase (TH) levels were measured in gestationally saline- and MA-treated neonatal mice. C57BL/6J x DBA/2J F1 mice were mated, and dams were administered MA for 19 days of gestation. Mice were first trained to swim to a visible platform in each quadrant of the water maze over 2 days, and then over 2 days to a "hidden" platform submerged immediately below the surface of the water in a target quadrant, where room cues could be used for navigation. Following the 4 days of training, a probe trial (no platform) tested spatial memory retention. Subsequently, over the next 2 days, mice were trained to find the hidden platform in a new location ("reversal" training), and were tested the following day in a reversal probe trial for recall of the new target location. There were no significant effects of gestational MA on distance traveled or swimming velocity in either probe trial or on body weight. Adult mice that had been exposed to MA during gestation tended to spend less time in the target quadrant than saline-treated mice in the first probe trial ($p=.07$), and spent significantly less time in the new target quadrant ($p=.03$) and the platform zone within the new target quadrant ($p=.008$) during the reversal probe test than saline-treated mice. Furthermore in the reversal probe trial, male MA-exposed mice spent a significantly greater percentage of time ($p=.0006$) in the original target quadrant, compared to saline-treated mice. These findings indicate that prenatal MA exposure may have long-term effects on spatial cognitive ability, and may significantly affect the plasticity of learning in these tasks. This regimen of MA exposure significantly decreased brain TH levels of postnatal day 1 pups to about 50% of levels in saline-treated controls ($p<.05$). Here we report prenatal MA exposure effects on brain TH levels, and lasting effects on spatial cognitive ability. This model of prenatal MA exposure should be useful for studying the effects of prenatal MA exposure without the confound of possible effects of other drugs, which is difficult to accomplish in humans.

Disclosures: J.H. Harkness: None. **E. Eastwood:** None. **J.M. Loftis:** None. **J. Raber:** None. **T.J. Phillips:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA T32DA007262

NIDA F31DA026243

Title: Developmental methamphetamine exposure decreases the number of vasopressin-immunoreactive cells in the hypothalamic paraventricular nucleus

Authors: *D. G. ZULOAGA[1], J. A. SIEGEL[2], J. RABER[1]
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Abstract: The psychostimulant methamphetamine is abused by approximately 1.2 million individuals over the age of 12. Women that abuse methamphetamine might also do so while pregnant. Exposure to methamphetamine during brain development can lead to long-term behavioral and cognitive deficits. Methamphetamine can induce detrimental effects by increasing circulating levels of glucocorticoids. This can lead to long-term disruptions in the hypothalamic pituitary adrenal (HPA) axis, which plays a critical role in the regulation of stress responses. Furthermore, alterations in proteins and neuropeptides associated with the HPA axis, including vasopressin and glucocorticoid receptors, are correlated with disorders of anxiety, depression and cognition. We hypothesized that methamphetamine exposure during the stress hyporesponsive period would result in lasting alterations in the expression of HPA axis-associated proteins and/or neuropeptides and that these effects may contribute to alterations in stress responses reported in adult rodents treated with methamphetamine during brain development. To begin testing this hypothesis, male mice were treated with methamphetamine (5 mg/kg daily) or saline from postnatal day 11-20, a period of development in mice that models a similar period of brain development in humans that occurs in utero during the third trimester. In adulthood, mice were perfused for immunohistochemical detection of the glucocorticoid receptor and vasopressin within brain regions associated with the HPA axis. Our results indicate a decrease in the number of vasopressin-immunoreactive cells within the hypothalamic paraventricular nucleus (PVN) of mice treated with methamphetamine. As vasopressin also contributes to the regulation of cognition, decreased vasopressin may be one mechanism through which developmental methamphetamine exposure contributes long-term deficits in this function. No significant differences in glucocorticoid receptor expression were found in the hippocampus, PVN, or central amygdala. Thus, developmental exposure to methamphetamine can result in lasting

alterations in the vasopressin system, which may in turn affect stress-related behaviors, including those following chronic stress, and cognition.

Disclosures: D.G. Zuloaga: None. **J.A. Siegel:** None. **J. Raber:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

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Title: Cocaine intravenous self-administration (IVSA) in adult Long-Evans rats exposed to polychlorinated biphenyls throughout gestation and lactation

Authors: *M. M. MILLER, A. E. MEYER, J. L. NELMS, P. E. DICKSON, M. A. WARD, M. CALTON, H. J. K. SABLE
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Abstract: Polychlorinated biphenyls (PCBs) are ubiquitous and persistent in the environment and pose a serious human health threat. Developmental exposure to PCBs in rats has been shown to produce alterations in brain dopamine (DA) concentrations that persist into adulthood. These alterations suggest that the reinforcing properties of drugs of abuse which act on the DA system may be affected by developmental PCB exposure. Our previous research has revealed that stimulation-evoked DA efflux in the nucleus accumbens (NAc) following stimulation of the medial forebrain bundle was significantly higher in rats developmentally exposed to PCBs following acute injections of cocaine, but attenuated (relative to non-exposed controls) following chronic cocaine injections. Thus, given that PCB exposure altered the dopaminergic response to cocaine, it was hypothesized that PCB exposure would influence cocaine self-administration (IVSA). In particular, the enhanced DA release seen in animals exposed to PCBs during gestation and lactation was expected to promote faster acquisition of cocaine self-administration during adulthood. Female Long-Evans rats were orally exposed to 0, 3 or 6 mg/kg/day of an environmentally relevant mixture of PCBs beginning 4 weeks prior to breeding and continuing until litters were weaned on postnatal day 21. One male and female per litter were food deprived around PND 90 to 85% of their free-feeding weight and trained to lever press (FR 5) for a food

reinforcer. Between PND 120-180, rats were surgically implanted with a jugular catheter and given a minimum of 48 hours for recovery. After the lever-press response to food was re-established, the reinforcer was changed to a 50µl infusion of 250µl of cocaine. The criterion for meeting acquisition was defined as <15% variability in reinforcers delivered over three consecutive days of testing. Preliminary data indicates that male rats developmentally exposed to 6 mg/kg/day PCBs reach criterion for cocaine IVSA in about half as many sessions as non-exposed controls. A similar but more variable trend occurs in the females. Together, these findings suggest that developmental exposure to PCBs can modify DA pharmacology in a manner that may promote cocaine drug-seeking behavior.

Disclosures: M.M. Miller: None. **A.E. Meyer:** None. **J.L. Nelms:** None. **P.E. Dickson:** None. **M.A. Ward:** None. **M. Calton:** None. **H.J.K. Sable:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA Intramural Research Program (AA 11034 & AA07574, AA07611)

Title: Effect of chronic oral methylphenidate treatment and abstinence on dopamine transporter (DAT), D1R, and D2R binding in young rats

Authors: *L. S. ROBISON[1,2,3], M. ANANTH[1], J. M. SWANSON[4], J. K. ROBINSON[3], G.-J. WANG[1], N. D. VOLKOW[2], P. K. THANOS[1,2,3]
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Abstract: Methylphenidate (MP) is widely used in the treatment of children and adolescents with ADHD. Since MP's mechanism of action is partly mediated by its ability to block DA transporters (DAT) and increase levels of extracellular dopamine in the striatum and nucleus accumbens (Volkow, et al. 2001); (Volkow et al. 2002a); (Volkow et al. 2002b), this has raised concerns regarding neuroadaptations in dopamine signaling that could increase their risk for substance use disorders in adulthood. Here, we investigate the effect of chronic MP treatment initiated in adolescence on dopamine receptor levels (D1 and D2) and DAT in adulthood.

Male Sprague-Dawley rats were split into 3 groups (*n=16/group)* at 4 weeks old: control (water), low dose MP (LD), and high dose MP (HD). A two-bottle 8-hour limited access drinking paradigm was utilized, producing an MP pharmacokinetic profile similar to human patients (Steier et al., 2008). Rats drank from the first bottle [4 mg/kg MP (LD) or 30 mg/kg MP (HD)] for the first hour. This bottle was replaced with a second bottle [10 mg/kg (LD) or 60 mg/kg MP (HD)], which was available for hours 2-8. Control rats were given water in both bottles. After 13 weeks of treatment, half of the rats in each group were sacrificed (n=8/group), and the remaining rats (n=8/group) underwent 5 weeks of abstinence (given water for the 8h drinking period) and were then sacrificed. Brains were harvested, flash-frozen, sectioned at 14µm, and stored at -80 °C. *In vitro* autoradiography was performed to assess MP's effects on levels of DAT and dopamine receptors D1R and D2R, using [^{3}H] WIN35,428, [^{3}H] SCH23390, and [^{3}H] spiperone, respectively.

MP treatment resulted in increased D1R density in the olfactory tubercle (LD: 5.1%, $p<0.05$; HD: 10.4%, $p<0.001$) and caudal portion of the dorsal CPu (HD: 37.1%, $p<0.001$), and decreased D1R binding in the ventrolateral CPu (rostral portion; LD: 5.0%, $p<0.05$). These effects were not observed in rats that had undergone subsequent abstinence from MP. MP was previously found to result in decreased D1R density in the CPu and olfactory tubercle (Carey et al., 1998); however, while treatment began at 4 weeks of age, dose, route of administration (3 mg/kg i.p.), and treatment length (2 weeks) differed from our study. Neither dose of MP changed D2R density, similar to prior findings (Carey et al., 1998; Randall et al., 1999) but distinct from prior results in which we showed that HD MP treatment (8 weeks) decreased D2R availability in the CPu, using microPET with [^{11}C] raclopride (Hwang et al., 2008). This discrepancy could result from increased dopamine release after chronic MP, rather than a reduction in D2R levels. Analysis of the DAT binding data is ongoing.

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Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

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Title: 3-dimensional diffusion tensor imaging (dti) atlas of the rat brain in postnatal day 5, 14, and adulthood.

Authors: *A. RUMPLE[1], P. MAKAM[1], M. RADCLIFFE[1], J. LAUDER[1], M. MCMURRAY[2], I. OGUZ[1], J. JOHNS[1]
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Abstract: Anatomical atlases play an important role in the analysis of neuroimaging data in rodent neuroimaging studies. Having a high resolution, detailed atlas can, not only expand understanding of rodent brain anatomy, but also enables automatic segmentation of new images, thus greatly increasing the efficiency of future analysis when applied to new data. This project seeks to develop and produce a set of detailed 3D anatomical atlases of the rat brain at postnatal day five (P5), fourteen (P14), and adult dams to be used for analysis of anatomy in a study focused on intrauterine cocaine abuse in relation to neurodevelopment as well as for use with future data sets.

Our methods consisted of first creating a template image based on fixed scans of control rats, then manually segmenting various individual brain regions on the template. The subjects were Sprague-Dawley rats that served as controls from a study focused on intrauterine cocaine exposure and development. They were euthanized and underwent fixation before being scanned overnight for approximately fifteen hours. For each atlas, the template image was an average of fixed scans of control rats for that age. The P5 and P14 templates were made from scans of twenty rats each (ten female and ten male). The adult dam template was made from the scans of six female rats. All DTI scans were acquired on a 9.4T Bruker scanner. The resolution for the adult dam scans was (0.16 x 0.125 x 0.16)mm^3 with twelve gradient directions and two baseline images; the resolution of the P5 and P14 images was (0.12 x 0.07 x 0.12)mm^3 with twenty-one gradient directions and three baseline images. The individual cases were first resampled to isotropic resolution, then manually skull-stripped, then deformably registered via an unbiased atlas building method to create a single template image. This template was used to segment the individual brain structures.

Using itkSNAP software, subcortical regions, including both white matter and gray matter structures, were manually segmented in the axial, sagittal, and coronal planes. The adult dam, P14, and P5 atlases had twenty-nine, forty-five, and thirty-nine regions segmented out, respectively.

We created a high-resolution anatomical atlas of pup and adult rat brains. This atlas can now be used to analyze images from live scans of individual cases; it can be easily used on lower resolution images so that the segmentation of future images can be completed more efficiently and robustly. Currently, we are working on applying these atlases to the live images generated from this study and are acquiring results of cocaine's effects on development. These atlases will be made available to the broader research community.

Disclosures: A. Rumple: None. **P. Makam:** None. **M. Radcliffe:** None. **J. Lauder:** None. **M. McMurray:** None. **I. Oguz:** None. **J. Johns:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:871.09/U1

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA

College of Arts and Sciences

Title: Preadolescent behavioral responsivity to an acute injection of cocaine

Authors: A. URUENA[1], *C. L. KIRSTEIN[2]
[1]USF, Tampa, FL; [2]Univ. South Florida Tampa, Tampa, FL

Abstract: It has become increasingly well known that exposure to drugs of abuse during early developmental time periods impacts neurodevelopmental processes. Such drug-induced alterations may contribute to the observed vulnerability in developing drug addiction later in life. Past investigations have demonstrated age- and dose-dependent differences in acute cocaine-induced behavioral responsivity. Recent reports have shown a dose-dependent augmentation in locomotor activity in response to acute cocaine administration in naïve adolescent rats, as compared to naïve adult rats. Such findings demonstrate drug sensitivity is greater during adolescence than during adulthood, suggesting the rewarding properties of cocaine may be greater during different developmental time periods. In order further assess ontogenetic differences in drug sensitivity, established experimental procedures were employed to investigate the behavioral profile of preadolescent rats exposed to an acute injection of cocaine. Naïve male preadolescent rats (postnatal (PND) 25) were habituated to a novel open-field arena for 20 minutes. Following habituation, rats were administered either cocaine (20 mg/kg, i.p.) or a saline vehicle. The 20 mg/kg dose of cocaine was used as this dose has been shown to effectively increase locomotor activity in all several age groups. Preliminary results show cocaine-induced hyperlocomotion as a result of a decrease in the frequency of immobile episodes (start/stops). When results were compared to rats previously tested at age PND 35, 45 and 60 (i.e. early and late adolescence, or adulthood, respectively), preadolescent rats demonstrated greater behavioral responsivity to acute cocaine administration compared to additional age groups. Further experimentation is warranted as such observations suggest a unique susceptibility to drug addiction as a function of increased drug sensitivity and drug reward during preadolescence.

Disclosures: A. Uruena: None. **C.L. Kirstein:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:871.10/U2

Topic: C.17. Drugs of Abuse and Addiction

Support: R01 DA14020

Title: Cocaine and sucrose self-administration and cue-induced reward seeking in adolescent versus adult rats.

Authors: *D. S. COUNOTTE[1], K. KONKA[1], Y. SHAHAM[2], P. O'DONNELL[1]
[1]Anat. & Neurobio., Univ. of Maryland Baltimore, Baltimore, MD; [2]Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Adolescence is a critical window of continued brain development, and a period during which individuals first come into contact with drugs of abuse. Epidemiological research suggests that early use of drugs is associated with more severe drug addiction in adulthood. Here, we investigated in rats whether adolescent cocaine self-administration followed by forced abstinence leads to enhanced drug seeking compared to rats that self-administered cocaine during adulthood. In a separate group of rats, we did a similar experiment with oral sucrose instead of cocaine.
For cocaine self-administration, adolescent rats were implanted with jugular vein catheters at P35 and adult rats were implanted at P65. They were allowed to recover for one week and started cocaine self-administration at P42 and P72, respectively. Active lever presses led to cocaine infusions (0.4 mg/kg/infusion) that were paired with a compound tone-light cue. After 10 days of cocaine self-administration, the rats were left in their home cage for four weeks until cue-induced cocaine seeking was assessed in a single extinction session in which an active lever-press led to presentation of the tone-light cue but not cocaine.
For sucrose self-administration we used a similar procedure, except that lever-presses lead to the delivery of 0.6 ml of 10% sucrose solution. Preliminary data indicate that young adolescent rats (starting at P35) self-administered less sucrose than adult and older adolescent (starting at P42) rats, even when the amount of sucrose was adjusted to body weight. Data on age differences in cocaine self-administration and cue-induced cocaine and sucrose seeking after a prolonged abstinence period will be presented at the meeting.

Disclosures: D.S. Counotte: None. **K. Konka:** None. **Y. Shaham:** None. **P. O'Donnell:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:871.11/U3

Topic: C.17. Drugs of Abuse and Addiction

Title: Sex differences in induction and expression of methylphenidate sensitization and brain-derived neurotrophic factor (BDNF) in adolescent rats.

Authors: *R. ROEDING[1], M. K. PERNA[2], S. B. GRIFFIN[3], R. BECKER[3], R. W. BROWN[3]
[1]Psychology, [2]Quillen Col. of Med., [3]East Tennessee State Univ., Johnson City, TN

Abstract: Methylphenidate (MPH) is a psychostimulant that is used for the treatment of attention-deficit hyperactivity disorder that is often recreationally abused. Past studies have primarily analyzed the effects of MPH on behavior and BDNF using males as subjects, with studies showing a lack of behavioral sensitization, although the effect of MPH on BDNF has yielded contradictory results. BDNF is a neurotrophin ubiquitously found throughout the brain that plays an important role in synaptic maintenance and development and has been implicated in addiction. This study analyzed sex differences in induction and expression of MPH locomotor sensitization in adolescent male and female rats as well as the effects of MPH on BDNF protein in two brain areas of drug reward: the nucleus accumbens (NAcc) and striatum (STR), after both induction and expression of sensitization. After habituation to a locomotor arena, animals (N=6-8 per group) were administered MPH (5 mg/kg) or saline every other day from postnatal day (P)33 to 49 and tested for 30 min in the same arena with activity counts recorded. In one group, brain tissue was removed one day following testing and the NAcc and STR assayed for BDNF at P50. A different group of animals was raised to P60 and given an MPH (or saline) challenge. One day following the challenge, brain tissue was removed and the NAcc and STR were assayed for BDNF at P61. Females administered MPH demonstrated behavioral sensitization from P33 to P41, and then decreased in activity from P41 to P49. Females demonstrated a robust increase in locomotor activation as compared to males, which failed to demonstrate sensitization to MPH. However, both groups given MPH demonstrated an increase in activity compared to controls throughout sensitization testing. On the challenge at P60, females administered MPH demonstrated higher levels of activity compared to all other groups and were equivalent to their final day of sensitization. Males administered MPH also expressed sensitization, as they demonstrated increased behavioral activation as compared to saline-treated controls. Neurochemical analyses at P50 revealed that MPH produced a significant increase in striatal BDNF in males, but a significant decrease in striatal BDNF in females. There were no changes in

the NAcc. At P61, BDNF was increased in both STR and NAcc in males, and female data will be presented. These data demonstrate robust sex differences in behavioral activation and sensitization to MPH that is both induced and expressed in females, but only expressed in males. Further, MPH produces sex-dependent effects on BDNF, indicating sex differences in the brain plasticity response to MPH in adolescence.

Disclosures: R. Roeding: None. **M.K. Perna:** None. **S.B. Griffin:** None. **R. Becker:** None. **R.W. Brown:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:871.12/U4

Topic: C.17. Drugs of Abuse and Addiction

Support: Ronald E. McNair Program

Title: Methylphenidate conditioned place preference in adolescent male and female rats

Authors: E. D. CUMMINS, S. B. GRIFFIN, R. L. ROEDING, J. H. THOMAS, *R. W. BROWN
East TN State Univ., JOHNSON CITY, TN

Abstract: Methylphenidate (MPH) is a psychostimulant drug that is commonly prescribed for Attention Deficit-Hyperactivity Disorder (ADHD), however, this drug is also abused. The primary pharmacological action of MPH is similar to that of cocaine, in that the drug blocks the dopamine transporter and enhances dopamine neurotransmission. The similarity to cocaine has brought to light legitimate concerns at the abuse liability of MPH. The focus of the present study was to analyze MPH conditioned place preference (CPP) in adolescent rats, with the focus on sex differences in this effect. CPP is a commonly used measure of drug reward in rats. Animals were also tested at two different time periods, from postnatal day (P)32-38 or P43-49, representing periods during adolescence both before and after the commencement of the estrous cycle in females, which begins on approximately P41. After an initial preference test on P32 or 43 revealed no significant preference for either context, an unbiased procedure was used. Beginning on P33 or P44, MPH (5 mg/kg) was paired with either context in animals in the drug condition, balanced across males and females. Controls were given saline in both contexts (N=8-9 in all groups). A preference test was given on P38 or P49 with dividers removed. Results revealed no sex or age difference, but MPH induced a robust increased preference for the paired context in both males and females on the post-conditioning preference test, which was significantly greater

than male and female controls. Interestingly, males demonstrated significantly higher levels of activity and a higher number of entries made into the paired context when tested at the younger age, but there were no sex differences in these responses in older animals. Brain tissue is currently being analyzed for levels of the dopamine transporter (DAT), and this data will be presented. Past studies have shown that females have a higher number of DAT in the striatum compared to males in adulthood, but this has never been established in adolescence. In conclusion, it appears that a 5 mg/kg dose of MPH is capable of inducing drug reward in adolescent rats, and unlike recent results from our laboratory regarding behavioral sensitization, there are no sex differences in this response.

Disclosures: E.D. Cummins: None. **S.B. Griffin:** None. **R.L. Roeding:** None. **J.H. Thomas:** None. **R.W. Brown:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:871.13/U5

Topic: C.17. Drugs of Abuse and Addiction

Support: FAP: Arnaldo Vieira de Carvalho Foundation

Title: Variable chronic cocaine treatment induces an increased food intake

Authors: *H. R. SILVA, P. S. SANZOVO, S. A. BARBOSA, C. LOPES, C. A. TIEPPO
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Abstract: The continuous and chronic treatment with cocaine (COCA) provokes anorexigenic effects, such as a diminished food-search behavior in animals and an appetite reduction in humans. Nevertheless, the majority of animal treatment protocols with COCA disregard the study of the abstinence periods, which are usually found in recreational use by humans. This work objective was to evaluate the food intake and body mass progression of animals submitted to a COCA chronic treatment that simulates the recreational use of this drug by humans. Forty male Wistar rats of ≈ 200 g were divided into 4 groups and submitted to 2 different protocols: a variable-dose administration protocol of COCA (VAR) and a continuous and fixed-dose administration protocol of COCA (CONT). Regarding VAR, 10 animals received variable doses of COCA (2 to 15 mg/kg, ip), and other 10 received saline (SAL), during 45 days, with alternating periods of abstinence and treatments, which could have from 2 to 7 days of COCA administration. The treatment period and established doses were determined by random numbers generated by an EXCEL spreadsheet. Regarding CONT, 10 animals received daily the average

dose used in VAR (4 mg/kg, ip) of COCA, during 45 days; and other 10 animals SAL. During the VAR treatment, the body mass was measured at the beginning and at the end of the abstinence or treatment periods. The body mass of the CONT group was measured weekly. The food intake was followed daily with the measurement of the initial ration mass minus the final ration mass. Regarding the VAR treatment, the COCA animals had an increased final food intake (COCA: 511.67 ± 25.39 g; SAL: 466.72 ± 15.28; $p \leq 0.0001$) and an enhanced body mass gain (COCA: Δ Body Mass 305 ± 36.06 g; SAL: 277.4 ± 24.86; $p \leq 0.010$), when compared to SAL. Nevertheless, the COCA animals submitted to CONT expressed a decreased final food intake (COCA: 453.67 ± 17.93 g; SAL: 491.5 ± 22.37; $p \leq 0.0001$), when compared to SAL. There were no significant differences regarding the body mass gain. The increased food intake and the enhanced body mass gain, regarding the COCA treated animals of both protocols, unveiled the lack of the anorexigenic effect classically observed in cocaine chronic treatments. The occurrence of intermittent abstinence periods and variable-dose administrations may be responsible for the plastic changes in the catecholaminergic systems related to the food intake control. Since the variable-dose treatment protocol with cocaine was developed to simulate the recreational use standards of the drug by humans, these results indicate that the VAR protocol may be useful to the understanding of the neuroplastic phenomena found in the abstinence of psychostimulant drugs.

Disclosures: H.R. Silva: None. **P.S. Sanzovo:** None. **S.A. Barbosa:** None. **C. Lopes:** None. **C.A. Tieppo:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:871.14/U6

Topic: C.17. Drugs of Abuse and Addiction

Support: NCMHD Grant P20 MD003942

NHSN

Title: Methylphenidate exposure prior to adolescence (postnatal days 11-20) enhances cocaine reward in early, but not late adolescent rats

Authors: C. B. SHIN, R. FLORES, D. SAVEAU, D. F. COLLIN, J. GIANG, *A. R. ZAVALA
California State Univ., LONG BEACH, CA

Abstract: Methylphenidate is the most prescribed treatment for attention deficit hyperactivity disorder. Surprisingly, little research has been done examining the long-term effects of this drug, particularly when it is prescribed to preschool age children. In rats, exposure to methylphenidate early in development has been shown to produce long-lasting effects on the sensitivity to cocaine when animals are tested as adults. However, the effects of methylphenidate exposure on the sensitivity to cocaine in adolescent rats have not been previously investigated. Using the conditioned place preference (CPP) paradigm, we examined the effects of early methylphenidate exposure on cocaine reward in early and late adolescent rats. Male and female Sprague-Dawley rats were treated twice daily with methylphenidate (0, 2, or 4 mg/kg) from postnatal days (PDs) 11-20. Rats were then assessed for cocaine-induced CPP (2.5 mg/kg) beginning on PD 27 (early adolescence) or PD 41 (late adolescence). A 6-day CPP procedure was used, which included 1 preconditioning day, 4 conditioning days, and 1 test day. Results showed that in males, early methylphenidate (2 mg/kg) enhanced cocaine-induced CPP when animals were tested during early adolescence (PD 34). In contrast, methylphenidate pretreatment did not affect cocaine-induced CPP when male rats were tested during late adolescence (PD 48). In females, early exposure to methylphenidate at either dose increased cocaine-induced CPP during early (PD 34), but not during late adolescence (PD 48). The present data adds to a growing body of evidence that early methylphenidate enhances the rewarding value of cocaine, particularly during early periods of development.

Disclosures: C.B. Shin: None. **R. Flores:** None. **D. Saveau:** None. **D.F. Collin:** None. **J. Giang:** None. **A.R. Zavala:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:871.15/U7

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA009079

Title: Sex and developmental differences in dopamine transporter and autoreceptor regulation of phasic dopamine in nucleus accumbens of rats.

Authors: *Q. D. WALKER, C. M. KUHN
Pharmacol. & Cancer Biol., Duke Univ. Med. Ctr., DURHAM, NC

Abstract: Illicit drug use and the development of addiction exhibit important sex- and age-specific differences. The rat may be a useful animal model to study biologic sex and age

differences in the dopamine system that might mediate these observed human patterns. We have shown that cocaine induces less behavioral stimulation and electrically-stimulated dopamine efflux in dorsal striatum in the adult male rat relative to adult females and adolescent male and female rats. The present studies investigated sex and age differences in spontaneous dopamine transients and tonic dopamine levels in nucleus accumbens shell using cyclic voltammetry in prepubertal (PN28) and adult (PN70+) urethane-anesthetized rats. Basal transient frequency and duration were greater in adult than adolescent accumbens shell, while amplitude trended higher in females than males. Injection of 15 mg/kg cocaine i.p. increased transient frequency and amplitude more in adolescent than adult animals and adult males were less affected than females. The D2 antagonist, 2 mg/kg raclopride s.c., increased dopamine transients most in adult males and least in adult females. Cocaine enhanced phasic dopamine more rapidly in adolescents than adults. Simultaneous change in tonic dopamine over 15 or 30 min following systemic drug administration was determined by principal components regression. Cocaine increased extracellular dopamine more in both groups of adolescents than adults (1 µM vs 0.5 µM) and acute raclopride induced the biggest increase in adult males. Interestingly, raclopride pretreatment tripled the tonic dopamine increase by cocaine in all groups except adult males, where little enhancement was observed. Tonic increases induced by cocaine were also more rapid in adolescents. The greater cocaine-stimulated motor effects in female than male adult rats were better reflected in the present robust sex differences in cocaine effects on phasic dopamine. D2 autoreceptor regulation of dopamine transients increased during development in males but decreased in females, indicating sex-specific development of autoreceptor regulation. The present results showed that spontaneous dopamine release events mirrored, and suggest that they may mediate (at least partly), the observed sex and age differences in the behavioral effects of cocaine in rats.

Disclosures: Q.D. Walker: None. **C.M. Kuhn:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:871.16/U8

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA25674

Title: Transgenerational epigenetic modifications in response to adolescent morphine exposure in female rats.

Authors: *E. M. BYRNES[1], N. L. JOHNSON[1], J.-E. OH[2], J. GAL-TOTH[4], N. CHAMBWE[3], F. CAMPAGNE[3], M. TOTH[2]
[1]Tufts Univ. Cummings Sch. Vet Med., North Grafton, MA; [2]Pharmacol., [3]Inst. for Computat. Biomedicine, Weill Cornell Med. Col., New York, NY; [4]The Rockefeller Univ., New York, NY

Abstract: Adolescent opiate use has increased over the past decade with unknown consequences on development during this critical period. For several years we have used the rat to identify transgenerational consequences of female adolescent opiate exposure with morphine serving as the prototypical opiate. These studies have revealed significant neural, endocrine and behavioral effects in the F1 generation of adolescent morphine exposed females (F0 MOR) when compared to the offspring of adolescent saline-exposed females (F0 SAL). It is important to note that all females are drug-free for several weeks prior to mating and thus direct effects of morphine on the developing fetus are not possible. Recent behavioral data observed alterations in anxiety-like behavior and significant disruption of object recognition in F1 MOR females. Moreover, these effects continued to be observed in F2 MOR females as well as in F1 MOR females that were reared by an untreated donor mother. These behavioral findings suggest that adolescent morphine exposure leads to epigenetic modifications that are maintained beyond the F1 generation. To begin to elucidate the mechanisms underlying these transgenerational effects, methylation patterns were determine in the dorsal CA1 region of the hippocampus, a region that regulates both anxiety-like behavior and recognition memory. Differential methylation at CpG sites in dorsal CA1 neurons was determined by "reduced representation bisulfate sequencing (RRBS)" comparing adult MOR and SAL female offspring from both the F1 and F2 generation. We profiled approximately 2 million CpG sites at 35x coverage (approx. 10% of all CpG sites in the mouse genome). We found significant methylation changes (BH corrected p<0.05 and delta methylation rate >20%) at approx. 100K sites in F1 MOR and at approx. 30K sites in F2 MOR samples. This indicates a substantial genome-wide effect of adolescent morphine in the F1 offspring that can reach the F2 generation. Importantly, there was a significant overlap between the F1 and F2 changes (at approx. 6K sites) suggesting that a fraction of the F1 methylation changes are reproduced across generations. Co-segregation of these methylation changes with the behavioral changes may indicate their role in the transgenerational effect of adolescent MOR.

Disclosures: E.M. Byrnes: None. **N.L. Johnson:** None. **J. Oh:** None. **J. Gal-Toth:** None. **N. Chambwe:** None. **F. Campagne:** None. **M. Toth:** None.

Poster

871. Cocaine, Amphetamine and Related Drugs: Developmental Effects

Location: Hall F-J

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA07432

NIH Grant R01 DA021059

NIH Grant R03 DA0291113

NIDA IRP

Title: Response inhibition and Brodmann Area 45 volume in adolescents with prenatal drug exposure.

Authors: *K. A. CACIC[1,2], B. J. SALMERON[1], T. RIGGINS[2], S. BUCKINGHAM-HOWES[3], L. SCALETTI[3], T. J. ROSS[1], M. M. BLACK[3]
[1]NeuroImaging Res. Br., Natl. Inst. On Drug Abuse, Baltimore, MD; [2]Univ. of Maryland, College Park, MD; [3]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The objective of the present study was to examine the influence of prenatal drug exposure on cortical frontal volumes involved in cognitive performance in a well-characterized sample of 51 adolescents (27 with a history of PDE and 24 from a comparison group of community controls (CC); mean age = 14 years). Inclusion criteria for the PDE group included a positive maternal or infant toxicology screen at time of birth for cocaine and/or heroin or reported use of either substance during the last 6 months of pregnancy. PDE participants were recruited at birth while CC participants were born at the same hospital and recruited from the same primary care clinic in which all participants were followed. The groups were well-matched on socioeconomic measures. Data was collected on a 3-T Siemens Allegra scanner with whole-brain oblique axial T1-weighted images (1-mm3 isotropic voxels: TR=2.5 s, TE=4.38 ms, FA = 80°). Cortical reconstruction and volumetric segmentation were completed in Freesurfer. Initial analysis with total cortical gray matter as the only covariate revealed that bilateral BA 45 differed between groups such that PDE adolescents had smaller volumes than CC adolescents. The BA 45 has previously shown involvement in response inhibition and cognitive control tasks. Therefore, differences in Continuous Performance Task (CPT) performance were examined in conjunction with the volumetric difference. Results revealed adolescents with a history of PDE had a “riskier” response style than controls on a CPT response inhibition task (tended towards more errors of commission than omission). Further analyses considered: age at time of scan, gender, IQ, prenatal exposure to alcohol and tobacco, indices of early childhood environment (i.e. caregiver depression, primary caregiver, caregiver changes through age 7) and primary caregiver at the time of assessment. After multiple covariates adjustment, significant differences remained between right BA 45 volumes and CPT response style. Furthermore, right BA 45 volume was positively correlated with CPT response style such that larger volumes were associated with higher response style scores, indicating progressively “safer” response styles.

These findings provide support for long-terms effects of PDE on response inhibition and point to neural mechanisms that may underlie these outcomes.

Disclosures: K.A. Cacic: None. **B.J. Salmeron:** None. **T. Riggins:** None. **S. Buckingham-Howes:** None. **L. Scaletti:** None. **T.J. Ross:** None. **M.M. Black:** None.

Poster

872. Opioid Reward and Addiction

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:872.01/U10

Topic: C.17. Drugs of Abuse and Addiction

Support: DOH98-TD-I-111-DD004 (R-BL)

Title: Dextromethorphan attenuate inflammatory and dependence in opioid dependent patients

Authors: *S. CHEN[1], S. LEE[2], M. WEN[2], R. LU[2]
[1]Departmen of Psychiatry, Natl. Cheng Kung Univ., Tainan, Taiwan; [2]Psychiatry, Col. of Med. & Hospital, Natl. Cheng-Kung Univ., Tainan, Taiwan

Abstract: Abstract Recent studies show that overactive glia cells and their secretions, especially proinflammatory cytokines, might be related to the development of opioid dependence. We evaluated whether the inflammatory process is related to the progression of opioid dependence. A double-blind, randomly stratified clinical trial to investigate whether add-on dextromethorphan (DM, 60-120 mg/day), attenuated inflammation and dependence in opioid abused patients with methadone maintenance therapy (MMT). All participants (107 opioid dependent patients and 113 nondependent healthy controls) were recruited from National Cheng Kung University Hospital. Their plasma cytokine levels and opioid concentration were measured to evaluate the effect of add-on DM. Plasma TNF-α and IL-8 levels were significantly higher in long-term opioid dependent patients than in healthy controls ($p < 0.001$). We also found that the plasma cytokines levels were significantly positive correlated to the heroin abused years, which indicates the possibility of an inflammatory process after long-term opioid use. These patients were then accepted for the MMT combined with placebo or DM treatment. After 12 weeks of treatment, plasma cytokine TNF-α and IL-8 levels were significantly attenuated in both the DM 60 mg and the DM 120 mg group ($p < 0.05$), but not in the placebo group. In addition, the methadone oral dose in the placebo group gradually increased from 100% to 117 ± 9.9% at 12 weeks, which suggests that MMT patients become tolerant to methadone when it is used alone. The methadone doses in the DM 60 mg and DM 120 mg groups, however, decreased from 100% to 91.6±5.2% and 93.2±5.3%, respectively, at 12 weeks. Moreover, analyses of urinary and plasma morphine

levels showed that the combined used of opioid was worsened in the placebo group, but that it did attenuate in the two add-on DM groups. These results suggested that dextromethorphan may be used to attenuate inflammatory process, inhibit tolerance of methadone and opioid abuse in opioid dependent patients.

Disclosures: S. Chen: None. **S. Lee:** None. **M. Wen:** None. **R. Lu:** None.

Poster

872. Opioid Reward and Addiction

Location: Hall F-J

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Program#/Poster#:872.02/U11

Topic: C.17. Drugs of Abuse and Addiction

Support: DA023069

DA027541

Title: AMPA receptors in central amygdala mediate pain-induced suppression of opioid reward

Authors: *Y.-Y. HOU, Z. ZHANG, Y.-Q. CAI, Y. XIA, W. WANG, Z. PAN
Pain Med., MD Aderson Cancer Ctr., Houston, TX

Abstract: Pain and reward have opposite emotional, motivational effects and functionally influence each other. Opioids are powerful pain-relieving drugs that also have strong rewarding effects for opioid addiction, which limit their optimal prescription use for control of chronic pain. The neurobiological mechanisms underlying functional interactions between chronic pain and opioid reward are unclear. We examined the role of GluR1 and GluR2 subunits of AMPA receptors in the central nucleus of the amygdala (CeA) in regulation of pain impact on the rewarding properties of opioids. We found that CFA-induced persistent pain time-dependently decreased morphine craving in rats self-administrating morphine. Pain relief by pretreatment with indomethacin, a non-steroidal anti-inflammatory drug, largely reversed CFA-induced suppression of morphine intake. Western blotting revealed that morphine self-administration, but not passive morphine injection, increased synaptic GluR1 level and decreased synaptic GluR2 level in the CeA, whereas total GluR1 and GluR2 protein levels were unchanged, indicating morphine self-administration-induced membrane trafficking of GluR2-lacking AMPA receptors. In contrast, CFA-induced pain elevated both synaptic GluR1 and GluR2 levels in saline control rats. However, in morphine self-administrating rats, CFA-induced pain significantly decreased both GluR1 and GluR2 levels in the CeA, which was reversed by indomethacin pretreatment. These data indicate that GluR2-lacking AMPA receptors in the CeA play an important role in

mediating opioid reward and the impact of persistent pain on opioid reward may depend on the emotional state and the time course of pain development through regulation of relative synaptic expression of CeA GluR1 subunits.

Disclosures: Y. Hou: None. **Z. Zhang:** None. **Y. Cai:** None. **Y. Xia:** None. **W. Wang:** None. **Z. Pan:** None.

Poster

872. Opioid Reward and Addiction

Location: Hall F-J

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Program#/Poster#:872.03/U12

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R01 DA025036

Title: NMDA receptors in the hippocampus underly morphine-induced alterations in synaptic plasticity.

Authors: *A. K. FAKIRA, G. S. PORTUGAL, B. CARUSILLO, Z. MEYLAN, J. MORON CONCEPCION
Anesthesiol., Columbia Univ. Med. Ctr., New York, NY

Abstract: Previous studies in our laboratory demonstrate that escalating doses of morphine paired with a novel context produced robust locomotor sensitization and impaired long term potentiation (LTP) in the hippocampus. Whereas behavioral sensitization was attenuated by mutating Ca2+ permeable AMPA receptors in the hippocampus LTP remained impaired. Ca2+ permeable NMDA receptors, composed of NR2A, NR2B and NR1 subunits, are negatively regulated by Ca2+-activated small-conductance potassium channels (SK channels) and modulation of this feedback loop can affect long-term potentiation (LTP). In this study we examined the role NMDA receptors (NMDAR) and SK2 channels in the mechanisms underlying morphine-induced alterations in synaptic plasticity. Mice displaying behavioral sensitization to escalating doses of morphine (5, 8, 10 and 15 mg/kg) after receiving a morphine challenge (5mg/kg; Mor + Mor) one week later demonstrated increased NR2A subunit trafficking to the hippocampal PSD and whole cell patch-clamp recordings from CA1 pyramidal cells showed that sensitivity of EPSCs to NR2A subunit blocker NVP is increased suggesting an increase in NR2A containing NMDA receptors. Moreover, sensitivity to NR2B blocker Ro 25-6981 is decreased in morphine treated animals suggesting morphine-induced changes in NMDAR composition. In addition to alterations in NMDARs, expression of SK2 channels is increased in the hippocampus of Mor + Mor mice. Furthermore, bath application of SK channel blocker apamin produced a

higher increase of NMDAR-EPSCs in Mor + Mor group compared to Sal + Mor, indicating enhanced activation of SK-channel-NMDAR feedback loop in morphine treated animals. Finally, we found that apamin application can restore the ability of CA1 cells to produce LTP.
Our data suggest that morphine-induced changes in NMDAR composition can affect SK channel-NMDAR loop leading to down-regulation of LTP. Therefore, we provide an evidence for a new key player to the mechanism of impaired synaptic plasticity after morphine-induced sensitization.

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Poster

872. Opioid Reward and Addiction

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Program#/Poster#:872.04/U13

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA09082

Title: Morphine effects on opioid, cannabinoid, and stress-related receptors in the locus coeruleus

Authors: *K. M. JAREMKO[1], B. A. S. REYES[1], A. S. MENKO[2], E. J. VAN BOCKSTAELE[1]
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Abstract: Opiate addiction contributes to the 193 billion dollars spent yearly in the United States on drug abuse, with approximately 2 million people currently addicted to heroin or non-medical prescription opiates. Chronic stress is known to negatively impact addiction recovery and the interaction between the stress axis and opioid circuitry has been demonstrated in the locus coeruleus (LC). The LC serves as the primary source of norepinephrine (NE) with diffuse projections throughout the brain and is reciprocally regulated by endogenous opioids and the stress-related neuropeptide, corticotropin releasing factor (CRF). Previous work has shown that chronic morphine sensitizes the LC to the stimulatory effects of CRF (Xu et al., J Neurosci, 24: 8193-8197, 2004). We previously showed that the mu-opioid receptor (MOR) co-localizes with LC dendrites that contain the CRF receptor (CRFr) (Xu et al., J Neurosci, 24: 8193-8197, 2004). Additionally we have shown that the cannabinoid-1 receptor (CB1r) is found within MOR labeled somatodendritic profiles, as well as within axon terminals contacting MOR dendrites in the LC (Scavone et al., Brain Research 1312**:** 18-31, 2010). The LC is uniquely poised to be a

site of convergent actions of opioid, cannabinoid, and stress pathways that modulate NE output. In the present study, we investigated protein expression levels for MOR, phosphorylated MOR, CB1r, and CRFr in the LC following acute exposure to morphine. Male Sprague Dawley rats were injected systemically with morphine or saline and euthanized following a period of 1 hour. Western blot analysis revealed no significant difference in expression levels for CRFr, MOR or phosphorylated MOR following acute exposure to morphine in LC extracts when compared to control. In a separate cohort, rats were implanted with morphine or placebo pellets for 7 days and withdrawal was elicited by subcutaneous injection of naltrexone, a MOR antagonist. Co-immunoprecipitation analysis of MOR and CB1r in LC extracts following withdrawal revealed an unexpected association between the two receptors in rats subjected to antagonist precipitated withdrawal when compared to control. Investigating the intersection between opioid, cannabinoid, and stress signaling systems in the LC after morphine exposure may uncover broader circuit-based interactions that impact noradrenergic signaling and potentially elucidate novel targets for the treatment of opiate addiction.

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Poster

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Support: National Basic Research Program of China (no. 2009CB522000)

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National Natural Science Foundation of China (no. 81071079)

Title: Effects of ZNF804A genetic polymorphism on neurocognitive performances and gray matter volume in heroin addicts

Authors: *J. SHI[1], Y. SUN[1], L. Y. ZHAO[1], L. LU[1], G. B. WANG[1], J. L. LI[1], N. CHEN[1], L. L. SUN[1], W. H. YUE[2], Y. HE[3], N. SHU[3], J. WANG[3], Y. D. TANG[4], S. X. LIU[4], G. F. DENG[5], J. H. DIAO[5], F. WANG[1]
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Abstract: Evidence has shown that genetic factors may interact with environmental modulators to promote heroin addiction and relapse after withdrawal. ZNF804A (zinc finger protein 804A) rs1344706 is a schizophrenia risk gene that was first identified by genome-wide association studies and associated with a large set of psychiatric disorders. Using structural magnetic resonance imaging and behavioral tasks, we investigated the effect of a ZNF804A rs1344706 polymorphism on gray matter volume and neurocognitive performance in heroin addicts and healthy controls. We found a significantly high frequency of the T allele in heroin addicts. Heroin addicts showed impaired decision-making on the Iowa Gambling Task, and the impairment was aggravated as the T allele dose increased. Brain imaging data were based on a subset of 76 heroin addicts and 78 healthy controls. The significant main effects of ZNF804A indicated that subjects who carried the T allele had larger gray matter volume in the temporal gyrus, parietal gyrus and frontal-insular cortex. Furthermore, a significant interaction between heroin addiction and genotype showed that gray matter volume in the dorsolateral prefrontal cortex exhibited a U-shaped curve as the T allele increased in addicts, but opposite changes were observed in controls. Overall, these findings might reflect differential genotypic effects of ZNF804A on brain morphology and neurocognitive performance in heroin addicts and healthy controls, suggesting a possible role of brain morphology as an endophenotype for future genetic studies on heroin addiction.

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Poster

872. Opioid Reward and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: USPHS Award DA026451

USPHS Award MH073930

Title: Knockdown mu-opioid receptors in the ventral tegmental area: Critical effect on BDNF expression after intermittent social defeat stress

Authors: *C. E. JOHNSTON[1], A. W. LASEK[2], U. HEBERLEIN[3], R. P. HAMMER, Jr.[1,4], E. M. NIKULINA[4]
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Abstract: Intermittent social defeat stress produces an upregulation of mu opioid receptors (MOR) in the ventral tegmental area (VTA). This phenomenon also results in increased social avoidance and augmented locomotor responses, or sensitization, following amphetamine challenge. Research has shown that intermittent social defeat stress produces a prolonged induction of brain-derived neurotrophic factor (BDNF) in the VTA. Increased VTA BDNF expression has been tied to both enhanced susceptibility to stress-related behaviors and locomotor response to stimulants. Recently we have shown that MOR knockdown in the VTA is sufficient to prevent social defeat stress-induced amphetamine sensitization. In this study, we hypothesize that VTA MOR knockdown prevents social defeat stress-induced changes in the expression of BDNF. To test our hypothesis, lentivirus-mediated gene transfer and RNA interference were used to produce persistent depletion of MORs in the VTA of adult male Sprague Dawley rats. Bilateral infusions of either lentivirus-GFP-shRNA targeting MORs or a viral construct expressing a scrambled control sequence were made to the VTA. Starting one week later, social defeat stress and control handling procedures were administered four times in 10 days; social defeat stress was induced by a short confrontation with an aggressive male rat. Seven days after the last episode of social defeat stress, rats received an amphetamine challenge (1.0 mg/kg, i.p.) and their locomotor activity was measured. Brains were collected 10 days after amphetamine challenge. Immunohistochemistry was performed to quantify BDNF expression in the VTA. Tissue punches were taken for Western blot analyses in the nucleus accumbens (NAc) and prefrontal cortex. VTA MOR knockdown significantly reduced amphetamine cross-sensitization, which is consistent with our previous findings. BDNF expression increased significantly in the VTA after repeated social defeat stress in rats that received infusions of the scrambled control virus, however BDNF induction was prevented by intra-VTA MOR depletion. Western blots revealed a trend toward increased NAc BDNF expression in animals with intact MORs only after social defeat. There were no differences in BDNF levels in the prefrontal cortex. In conclusion, VTA MOR depletion was sufficient to prevent social defeat-induced increases of BDNF. Thus, we suggest that the VTA MORs serve as the starting point for the persistent effects of intermittent social defeat stress mediated by BDNF. These findings may have implications for stress-induced neuroplasticity and its relationship to substance abuse and mental disorders.

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Poster

872. Opioid Reward and Addiction

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Support: R01DA025036

R00DA025182

Title: Morphine conditioned place preference alters basal synaptic transmission in the hippocampus and amygdala and is associated with decreased synaptic NMDA receptor subunit expression.

Authors: *G. S. PORTUGAL[1], R. HASANI[2], M. R. BRUCHAS[2], J. A. MORON CONCEPCION[1]
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Abstract: Associations between the rewarding properties of morphine and contextual cues can lead to cravings and increased drug-seeking behavior, which in turn may promote continued use and facilitate relapse. Glutamatergic systems, including AMPA and NMDA receptors, may play an important role in morphine-induced drug-seeking behavior such as morphine conditioned place preference (CPP), and we have previously demonstrated that context-dependent morphine treatment produces changes in AMPA receptor expression and synaptic plasticity in the hippocampus. In the present study, we used immunoblotting, electrophysiological, and behavioral techniques to investigate the effects of repeated morphine administration on AMPA and NMDA receptor expression, synaptic plasticity, and morphine CPP. Repeated morphine treatment produced a conditioned place preference for morphine when mice were tested 24 hours or one week following conditioning. This behavioral effect was associated with changes in basal synaptic transmission in the CA1 region of the hippocampus and the central nucleus of the amygdala (CeA), and impaired long-term potentiation in the hippocampus. Furthermore, preliminary results suggest that morphine CPP is associated with reduced synaptic expression of NMDA receptor subunits (NR1, NR2a, and NR2b) and additional experiments will examine alterations of AMPA and NMDA expression in the hippocampus and amygdala. These data suggest that glutamatergic synaptic transmission in the amygdala and hippocampus may play an important role in the acquisition and/or expression of morphine CPP, and future experiments will use optogenetic techniques to investigate functional interactions between the hippocampus and amygdala.
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Poster

872. Opioid Reward and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: MH063266

Title: Development and characterization of kappa-opioid receptor (KOR) mutant mice

Authors: A. J. BECHTHOLT-GOMPF[1], A. VAN'T VEER[1], S. ONVANI[1], D. POTTER[1], Y. WANG[2], E. H. CHARTOFF[1], U. RUDOLPH[1], L.-Y. LIU-CHEN[2], *B. M. COHEN[1], W. A. CARLEZON, Jr[1]
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Abstract: Accumulating evidence indicates that kappa-opioid receptor (KOR) antagonists have antidepressant- and anxiolytic-like effects in animal models of psychiatric illness. However, prototypical KOR antagonists have unusual pharmacological properties, including a delayed and exceptionally long duration of action, that complicate data interpretation. To further evaluate the role of KORs in complex behavior, we generated KOR-mutant mice. Our mutant lines offer some advantages over those described previously because they enable (1) constitutive ablation of KORs or (2) inducible ablation in a time and region-specific manner. We flanked exon 3 (encoding transmembrane regions 2-4) of the KOR gene (Oprk1) with loxP sites. Protein lacking this region is non-functional and the excision shifts the open reading frame, ensuring that full-length protein is not made. Balb/c blastocysts were injected with IC1 C57BL/6 embryonic stem cells transfected with the targeting vector (inGenious Targeting Laboratory). After breeding out the Neo cassette (using FLP-FRT recombination) and the FLP transgene, we backcrossed the Oprk1-floxed mice (Oprk1-F/F) to C57BL/6J to congenicity (>10 generations). We then crossed these mice with mice expressing Cre recombinase ubiquitously (EIIa-Cre) and mice expressing Cre in dopamine transporter containing cells (DAT-Cre). This approach enabled us to generate global knockout mice (KOR-KO) and mice lacking KOR in DAT expressing cells (DAT/KOR-KO). We verified ablation using radioligand binding autoradiography and qPCR. As another method of verification, both lines were tested in the tail flick assay, which is sensitive to opioid-related manipulations. KOR-KO but not DAT/KOR-KO mice had decreased sensitivity to

a KOR agonist. We also tested their suitability for more complex behavioral tests by measuring visual acuity in the visual cliff assay, hearing in an acoustic startle paradigm, baseline locomotor behavior, and body weight from weaning to adulthood. KOR-KO and DAT/KOR-KO mice were normal on these measures compared to their respective wildtype (WT) littermates. Because KORs are strongly implicated in addiction, we tested both lines for sensitivity to cocaine in conditioned place preference (CPP) and locomotor sensitization tests. Although KOR ablation had no effect on cocaine-induced CPP, DAT/KOR-KO but not KOR-KO mice showed increased sensitization compared to WT controls. Our data validate our ablation technique and suggest that KORs localized on DAT-expressing neurons may normally inhibit some types of cocaine-induced plasticity.

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Poster

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Title: ^{1}H-mrs analysis of neurochemical response after acute treatment with the μ -opioid agonist fentanyl in mice lacking adenylyl cyclase isoforms 1 and 8

Authors: *F. GHODDOUSSI[1], N. SAWAF[2], J. LOWING[3], L. SUSICK[3], A. C. CONTI[3], M. P. GALLOWAY[1]
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Abstract: Introduction: G protein coupled receptors can modulate at least 8 isoforms of adenylyl cyclases (AC) and 2 of these isoforms (AC1, AC8) are uniquely stimulated by calcium-calmodulin. Mice with a genetic deficit in AC1/8 (DKO) show an impaired behavioral response to repeated cocaine (DiRocco *et al.* 2009), methamphetamine, or morphine (Li *et al.* 2006; Zachariou *et al.* 2008). The DKO also shows prolonged ethanol-induced sedation or increased behavioral sensitivity at high doses. The fetal DKO in comparison to WT also shows increased striatal apoptosis in response to ethanol (Conti *et al.* 2009, 2010). Using ^{1}H-MRS, we investigated the acute neurochemical response to fentanyl, a potent μ-opioid agonist, in the striatum of the DKO mice.
Methods: WT and AC1/8 null mice, generated as previously described (Conti *et al.* 2009) were treated with fentanyl (25 μg/kg sc) or saline (4groups, n=6 in each group), then sacrificed 45 min later. Brains were rapidly removed, 2 mm coronal slices obtained and 1.5 mm diameter punches (3-4 mg)containing a region of interest were analyzed with magic angle spinning HR-MAS ^{1}H-MRS on an 11.7T Bruker magnet.
Results: In Rostral Medial Dorsal Striatum, in the DKO, but not WT, fentanyl decreased GABA (-26%) and glutamate (GLU, -22%) Additionally, significant decreases were noted in creatine(-17%), lactate(-25%), N-acetylaspartate(-26%) and taurine(-23%). Glutamine/GLU was increased in the DKO after Fentanyl in the same region.
Discussion: The striatal GABA and GLU response to the μ opioid agonist Fentanyl in AC1/8 null mice may be related to some of the behavioral phenotypes (e.g. hypo-activity in males) by DKO mice (described by Krishnan et al. 2008). Decreased GABA is also consistent with a key role for an AC1/8 mediated pathway regulating GABA synthesis. Moreover, our observations may be a prelude to deleterious consequences since AC1/8 are thought to provide a protective effect on synaptic homeostasis (Conti *et al.* 2010). Alternatively, decreased GABA and GLU may reflect the action of inhibitory μ opioid heteroreceptors on cortico-striatal GLU terminals or recurrent collaterals of GABA medium spiny neurons.

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Poster

872. Opioid Reward and Addiction

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Title: Role of the kappa opioid system in the modulation of CeA GABAergic transmission in rats following extended access to cocaine self-administration

Authors: *M. KALLUPI[1,2], S. WEE[2], S. EDWARDS[2], T. WHITFIELD[2], C. S. OLEATA[2], G. LUU[2], G. F. KOOB[2], M. ROBERTO[2]
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Abstract: It is well established that the kappa opioid system is recruited during cocaine withdrawal and is implicated in multiple withdrawal-induced anxiety-related behaviors including dysphoria and stress-induced relapse to compulsive cocaine seeking. However, little information is available about the direct interaction between the kappa opioid system and GABAergic neurotransmission in cocaine self-administering animals. Studies in our lab and others have demonstrated a keyrole of the central amygdala (CeA) in addiction. Here, using in vitro electrophysiological technique, we investigated the effects of acute cocaine and kappa opioid receptor (KOR) ligands on the GABAergic transmission in CeA of rats following limited (non compulsive ShA, 1 h/d) or extended (compulsive LgA, 6 h/d) access to cocaine self-administration. Pharmacological studies in animals with intra-CeA cannula were also employed. We found that 1) baseline evoked GABAergic transmission was enhanced in the CeA from both ShA and LgA rats compared to cocaine-naïve rats, 2) Acute cocaine application significantly decreased GABAergic responses via decreasing GABA release in both naïve and LgA rats 3) U50488, a KOR agonist, significantly decreased CeA GABAergic transmission in naïve rats, but increased their amplitudes in LgA rats, 4) nor-BNI, a KOR antagonist, significantly increased CeA GABAergic responses in naïve rats, but decreased their amplitudes in LgA rats, 5) CeA microinfusions of nor-BNI blocked cocaine-induced locomotor sensitization and 6) attenuated the heightened anxiety-like behavior observed during withdrawal from chronic cocaine exposure in the defensive burying paradigm. Our combined findings demonstrate important neuroadaptive changes induced by cocaine on the GABAergic system within the CeA and the critical role of the kappa system in cocaine sensitization and withdrawal-related behaviors. These results suggest that cocaine dynamically regulates dynorphin KOR signaling in the CeA of rats, and provides evidence that the CeA may be a key brain region in mediating the anxiolytic-like effects of KOR antagonists. Finally, the CeA kappa system may also play a role in the incentive motivational properties of cocaine.

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Poster

872. Opioid Reward and Addiction

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Program#/Poster#:872.11/U20

Topic: F.02. Animal Cognition and Behavior

Title: Cocaine- and morphine-induced conditioned analgesia in preweanling rats

Authors: *D. E. HUMPHREY, C. M. MARTINEZ, G. J. KAPLAN, K. L. WHITTENBURG, C. M. NUQUI, S. A. MCDOUGALL
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Abstract: Mu-opioid agonists (e.g., morphine) and indirect dopamine agonists (e.g., cocaine) produce unconditioned analgesia in preweanling and adult rats. Various research groups have shown that this analgesia is mediated by both spinal and supraspinal mechanisms. When an odor cue is used, morphine will also induce conditioned analgesia in adult rats. In this paradigm, rats are repeatedly treated with saline or morphine (i.e., the unconditioned stimulus, US) in the presence of a novel odor (i.e., the conditioned stimulus, CS). On the test day, rats are exposed to the CS with no drug being administered. The presence of an analgesic response is then assessed using a standard antinociceptive test. Although it is well established that adult rats show conditioned analgesia after multiple odor-morphine pairings, it is unclear whether cocaine will induce conditioned analgesia or if this phenomenon is even evident during early ontogeny. In Experiment 1, the unconditioned analgesic effects of acutely administered cocaine (15 or 30 mg/kg, ip) and morphine (20 mg/kg, ip) were measured using a hot plate task on postnatal day (PD) 20. In Experiment 2, cocaine-induced conditioned analgesia was assessed after either one (PD 19) or four (PD 16-PD 19) conditioning days. During the conditioning phase, rats were placed in a chamber and exposed to the compound odor (i.e., the CS) of pine bedding sprinkled with 1.5 ml of banana extract. After 15 min, rats were injected with saline or cocaine (15 or 30 mg/kg) and exposed to the odor for an additional 30 min. Rats were then returned to the home cage. On the test day, which occurred 24 h later (i.e., on PD 20), all rats were injected with saline and exposed to the odor CS for 30 min. Rats were then taken to a separate room and tested on a hot plate, with paw-lick latencies and avoidance responses being recorded. Experiment 3 was conducted as just described, with the exception that saline or morphine (20 mg/kg) were injected on the conditioning days. Results showed that acutely administered cocaine (15 or 30 mg/kg) and morphine (20 mg/kg) produced unconditioned analgesia on PD 20. Morphine-induced conditioned analgesia was also evident, because rats given odor-morphine pairings on PD 19 or PD 16-PD 19 had longer paw-lick latencies than rats given odor-saline pairings. Similarly,

cocaine (15 or 30 mg/kg) produced conditioned analgesia on PD 20, and this effect occurred regardless of whether one or four odor-cocaine pairings were provided. In conclusion, rats are capable of showing cocaine- and morphine-induced conditioned analgesia during early ontogeny, with these effects being evident after a single odor-drug pairing.

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Poster

872. Opioid Reward and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: 1P01 DA031656-01 (F030645) to BJA

Title: Novel characterization of dopamine neurotransmission following oxycodone and morphine as determined by multiple *in vivo* measurement technologies

Authors: *C. M. VANDER WEELE[1], O. S. MABROUK[2], K. A. PORTER-STANSKY[1], B. E. COUTURIER[1], V. LOVIC[1], R. T. KENNEDY[2], B. J. ARAGONA[1]
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Abstract: In vivo electrochemical recordings, using fast-scan cyclic voltammetry (FSCV) to measure sub-second changes in dopamine (DA) concentration in response to intravenous opiate administration, have revealed interesting differences across drugs. Oxycodone, an opiate that acts at mu-opioid receptors, elicits the predicted increase in DA transmission within the nucleus accumbens (NAc), by robustly increasing DA concentration, transient frequency, and transient amplitude; and DA levels remain elevated for the duration of the recording period. However, morphine (another opiate and mu-opioid receptor agonist) causes changes in DA transmission that are more complex and unexpected. Within ~ 5 seconds, morphine caused an increase in DA concentration in the NAc core and shell that was quite high during the first minute; however, after this initial increase, DA concentration decreased to baseline levels (or below) soon after the infusion. Furthermore, DA concentration remained at, or below basal levels for the duration of the 15-minute recording period. This was unexpected considering previous studies using microdialysis have found that morphine generally increases DA in the NAc. However, these early microdialysis studies used longer sampling intervals. To further explore differences in DA transmission between these opiates, we utilized microdialysis with rapid sampling periods (60 s samples) coupled to novel LC-MS technology for dialysate analyses. Doses that caused similar

initial increases in overall DA concentration, as determined by our FSCV studies, were chosen (i.v.; morphine, 0.5mg/kg; oxycodone, 0.5 mg/kg). When DA measurements were analyzed with similar time averaging, the results from fast microdialysis were consistent with FSCV findings in that oxycodone elicits a rapid, long-lasting increase in DA, whereas morphine increases dopamine concentration briefly before returning to basal levels. The longer term microdialysis recording also revealed a second peak in DA concentration ~ 20 to 40 minutes post-infusion. Currently we are using FSCV to measure for a longer period of time post-morphine infusion to determine if we can detect the second DA peak revealed by microdialysis. Further, the LC-MS technology used to analyze dialysates allows analysis of additional neurochemicals that are currently being analyzed. Thus, converging evidence from two in vivo recording techniques with fast temporal resolution have revealed previously unresolved changes in DA transmission dynamics caused by morphine and demonstrates that complementary in vivo technology can powerfully address novel characterization of neurotransmission dynamics.

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Poster

872. Opioid Reward and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

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Title: *In vitro* identification of carbon-fiber micro-electrodes with sensitivity sufficient for voltammetric detection of dopamine transients *in vivo*

Authors: *B. E. COUTURIER, C. M. VANDER WEELE, B. T. SAUNDERS, B. J. ARAGONA
Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Fast-scan cyclic voltammetry (FSCV) utilizes carbon-fiber micro-electrodes to measure phasic changes in dopamine (DA) concentration in freely moving animals. In traditional FSCV experiments, phasic DA release has been evoked by electrical stimulation or, more recently, by presentation of rewards or reward predictive cues. Advances in this technology have now made it possible to detect naturally occurring changes in DA concentration in freely moving animals that have no obvious environmental cause. We refer to these surges in DA concentration as DA ‘transients’. Among the important aspects of detecting DA transients is that when one

uses an electrode sensitive enough to detect transients during the baseline recording period of an experiment, higher quality, more reliable data are collected. One technical difficulty currently in the FSCV field is that there is no standard in vitro method to quantify an electrode's sensitivity that will predict its ability to measure in vivo transients. Electrodes that detect signals with an equal signal to noise ratio in response to DA concentrations within the 1μM range (either in vitro or in vivo) are not always capable of detecting DA transients during in vivo recordings, which have an average concentration value of approximately 40 nM. Therefore, we hypothesize that in vitro pre-calibration of electrodes at low nanomolar concentrations (i.e., within the range of DA transients) will increase the ability to identify electrodes capable of detecting transients in vivo. Our preliminary data show that we can indeed separate electrodes into two sub-populations (based on their flow cell performance) that show equal signal to noise at 1μM DA concentration but differ significantly in these measures at lower DA concentrations. Our next step is to compare how electrodes from these two different populations perform in awake, freely moving rats, to test the hypothesis that electrodes with excellent performance at low concentrations at the pre-calibration stage will perform better in in vivo testing conditions. This will identify a 'performance threshold' at a low DA concentration (e.g., 50 to 150 nM) that will allow us to identify electrodes that are more likely to detect transients in vivo, thus greatly improving the efficiency of FSCV experiments.

Disclosures: B.E. Couturier: None. **C.M. Vander Weele:** None. **B.T. Saunders:** None. **B.J. Aragona:** None.

Poster

873. Addiction and Genetics

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:873.01/V3

Topic: C.17. Drugs of Abuse and Addiction

Support: Intramural NIH (NIDA)

Title: Cadherin 13, addiction and related phenotypes: Human studies

Authors: *G. R. UHL, D. WALTHER
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Abstract: Cadherin 13 (CDH13) is a glycophosphoinositol-anchored cell adhesion molecule that is expressed by monoaminergic and cholinergic neurons implicated in addictions, in habenula regions implicated in aversive features of addictive substances and in other brain regions implicated in addiction. CDH13-CDH13 recognition can attenuate process outgrowth when

expressing neurons contact other expressing cells. Clusters of nearby single nucleotide polymorphism (SNP) markers are significantly (10-2 > p > 10-8) associated with a) dependence on at least one addictive substance (7 of 8 datasets; p > 10-11) and b) smoking cessation success (5 of 6 datasets; p > 10-8) and c) attention deficit hyperactivity disorder, a major addiction comorbidity that also influences smoking cessation success (Neale et al, 2010). Clusters of significantly-associated SNPs lie in several regions of the CDH13 gene, including its first and seventh introns. We have identified robust expression of CDH13 mRNA in cerebral cortical and hippocampal specimens from human postmortem brains. In initial samples, and in a replication sample, there was association between level of CDH13 expression and haplotypes marked by intron 1 CDH13 SNP markers that reached the margin of statistical significance. Though there were few homozygotes for the minor A allele of rs8056579, for example, there was about a 50% difference in CDH13 mRNA in cerebral cortex between AA and GG homozygotes, with heterozygotes falling intermediate. These data support the validity of mice with altered levels of CDH13 expression (see adjacent abstract) as models for some of the influences of common human CDH13 variants on drug responses relevant to addiction and smoking cessation. Support: NIH IRP (NIDA).

Disclosures: G.R. Uhl: None. **D. Walther:** None.

Poster

873. Addiction and Genetics

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:873.02/V4

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH HD25938

Title: Cadherin 13 and cocaine: Mouse studies

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Abstract: Data from genome wide association studies support significant roles for variation in the cell adhesion molecule cadherin 13 (CDH13, T-cadherin) in dependence on substances that include psychostimulants (*see Uhl at al. abstract*). CDH13 mRNA is expressed in monoaminergic and cholinergic neurons implicated in actions of stimulants, in habenula regions implicated in aversive features of addictive substances and in other brain regions associated with addiction. We now report significant upregulation of CDH13 in cerebral cortex and hippocampus

of mice sacrificed 24 hours following acute or chronic/challenge cocaine administration. Conditioned place preference (CPP) studies of mice with reduced CDH13 expression reveal their increased sensitivity to the rewarding effects of the drug: enhanced preference for places associated with 5 mg/kg cocaine and reduced preference for places associated with 10 mg/kg cocaine. Strikingly, locomotion induced by 10 mg/kg cocaine doses during conditioning sessions was similar in wildtype and knockout mice, supporting specificity of this effect. Knockout mice were also indistinguishable from wild type mice in performance in water maze mnemonic testing. These data support roles for CDH13 expression in modulating neuronal connections important for the rewarding and, perhaps, aversive actions of cocaine, with selective influences on ascending and descending limbs of the cocaine dose-response relationship.

Disclosures: J. Drgonova: None. **B. Ranscht:** None. **G.R. Uhl:** None.

Poster

873. Addiction and Genetics

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Program#/Poster#:873.03/V5

Topic: C.17. Drugs of Abuse and Addiction

Support: R01 MH097268-01

R01 AG040060-01

Title: A single-nucleotide polymorphism (SNP) in the delta-opioid receptor gene, OPRD1, is associated with differences in regional brain volumes in elderly adults

Authors: *F. F. ROUSSOTTE[1], N. JAHANSHAD[2], O. KOHANNIM[2], E. R. SOWELL[3], P. RAJAGOPALAN[2], A. W. TOGA[2], C. R. JACK, Jr[4], M. W. WEINER[5], P. M. THOMPSON[2]
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Abstract: Introduction
Delta-opioid receptors (DORs) play a key role in the reinforcing properties of drugs of abuse, and polymorphisms in OPRD1 (the gene encoding DORs) have been associated with drug addiction. DORs also play a role in protecting neurons against hypoxic and ischemic stress. Here, we hypothesized that common variants in OPRD1 would be associated with differences in brain structure, particularly in regions relevant to addictive (frontal and limbic lobes) and neurodegenerative (fronto-temporal areas) disorders. We also predicted that genotypes associated

with reduced volumes would predict CSF levels of markers of neurodegeneration.
Methods
738 Caucasian ADNI subjects were scanned with 1.5-Tesla MRI. Tensor-based morphometry was used to map voxelwise volumetric differences. Association p-values were obtained at each voxel for 5 SNPs spanning OPRD1. Sex and age were used as covariates, and p-value maps were corrected for multiple comparisons by controlling the false discovery rate (FDR). In 387 of our subjects, CSF biomarkers of neurodegeneration (total t-tau and the beta amyloid isoform Aβ42) were obtained. We tested how well the top hit (at the rs678849 locus) predicted CSF measures of t-tau and Aβ42 , adjusting for sex and age.
Results
One very common variant (rs678849, C/T, MAF: T=0.462) predicted differences in regional brain volumes. C allele carriers had smaller brain volumes in anterior cingulate, frontal (including right middle and left inferior frontal gyri), temporal (including inferior and middle temporal gyri) and occipital regions. Post hoc analyses revealed trends ($0.05<p<0.10$) for a main effect of genotype on CSF levels of biomarkers associated with neurodegeneration, and a genotype by diagnosis interaction. Therefore, we examined results within diagnosis groups and found a significant main effect of genotype ($p=0.027$) on t-tau / Aβ42 ratio in healthy elderly ($n=105$). Homozygous C allele carriers had higher ratios than participants with at least one T-allele. C homozygotes also showed a trend for higher t-tau levels ($p=0.063$) compared to T-carriers. However, genotype did not predict CSF levels of any biomarker in MCI ($n=182$) or AD ($n=100$) subjects.
Conclusion
This study is the first to report an association between brain structure and an opioid receptor genetic variant. One intronic SNP was broadly associated with regional brain volumes in a large elderly cohort. Carriers of these variants may be of interest in drug trials targeting DORs to treat addictive and degenerative brain disorders. If replicated, this SNP may help predict CSF biomarkers associated with early brain degeneration in the elderly.

Disclosures: F.F. Roussotte: None. **N. Jahanshad:** None. **O. Kohannim:** None. **E.R. Sowell:** None. **P. Rajagopalan:** None. **A.W. Toga:** None. **C.R. Jack:** None. **M.W. Weiner:** None. **P.M. Thompson:** None.

Poster

873. Addiction and Genetics

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Topic: C.17. Drugs of Abuse and Addiction

Support: R01AA019688 (EJV)

R01AA016828 (DMP)

K02DA025697 (GMM)

P51OD011103

Title: Advancing genetic models and tools for translational neuroscience: Comparative functional studies of the mu-opioid receptor

Authors: *E. J. VALLENDER, C. G. SWEENEY, Z. XIE, H. N. PANAS, D. M. PLATT, G. M. MILLER
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Abstract: Shared functional polymorphisms within populations and across species can be used for improved translational research. A prime example, the mu-opioid receptor is involved in many natural processes including stress response, pleasure, and pain. Mutations have also been associated with opiate and alcohol addictions as well as with responsivity to treatment. Two common and mutually exclusive polymorphisms have been identified in humans, A118G (N40D), found in non-African populations, and C17T (V6A), found in African populations. While A118G has been studied extensively, C17T is much less well understood. Using the endogenous agonist β-endorphin and the exogenous agonist morphine and antagonist naltrexone, we have undertaken direct binding assays using radiolabeled ligands, competition assays, and assays of downstream signaling. The T17 allele behaves in a functionally parallel manner to the G118 allele, conferring significant differences (K_d or EC_{50}) compared to the most common, "wild-type", allele C17/A118. Further, we have discovered common polymorphisms across non-human primate species in addition to C77G (P26R) already identified in rhesus macaques (*Macaca mulatta*): C140T (P47L) in cynomolgus macaques (*M. fascicularis*), G55C (D19H) in vervet monkeys (*Chlorocebus aethiops sabeus*), A111T (L37F) in marmosets (*Callitrhix jacchus*), and C55T (P19S) in squirrel monkeys (*Saimiri boliviensis peruviensis*). Like C77G, these variants may have similar *in vitro* functional effects to the human alleles. In alcohol self-administration studies in rhesus macaques the C77G allele has been shown to have not only a similar affect on alcohol consumption and preference, but also on response to naltrexone in attenuating these alcohol drinking behaviors. From these studies flow several important conclusions. First, subpopulations can harbor functionally equivalent polymorphisms, complicating association studies and having significant implications for minority health issues. Second, non-human primate models of human disease can be designed with shared molecular etiologies, allowing for separation of genetic and environmental factors in complex human association studies and greater translational relevancy in drug development. Third, evolutionary pressures have produced similar phenotypes across species, suggesting a shared genetic response and perhaps elucidating the mechanism by which these organism-environment interactions are

mediated physiologically and molecularly. These studies set the stage for future investigations of shared functional polymorphisms across species as a new genetic tool for translational research.

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Poster

873. Addiction and Genetics

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Program#/Poster#:873.05/V7

Topic: C.17. Drugs of Abuse and Addiction

Support: NWO 60-60600-97-207

Title: Serotonin transporter gene variation and compulsive cocaine self-administration in rats

Authors: *J. R. HOMBERG[1], M. VERHEIJ[2], L. NONKES[2], J. LATOUR[2], J. HANISCH[2], L. VENDRUSCOLO[3], S. EDWARDS[3], C. CONTET[3], G. KOOB[3]
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Abstract: Individuals
with cocaine addiction show increased negative emotional responses to stress, which is hypothesized to drive continuation of drug use and compulsivity. Because the serotonin transporter (5-HTT) promoter polymorphism in humans is associated with a negative emotional state and increased risk for cocaine dependence, we hypothesized that the serotonergic system may play an important role in negative reinforcement in cocaine dependence and the resulting compulsive cocaine seeking. However, inherited 5-HTT down-regulation may be associated with neurodevelopmental changes beyond increases in serotonin levels. To determine the contribution of serotonin-mediated neurodevelopmental changes *versus* increased serotonin levels to
anxiety and compulsive cocaine seeking, we compared our previously generated 5-HTT knock*out* rats to rats injected
with viral vectors designed to knock*down*
5-HTT in the dorsal raphe nucleus. Specifically, one group of 5-HTT knock*down* and knock*out* rats was tested for anxiety on the elevated plus maze test
under naive conditions. A separate group of 5-HTT knock*down* and knock*out* rats

was allowed to self-administer cocaine under short access (1 h per day) and long access (6 h per day) conditions, as well as under a progressive ratio schedule of reinforcement. 24 h into withdrawal following a short or long-access session these animals were tested for anxiety on the elevated plus maze. We found that both 5-HTT knock*out* and 5-HTT knock*down* rats showed increased anxiety under naive conditions. Furthermore, 5-HTT knock*out* rats self-administered higher amounts of cocaine under both short and long access conditions, whereas 5-HTT knock*down* rats showed an increase in cocaine self-administration only under long access conditions. In addition, 5-HTT knock*out* rats as well as 5-HTT knock*down* rats were more motivated to self-administer cocaine as measured under progressive ratio conditions 24 h after long access to cocaine. Finally, both 5-HTT knock*out* and 5-HTT knock*down* animals showed increased anxiety 24 h into withdrawal. In conclusion, we show that both constitutive 5-HTT knock*out* and adult 5-HTT knock*down* increase anxiety, which may be an important factor driving increased cocaine self-administration. These findings may have heuristic value for the treatment of cocaine dependence, because they suggest that increased serotonin levels, rather than serotonin-mediated neurodevelopmental changes, contribute to increased vulnerability to addiction in individuals with a polymorphism in the serotonin transporter region.

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Poster

873. Addiction and Genetics

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA05164

NIH Grant AA018779

Title: Adenosine transporter ENT1 regulates circadian home-cage activity and ethanol intake in female mice exposed to constant light

Authors: *C. L. RUBY[1], O. A. ABULSEOUD[2], C. A. ADAMS[1], D. L. WALKER[3], M. A. FRYE[2], D.-S. CHOI[1]
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Abstract: Circadian rhythm disruption is a common feature of mood disorders, addiction, and their frequent comorbid expression, constituting a significant barrier to treatment and a risk factor for relapse. Adenosine signaling has been implicated in alcoholism, mood and anxiety disorders, and circadian rhythm regulation, representing a possible link between them and a promising therapeutic target. Adenosine levels are controlled in part by equilibrative nucleoside transporters (ENTs). Compared to wildtypes, mice lacking ENT1 are less sensitive to ataxia and sedation from acute ethanol, drink more alcohol than wildtypes, and display some behavioral and neurochemical features of mania, including increased risk-taking, reduced behavioral despair, and elevated striatal dopamine levels. Consistently, a genetic variant of ENT1 was associated with mood dysregulation, chronodisruption, and alcoholism in women. However, the specific role of ENT1 in circadian rhythm regulation is unknown. Here, we examined circadian rhythm stability under a normal 12 h light:12 h dark (LD) photocycle in ethanol-naive and ethanol-exposed (2-bottle choice) female ENT1 null and wildtype mice using measures of entrainment, alpha (the duration of the active phase), bout distribution and intensity of activity. Ethanol drinking delayed the activity onset for both genotypes, but ENT1 null mice became active before wildtypes regardless of ethanol exposure. Ethanol reduced alpha only in wildtype mice, while ENT1 null mice were insensitive to this effect. ENT1 null mice showed less intense activity at night (active phase) compared to wildtypes, and ethanol reduced this measure in both genotypes. Interestingly, ethanol exposure affected bout length and intensity differentially between genotypes during the day (rest phase), reducing these measures in wildtypes, but increasing them in ENT1 null mice. As adenosine regulates circadian responses to photic input, we also examined ethanol drinking in the same mice under constant light (LL), which disrupts circadian rhythms and affects drinking behavior. LL lengthened free-running period similarly in both genotypes. However, only ENT1 null mice showed elevated ethanol consumption and preference in LL, with no such effect in wildtypes. Taken together, these data suggest that disruption in ENT1 levels or function may represent a risk factor for chronodisruption-related escalation in alcohol intake, and that this may be related to ethanol-induced stimulation, rather than suppression, of activity during the light-phase.

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Poster

873. Addiction and Genetics

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Topic: C.17. Drugs of Abuse and Addiction

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Michael J. Fox Foundation

Title: Signaling pathways targeting human dopamine transporter genes

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Abstract: Regulation of transcription determines where, when and extents to which a gene is expressed. Dopamine transporters (DATs), the plasma membrane DAT and vesicular monoamine transporter 2 (VMAT2), are the major regulators of dopamine (DA) transmission. The expressions of these transporters are highly circumscribed in discrete regions throughout the brain. Therefore, dissecting the pathways that regulate these transporter genes may help us understand DA-related brain functions (reward, learning and memory, motor function, rest, cognition, hormone production, and sociability), pharmacologic and genetic modulations of these functions and brain disorders (Parkinson's disease, attention deficit hyperactivity disorder, addiction, depression, schizophrenia and bipolar disorder), and develop rational medications. However, little is known about their regulatory pathways. We have used pharmacologic and genetic approaches in various *in vitro* systems to reveal the pathways for the two human transporter genes. We find that both DAT and VMAT2 genes are regulated by cAMP, G protein αs, protein kinase C, PI3 kinase and oxidative stress. There are many more VMAT2-responsive pathways than DAT-responsive ones and most regulations are affected by genetic variations in the genes. These findings compose an emerging picture on transcriptional regulation of the DATs.

Disclosures: Z. Lin: None. **Y. Zhao:** None. **N. Xiong:** None. **Y. Zhou:** None. **C. Chung:** None. **C.E. Glatt:** None. **O. Isacson:** None.

Poster

873. Addiction and Genetics

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R21DA027115

Title: Prolonged- and limited-access to cocaine self-administration at various periods of abstinence induces distinct patterns of DNA methylation.

Authors: *K. PLOENSE[1,2], A. CARR[3], T. W. BREDY[4], Y. E. SUN[5], T. E. KIPPIN[2]
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Abstract: DNA methylation is a key determinant of gene expression and is implicated in neuroplasticity relevant to addiction. Here, we examine DNA methylation within the medial prefrontal cortex (mPFC) following limited- or prolonged-access cocaine self-administration and varying periods of cocaine abstinence. Rats fitted with IV catheters lever pressed for saline, limited-access (1h/ day) to cocaine, or prolonged-access (6h/day) to cocaine for 15 sessions. The rats were then decapitated for tissue collection at 1, 14, and 60 days of abstinence from cocaine self-administration. As reported previously, only prolonged-access rats exhibited escalated cocaine intake across sessions. MeDIP-CHIP analyses of mPFC tissue revealed dramatic and extremely gene-specific changes in DNA methylation/demethylation within promoters across groups. Analyses of peak differences revealed significant learning-induced changes in DNA methylation with 993, 994, 880 genes exhibiting >1 or <-1 peak differences between limited- versus prolonged-access, saline versus limited-access, and saline versus prolonged-access, respectively, with the overall effect of cocaine-seeking appearing to bias towards de-methylation. A number of receptor and related genes involved in PFC neurotransmitter systems implicated in addiction exhibited altered DNA methylation. For example, the Homer2 promoter showed active changes in DNA methylation following prolonged-access but not in other conditions. Sodium bisulphite conversion followed by mass spectrophotometry interrogating a 1000bp sequence of the Homer2 promoter confirmed increased methylation induced by prolonged-access, including CpG sites within putative consensus sequences for GATA-1 and p300 and CRE-BP. These results demonstrate that cocaine self-administration alters DNA methylation, with prolonged-access leading to escalation of cocaine intake, which induces a

pattern of DNA methylation distinct from limited-access. Given the stability of DNA methylation, the present findings provide a possible molecular mechanism mediating the enduring nature of cocaine addiction.

Disclosures: K. Ploense: None. **A. Carr:** None. **T.W. Bredy:** None. **Y.E. Sun:** None. **T.E. Kippin:** None.

Poster

874. Neural Mechanisms of Stimulant Drug Abuse

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Support: PHS Grant P50 DA015369

PHS Grant F32 DA026692

Title: Medial prefrontal cortex afferents to the nucleus accumbens core, but not to the ventral tegmental area, are activated during cue-induced reinstatement of cocaine seeking.

Authors: *E. MCGLINCHEY, S. V. MAHLER, G. ASTON-JONES
Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: Drug addiction is a chronically relapsing disease; relapse often occurs when addicts are exposed to conditioned stimuli that initiate drug seeking behavior. Cues that provoke reinstatement of drug seeking induce Fos expression, a marker of neuronal stimulation, across a variety of reward-related structures including the prefrontal cortex (PFC), nucleus accumbens core (NAcc) and ventral tegmental area (VTA). Kalivas and colleagues have shown that the projection from dorsal PFC (prelimbic cortex, PL) to NAcc is required for reinstatement of extinguished cocaine seeking. In addition, our lab has shown that glutamate in VTA is needed for cued reinstatement of cocaine seeking. However, the specific circuit projections between subregions of PFC to NAcc or VTA that are activated in cue-induced reinstatement have yet to be elucidated. This study used behavioral and anatomical techniques to assess activation of PFC projections to NAcc or VTA during cue-induced reinstatement of cocaine seeking. All animals received jugular catheterizations followed by injections of the retrograde tracer cholera toxin B subunit (CTb), into either NAcc or VTA. These rats were then trained to intravenously self-administer 0.2mg/kg infusions of cocaine paired with a light/tone CS+ cue for 10 days and then extinguished of this behavior for 7+ days. Rats were then subjected to an additional extinction training day or a 2-hour reinstatement test session, in which the light/tone cues were presented

without cocaine infusions. Rats were perfused at the end of either session and brains were removed and processed with dual label immunohistochemistry for CTb and Fos. This allowed us to investigate Fos activation of NAcc- or VTA-projecting PFC neurons. Preliminary results indicate that PL neurons that project to NAcc are Fos-activated during cue-induced reinstatement behavior but not during extinction. This contrasts with PL projections to VTA, which were not activated during reinstatement. In addition, the percentages of NAcc-projecting PL neurons that were Fos activated were correlated with reinstatement behavior in individual animals. No Fos induction was observed for infralimbic cortex neurons that were retrogradely labeled from NAcc or VTA. These results demonstrate differences in Fos activation for projections to NAcc vs. VTA from different PFC subregions, further characterizing the circuits underlying cue-induced relapse in addiction. Supported by PHS grant P50 DA015369 and F32 DA026692.

Disclosures: E. McGlinchey: None. **S.V. Mahler:** None. **G. Aston-Jones:** None.

Poster

874. Neural Mechanisms of Stimulant Drug Abuse

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Support: NIH Grant 5T32DA7288-20

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Title: Selective deficits following long-access methamphetamine self-administration in rats

Authors: *B. M. COX[1], Z. A. COPE[1], A. PARSEGIAN[1], S. B. FLORESCO[2], G. ASTON-JONES[1], R. E. SEE[1]
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Abstract: Methamphetamine (meth) addiction is a chronically relapsing disorder that produces significant and persisting cognitive deficits. These deficits include decreased cognitive flexibility, which may impair addicts from altering their drug use and ultimately lead to increased relapse to drug seeking. Neuroimaging studies have demonstrated that meth addicts have decreased resting activity in the prefrontal cortex (PFC) that corresponds with decreased cognitive flexibility. Although several set-shifting tasks have been developed to assess cognitive

flexibility in rodents, the automated set shift task (ASST) used in the current study can selectively assess specific cognitive parameters commonly disrupted in human addicts. For example, excitotoxic lesions or reversible inactivation of the medial PFC selectively disrupts extra-dimensional shift (EDS), whereas orbitofrontal cortex (OFC) lesions selectively disrupt reversals. Further, the ASST provides more detailed analysis of these types of errors for more direct comparison and optimal face validity with human studies. Here, we determined whether extended access (6 hr/day) of meth self-administration, as compared to sucrose self-administration controls, would impair performance on the ASST. Although no differences were seen in EDS in rats with a chronic meth history, meth subjects showed increased perseverative errors during task reversal, an effect not seen in sucrose trained rats. These data indicate that long access meth self-administration may disrupt OFC function. Ongoing research will determine relationships between cognitive deficits in this task, meth intake history, and reinstatement to meth seeking within the same subjects.

Disclosures: B.M. Cox: None. **Z.A. Cope:** None. **A. Parsegian:** None. **S.B. Floresco:** None. **G. Aston-Jones:** None. **R.E. See:** None.

Poster

874. Neural Mechanisms of Stimulant Drug Abuse

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Topic: C.17. Drugs of Abuse and Addiction

Support: PHS grant R37-DA06214

Title: Decreased response of VTA dopamine neurons to tVTA activation after chronic morphine

Authors: *J. KAUFLING[1], G. ASTON JONES[2]
[1]Dept. of Neurosciences, Med. Univ. of South Carolina, Charleston, SC; [2]medical university of South Carolina, Charleston, SC

Abstract: Evidence indicates that GABAergic (GABA) neurons in the tail of the ventral tegmental area (tVTA), also called rostromedial tegmental nucleus (RMTg), control the mesolimbic dopaminergic (DA) system (Kaufling et al. 2009; Jhou et 2009) and express μ opioid-receptors. Opiates influence the DA system via VTA GABA interneurons, but the tVTA also could be a key player in opiate effects on VTA DA neurons. VTA GABA neurons have been studied extensively, but little is known about the activity of tVTA GABA neurons or their response to morphine (mor) or mor withdrawal.
Using in vivo recording in anaesthetized rats, we compared the activity of the tVTA GABA and

VTA DOPA neuron in response to acute mor, chronic mor (CM), or 14 days of withdrawal from CM (14dw).
Similar to Georges et al. (2006), acute morphine but not 14dw, increased the basal firing of VTA DA cells. Also, iv mor increased the firing rate of VTA DA neurons in naive, but not withdrawn rats. These data suggest a mor tolerance in VTA DA neurons.
These finding are to contrast to those obtained in the tVTA GABA neurons. We show that acute mor in naive rats decreased the firing of tVTA putative GABA neurons. We also found that the basal firing rate of tVTA GABA neurons was markedly lower in CM and 14dw rats than in naive rats. Also, an acute injection of morph in previously morph-exposed rats (CM or 14dw) or a local injection of morph in the tVTA still induced an inhibition of tVTA GABA cells.
In contrast to VTA DA neurons, these data reveal that tVTA GABA neurons do not show tolerance to mor. More interestingly, these results show that CM may induce an apparent functional disconnection between tVTA and VTA.
To further investigate the VTA/tVTA relationship in mor-exposed animals, we expressed a designer receptor (Designer Receptors Specifically Activated by Designer Drugs, DREADD) in tVTA neurons using a viral vector in vivo. This hM4D DREADD, an alternate strategy to optogenetics to control targeted neuronal populations, is a Gi-coupled receptor that inhibits activity selectively of expressing neurons when stimulated by its agonist, clozapine-N-dioxide (CNO). Rats received unilateral injections into tVTA of a lentivirus containing the hM4Di DREADD receptor gene downstream of a synapsin promoter. After recovery, rats were divided in two groups: naïve rats and 14dw rats.
To test VTA/tVTA disconnection, we are using in vivo unit recordings to compare the effect of tVTA inactivation on the firing rate of VTA DA cells by stimulating DREADDs with CNO administration. These studies are ongoing; we hypothesize a stronger activation of VTA DA cells after tVTA GABA neuronal inactivation in naive rats than in 14dw rats.

Disclosures: J. Kaufling: None. **G. Aston Jones:** None.

Poster

874. Neural Mechanisms of Stimulant Drug Abuse

Location: Hall F-J

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Program#/Poster#:874.04/V14

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant R37 DA06214

NIH grant F32 DA026692

Title: Just C NO to DREADDs: Inhibition of ventral pallidum projection to VTA blocks cue-triggered cocaine seeking.

Authors: *S. V. MAHLER[1], E. M. VAZEY[1], J. KAUFLING[1], B. L. ROTH[2], G. ASTON-JONES[1]
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Abstract: Designer receptors exclusively activated by designer drugs (DREADDs) are synthetic G-protein coupled receptors that are inert, except in the presence of their agonist, CNO (which is inert in the absence of DREADDs). DREADD-expressing neurons can therefore be experimentally controlled in a highly selective, "lock-and-key" manner.
Although light-activated opsins are preferable for experiments requiring precise temporal (milliseconds to seconds) control of neuronal activity, DREADDs can be preferable when extended periods of inactivation are desired (minutes to hours). In addition, DREADDs do not require tethering of animals to a light source, an advantage when animals perform complex behaviors.
Here, we examined the role of ventral pallidum (VP) and its projection to ventral tegmental area (VTA) in reinstatement of cocaine seeking. We used an hM4D Gi-coupled DREADD construct, expressed under control of a synapsin promoter via lentiviral neurotransduction in vivo in rats. First, we examined the necessity of VP for reinstatement elicited by either cocaine-associated cues or a cocaine priming injection. We bilaterally transfected VP neurons with the DREADD viral vector, trained rats on cocaine self-administration and extinction, and then systemically administered several doses of the DREADD agonist CNO during cue-induced or cocaine primed reinstatement sessions. We found that CNO dose-dependently blocked cue-induced but not cocaine-primed reinstatement of cocaine seeking, indicating that VP activity is required for cues to trigger cocaine seeking.
We previously observed that VP projections to VTA are robustly Fos activated during cue-induced reinstatement behavior, so we next sought to examine whether this specific projection is necessary for cue-induced reinstatement of cocaine seeking. For this, we again bilaterally transfected VP neurons with the hM4D DREADD, allowed time for DREADD transport to axonal terminals in VTA (2-3 weeks), and implanted bilateral cannulae into VTA. Prior to cue-induced or cocaine-primed reinstatement sessions, we microinjected CNO into VTA to specifically inhibit DREADD-expressing VP terminals. Again, this attenuated cue-induced, but not cocaine-primed reinstatement.
These findings demonstrate that i) DREADDs are a useful means of modulating neuronal activity in behavioral experiments, ii) DREADDs can be used to locally inhibit terminals of projections from transduced neurons, and iii) ventral pallidum projections to VTA are crucial for cue-induced, but not cocaine-primed reinstatement. Supported by PHS grants R37 06214 and F32 DA026692.

Disclosures: S.V. Mahler: None. **E.M. Vazey:** None. **J. Kaufling:** None. **B.L. Roth:** None. **G. Aston-Jones:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: PHS grant R37 DA06214

PHS grant T32 GM008716

Title: Behavioral economics for the neuroscientist: Demand curve analysis of the within-session threshold procedure

Authors: *B. BENTZLEY, K. FENDER, G. ASTON-JONES
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Abstract: Behavioral-economic demand curve analysis offers several useful measures of drug self-administration not available in typical behavioral analyses. Demand curves are used to mathematically model the consumption of drug as a function of the effort (lever presses) required to obtain a mg of drug. Generation of demand curves with this approach previously required multiple days, with a different fixed-ratio schedule for each day. This was a serious limitation for many neuroscience studies (e.g., local drug microinfusions, unit electrophysiology). However, recent within-session threshold procedures allow demand curve construction from a single 110-min cocaine self-administration session, making this analysis relevant to a broad range of self-administration experiments. There are several useful and important demand analysis parameters; some of these can be approximated via graphical estimation, but a mathematically fit demand curve is required to predict the 'essential value' parameter (α) and to derive accurate estimates of the maximum price (or effort; *Pmax*) subjects are willing to expend for drug. A mathematical approach to demand curve fitting has not been reported for the within-session threshold procedure. *Here,* we provide a method for fitting demand curves to single-session data for cocaine threshold procedure self-administration. Animals were trained to self-administer cocaine, and then tested with the threshold procedure in which the cocaine dose was sequentially decreased in successive 10-min bins during a single FR1 session. Critically, brain cocaine concentrations were estimated based on intake and response rates, and price points outside of stable brain cocaine concentrations were removed before curves were fit. Curve-fit accuracy was determined by the degree of correlation between graphical and calculated parameters for Q_0

(desired brain concentration at null effort) and *Pmax*. Our novel fitting method generated precise estimates of Q_0 and α, and resulted in *Pmax* values with significantly closer agreement with graphical *Pmax* than conventional methods. Our results show that the exponential demand equation can be fit to single-session data using the threshold procedure for cocaine self-administration. This method provides more accurate estimates of *Pmax* than graphical methods, and allows determination of α, permitting a more comprehensive analysis of drug self-administration via a behavioral-economic framework.

Disclosures: B. Bentzley: None. **K. Fender:** None. **G. Aston-Jones:** None.

Poster

874. Neural Mechanisms of Stimulant Drug Abuse

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Topic: C.17. Drugs of Abuse and Addiction

Support: DA024519

DA09815

Title: Addiction-prone OPRM1 118GG mice demonstrate less preference for naturally rewarding saccharin compared with 118AA mice

Authors: *C. S. FREET, P. S. GRIGSON
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Abstract: Rodents suppress intake of a saccharin conditioned stimulus (CS) when it is paired with a drug of abuse (Cappell et al., 1973; Carey and Goodall, 1974; Goudie et al., 1978). This phenomenon, termed reward comparison, is thought to be mediated by the rewarding properties of the drug and serves as a model for a number of behaviors observed in addiction including anticipation of reward, cue-induced withdrawal, and devaluation of natural rewards (Grigson, 1997, 2000, 2008). OPRM1 A118G is a single nucleotide polymorphism (SNP) of the mu-opioid receptor that has been linked to addiction in human alcoholics and is associated with greater alcohol and drug intake in humans, non-human primates, and mice (Bond et al., 1998; Miller et al., 2004; Ray and Hutchison, 2007; van den Wildenberg et al., 2007; Ramchandani et al., 2010). Similarly, OPRM1 118GG mice exhibit greater avoidance of a cocaine-paired saccharin cue compared to their 118AA wild type counterparts and it is believed that this facilitated effect is due, in part, to less preference for the naturally rewarding saccharin cue. To investigate this possibility, OPRM1 A118G mice (118AA: n=8; 118AG: n=9; 118GG: n=10) were evaluated in a

series of one-bottle and two-bottle intake tests for saccharin (0.02%, 0.15%, 0.30%) as well as the non-gustatory stimulus capsaicin (0.5µm, 1.0µm, 4.0µm). Results demonstrate that, while intake in one-bottle tests were similar between the genotypes for saccharin and capsaicin, intake of 0.15% saccharin against dH_2O in the two-bottle test was significantly greater in 118AA compared with 118GG mice.

Disclosures: C.S. Freet: None. **P.S. Grigson:** None.

Poster

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Chicago Biomedical Consortium Junior Scholarship

Title: Drug-unpaired environments modulate dendritic spine dynamics

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Abstract: Repeated exposure to amphetamine leads to the development of biochemical and neuroanatomical phenotypes that may underlie the formation of associative drug conditioning. Brief elevations in c-Fos expression in nucleus accumbens (NAcc) neurons following re-exposure to a drug-paired, but not unpaired, environment suggest that expression of this immediate early gene serves as a marker for neurons which process drug-context information. This correlation is supported by evidence showing that prevention of conditioning inhibits environmental regulation of c-Fos expression. In contrast, ΔFosB displays a long-lasting context-independent increase following repeated drug exposure, a neuronal expression pattern that overlaps with c-Fos activation following exposure to drug-paired stimuli. When conditioning accrues, a proportion of ΔFosB(+) cells may process this information although ΔFosB levels are also increased if conditioning is prevented. This transcription factor may thus have various functions and serve as a marker for cells encoding other information such as non-associative

behavioral sensitization that can manifest in the absence of associative conditioning. Previous research has revealed brain-region specific changes in dendritic architecture following repeated systemic amphetamine administration. Given the well documented relationship between conditioning and dendritic spine growth, we hypothesized that dendritic changes may be restricted to c-Fos(+) and ΔFosB(+) neurons that encode this behavioral information. Labeled cells were injected with the carbocyanine neuronal tracer DiI in order to characterize their dendritic spine morphology. One week after withdrawal from repeated amphetamine, NAcc FosB(+) cells showed an increase in the basal frequency (no challenge) of spines with medium head diameters. This increase was absent in c-Fos(+) neurons 30-minutes after re-exposing rats to a drug-unpaired environment, but preserved after exposure to a drug-paired environment. Consistent with these results, significant decreases in levels of the actin binding proteins Arp2 and p-cofilin were observed following exposure to the drug-unpaired environment. These results support the existence of long-lasting conditioning-related changes in the dendritic spine properties of NAcc neurons following repeated amphetamine and the inhibitory regulation of these changes by drug-unpaired environments.

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Poster

874. Neural Mechanisms of Stimulant Drug Abuse

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Topic: C.17. Drugs of Abuse and Addiction

Support: Environmental Foundation of Jamaica

Office of Graduate Studies & Research, UWI, Mona Campus

Title: A nutritional supplement regime diminished addictive behavior and mean arterial pressure without affecting serum catecholamines

Authors: *L. E. YOUNG, A. M. WEBBER-WAUGH, K. A. THAXTER
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Abstract: Current treatment strategies for drug addiction often result in relapse due in part to the anhedonic effects of drug withdrawal. Signs and symptoms of withdrawal are in turn due to increased sympathetic activity. This study investigated the effectiveness of a patented nutritional supplement regime consisting of fish oil and megavitamins (developed by Dr. A. Salako) in alleviating the withdrawal symptoms of amphetamine and cocaine addiction; and its effects on

serum catecholamines and cardiovascular indices. Thirty-six male Sprague-Dawley rats (250 - 350 g) were randomly divided into 3 groups (n = 12) and exposed to a biased conditioned place preference paradigm (CPP), utilizing a 3-chambered box and an 8-day conditioning schedule. On alternate days of conditioning, 2 groups of rats received either cocaine hydrochloride (20 mg/kg/ml, *i.p.*) or amphetamine hydrochloride (5 mg/kg/ml, *i.p.*) in the non-preferred white chamber, and saline (1 ml, *i.p.*) in the preferred black chamber. The third group (controls) received saline in both chambers. Post conditioning, each rat was placed in the neutral grey chamber and allowed to freely explore the CPP box for 20 minutes. Addicted rats spent at least 80% of their time in the drug-paired white chamber and were subsequently administered increasing dosage of cocaine (20-30 mg/kg/ml, *i.p.*) or amphetamine (5 -7 mg/kg/ml, *i.p.*) over an additional 6 days. Addicted rats and controls were then randomly subdivided equally into groups of rats fed chow containing the nutritional supplement or normal rat chow for 8 weeks, and their behavior re-examined in the CPP. ELISA analysis of tail blood samples for catecholamines (dopamine, norepinephrine and epinephrine) and mean arterial pressure (MAP) measurements were done before CPP exposure (baseline), after a 3-day drug-withdrawal period and following the 8-week supplementation period. Supplemented addicted rats showed significant decreases ($p<0.05$) in number of entries and time spent in the white chamber on repeat performance in the CPP; while non-supplemented addicted rats continued to show significant preference for this chamber. Nutritionally supplemented drug-exposed rats showed significantly lower MAP values ($p<0.05$) than their non-supplemented counterparts. Serum catecholamines measured at baseline, after withdrawal and following the supplementation period did not differ significantly from baseline. The nutritional supplementation-induced decline in addictive behavior to amphetamine and cocaine is thus likely to be a central effect. Further studies into the effects of the nutritional supplement on brain catecholamine levels and the mechanism of action are needed.

Disclosures: L.E. Young: None. **A.M. Webber-Waugh:** None. **K.A. Thaxter:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: Granted by The Guy Everett Laboratory Foundation

Title: Dopamine-beta-hydroxylase inhibition potentiates cocaine effect on dopamine release in the medial prefrontal cortex

Authors: P. DEVOTO[1,3], R. FRAU[1], L. FATTORE[4], *W. FRATTA[2], G. L. GESSA[3]
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Abstract: Several preclinical and clinical evidence support an important role for dopamine-beta-hydroxylase (DBH) inhibition in the ability of disulfiram in reducing cocaine use. The present study was aimed at characterizing the impact of the co-administration of DBH inhibitors and cocaine in either naïve and cocaine-experienced rats, by mean of neurochemical and behavioral approaches. In naïve rats, DBH inhibitors significantly increased extracellular dopamine levels in the medial prefrontal cortex (mPFC), but not in the nucleus accumbens, as measured by microdialysis. Conversely, DBH inhibitors decreased extracellular noradrenaline concentrations in both cerebral areas. Notably, the acute pre-treatment with DBH inhibitors before cocaine injection (10mg/kg, IP) dramatically potentiated the extracellular dopamine augmentation produced by cocaine alone, in the mPFC, but not in the nucleus accumbens. Notably, the dopamine imbalances measured in the mPFC of DBH inhibitors-cocaine treated rats were correlated with behavioral impairments related to cortical dopaminergic dysregulations, including sensorimotor gating deficits and reduced social investigations. Conversely, no significant difference on the motor activity parameters between cocaine- and cocaine-DBH inhibitors treated rats were found. Finally, in rats previously trained to intravenously self-administer cocaine, DBH inhibitors prevented cocaine-induced reinstatement of drug seeking and the neurochemical assays revealed that drug combination elicited an enhancement of the increase in the extracellular dopamine fluctuations in the mPFC equivalent to those observed in naïve rats. Collectively, these results suggest that DBH inhibitors prevent cocaine-seeking reinstatement by the enhancement of cocaine effects on dopamine release selectively in the mPFC, and that this elevated cortical dopamine level might exert a negative influences on perceptual and emotional processes. Moreover, they also may underpin the reported clinical observations of paranoia and other hallucinatory states in cocaine-addicted individuals under disulfiram therapy.

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Poster

874. Neural Mechanisms of Stimulant Drug Abuse

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PEDECIBA

Title: Behavioral sensitization elicited by Coca-paste, a drug widely consumed in Latin America, and the influence of caffeine as its main adulterant

Authors: *C. SCORZA[1], J. P. PRIETO[1], M. N. MEIKLE[1], X. LÓPEZ-HILL[1], M. GALVALISI[1], J. URBANAVICIUS[1], J. A. ABIN-CARRIQUIRY[2]
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Abstract: Coca-paste (CP) is an illicit drug of abuse. Despite its high prevalence and distribution throughout South America it has not been extensively studied. CP is an intermediate product of the cocaine alkaloid extraction process from coca leaves. It has a high content of cocaine base mixed with other chemical substances (impurities) and it is adulterated when it reaches the consumers. We have demonstrated that cocaine (base) and caffeine (as an active adulterant) are the main components which underlie the stimulant effect induced by acute injection of CP seized samples, and they act in an additive fashion when achieved specific proportions. However, their roles in a CP repeated administration have not been studied so far.
Firstly we evaluated the behavioral sensitization phenomenon elicited after a repeated administration of a CP seized sample in comparison with that induced by cocaine. Secondly and similarly to what was determined for acute treatment, the specific role of cocaine and caffeine in the induction of the CP behavioral sensitization was also investigated.
Male Wistar rats were intraperitoneally injected during 5 days with CP at an equivalent dose of 10 mg/kg of cocaine, cocaine (10 mg/kg) and vehicle. Additionally, other series of experiments were done in which animals were injected during 3 days with cocaine, the combination of cocaine and caffeine (at the same proportions usually found in CP seized samples) and vehicle. For both experiments, after 5 withdrawal days a challenge with each treatment was done. The animal motor activity was registered along the treatments by means of the Open Field paradigm and the Ethovision video-tracking software.
Our results showed that after 5 days of treatment, CP and cocaine induced the expression of behavioral sensitization. A 3 days cocaine treatment was not enough to elicit the expression of sensitization although a specific combination of cocaine and caffeine was.
These results highlight the relevance of caffeine in the development of addiction to CP since caffeine, as main active adulterant, could be able to accelerate the neural changes involved in the expression of the behavioral sensitization elicited by CP. These data may contribute to the understanding of the high dependence, craving and relapse in CP addicts.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

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Title: The effects of environmental enrichment on methamphetamine self-administration and reinstatement

Authors: *C. E. WILMOUTH, M. BARDO
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Abstract: While environmental enrichment is known to reduce the sensitivity to the reinforcing properties of various stimulant drugs, little is known about its effects on methamphetamine (METH) self-administration or reinstatement of METH seeking. In contrast to other stimulants such as cocaine and methylphenidate, a primary mechanism of action for METH is on the vesicular monoamine transporter in brain (VMAT2). The present experiment examined whether environmental enrichment alters METH self-administration, extinction and/or cue-induced reinstatement. Male Sprague-Dawley rats were reared in either enriched or isolated conditions from postnatal day 21 to 65 and then were implanted with jugular catheters. Following recovery, rats underwent an autoshaping procedure to learn to lever press for methamphetamine (0.1 mg/kg/infusion) for 10 days. All rats were then run in a dose-response experiment with 8 METH unit doses (0.0, 0.001, 0.01, 0.03, 0.05, 0.1, 0.3 or 0.5 mg/kg/infusion) presented in semi-random order. A 14-day extinction procedure then was conducted and followed immediately by cue-induced reinstatement. The intake of METH self-administered by enriched rats did not significantly differ from the isolated controls at any of the doses tested. During the extinction phase, however, enriched rats exhibited lower levels of drug seeking behavior and showed lower levels of cue-induced reinstatement compared to isolated controls. Thus, while enriched rearing does not attenuate the reinforcing effects of methamphetamine, it reduces methamphetamine seeking after a period of extinction. These preclinical findings suggest that environment-based therapeutic approaches may be useful for reducing relapse among METH abusers.

Disclosures: C.E. Wilmouth: None. **M. Bardo:** None.

Poster

874. Neural Mechanisms of Stimulant Drug Abuse

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant DA027985

NIGMS Grant GM083883

Title: Early ontogeny of d-amphetamine-induced one-trial behavioral sensitization

Authors: *C. M. NUQUI, A. T. QUIROZ, C. M. MARTINEZ, S. A. MCDOUGALL
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Abstract: Although adult rats of various ages exhibit one-trial behavioral sensitization, young rats only show one-trial sensitization during a narrow ontogenetic window that varies depending on the psychostimulant administered. For example, cocaine induces robust one-trial behavioral sensitization on postnatal day (PD) 21, whereas methamphetamine produces strong behavioral sensitization at only younger ages (PD 12-PD16). Because these psychostimulants differ in a variety of ways (e.g., brain half-life, selectivity at monoamine transporters, mechanism of action, etc.), it is uncertain what factors are responsible for the different sensitization profiles exhibited by young rats. Among amphetamine-like compounds, d-amphetamine and methamphetamine are readily dissociable in terms of pharmacokinetic factors, although they do share the same mechanism of action. If mechanism of action is the critical factor determining the occurrence of behavioral sensitization during early ontogeny, then it would be predicted that d-amphetamine should have a sensitization profile similar to methamphetamine and different from cocaine. To examine this issue, the ontogeny of d-amphetamine-induced one-trial behavioral sensitization was characterized using preweanling and preadolescent rats. In the first experiment, rats were injected with saline or d-amphetamine (4 or 8 mg/kg) in an activity chamber or home cage on PD 12, 16, 20, or 24. One day later, rats were challenged with 2 mg/kg d-amphetamine and distance traveled was measured in activity chambers for 120 min. In a separate experiment, a broad dose range of d-amphetamine was employed in order to ensure that the absence of sensitized responding was not the result of an inappropriate dosing regimen. Results showed that a single pretreatment injection of d-amphetamine caused a strong sensitized response at younger ages (i.e., on PD 13 and PD 17), which was not dependent on environmental context. Rats did not display d-amphetamine-induced sensitization on PD 21, nor was context-dependent sensitization evident on PD 25 even when a broad dose range of d-amphetamine was used. This pattern of d-amphetamine-induced one-trial behavioral sensitization is nearly identical to the pattern produced by methamphetamine, while the ontogeny of cocaine-induced one-trial sensitization is

distinctly different. The disparate sensitization profiles resulting from repeated d-amphetamine and cocaine treatment may be a consequence of their mechanism of action (i.e., d-amphetamine functions as a dopamine releaser, while cocaine is a transport inhibitor).

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Poster

874. Neural Mechanisms of Stimulant Drug Abuse

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA DA009815

Title: Using taste reactivity to track the development of responses to drug cues

Authors: *E. COLECHIO, P. S. GRIGSON
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Abstract: Addiction can be devastating for addicts, their families, and for society as a whole; the emotional and economic costs are astounding. Much of this damage occurs because addiction is a brain disease of chronic relapse. Stress, the drug itself, and drug-associated cues can repeatedly initiate drug-seeking and drug-taking, even following prolonged periods of abstinence. Drug-associated cues are particularly problematic as they utilize the innate learning and memory system – a system that is designed to remember associations and engage learned behaviors for a life time. It is, then, imperative that we understand how cues gain access to this system to engage drug-seeking and drug-taking behavior. Cues, it appears, can elicit drug-seeking in one of two ways. The cue can take on the positive reinforcing properties of the drug and thereby elicit approach (Wheeler et al., 2011). Alternatively, the cue can elicit a highly aversive opponent process which also elicits approach and drug-seeking, but in this case, in an effort to correct the aversive state (see Grigson, 2008; Wheeler et al., 2008). While important, these prior studies measured cue-induced positive and negative affect only at the end of training (i.e., after the 'addictive' behavior was acquired). Here we evaluated this behavior during acquisition, on a trial by trial basis. Specifically, rats received intraoral infusion of a saccharin cue once per min for 30 min. Thereafter, they were given 90 min to self-administer cocaine. Affect was assessed by measuring aversive taste reactivity behavior (i.e., gapes) throughout each daily intraoral infusion period. The results revealed large and small drug-takers following a single taste-drug pairing. As a group, large drug-takers exhibited significantly more gapes in response to the intraoral infusion

of the drug-paired saccharin cue. Thereafter, they were faster to take the first infusion of cocaine, they exhibited greater load-up (i.e., a longer run of rapid drug infusions at the start of the session), and they continued to seek drug even when drug was not available. Some rats, then, are more resilient and others more vulnerable. Vulnerable rats acquire the aversive opponent process very quickly and these rats exhibit greater seeking and taking of drug. This work was supported by DA009815.

Disclosures: E. Colechio: None. **P.S. Grigson:** None.

Poster

874. Neural Mechanisms of Stimulant Drug Abuse

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Program#/Poster#:874.14/W4

Topic: C.17. Drugs of Abuse and Addiction

Title: Inactivation of the basolateral amygdala blocks the expression of cue-induced hyperactivity

Authors: *G. W. DIEHL[1], T. A. PAINE[2]
[1]Oberlin, ; [2]Neurosci., Oberlin Col., Oberlin, OH

Abstract: Background: Presentation of drug-associated cues increases drug craving, which in turn increases the likelihood of relapse. Furthermore, drug-associated cues reinstate drug-seeking behavior in self-administration paradigms, reinstate place preferences and increase locomotor activity in non-human animal models of addiction. Drug-associated cues are thought to acquire salience through classical conditioning. Understanding the neural systems by which drug-associated cues acquire salience and promote relapse may enable us to develop more effective treatments for addiction. Here we examine the ability of basolateral amygdala (BLA) inactivation to block the expression of drug cue-induced locomotor activity.
Methods: Male Sprague Dawley rats were implanted with cannulae aimed at each BLA and allowed to recover. Next, rats were habituated to locomotor activity chambers over three 60-min sessions; these data were used to divide rats into cocaine-paired and saline-paired groups. Drug-cue pairing was then established over 12 conditioning sessions. There were two types of conditioning sessions, cue present (a flashing yellow bicycle light and a metronome; CS+) and cue absent (CS-), which occurred on alternating days. Prior to CS+ sessions, rats in the cocaine-paired groups received cocaine (15 mg/kg, IP) and rats in the saline-paired group received saline (1 ml/kg). Prior to CS- sessions all rats received saline (1 ml/kg). Two days after conditioning, testing began. Prior to test sessions all rats were treated with saline (IP) and received bilateral

intra-BLA infusions of either saline (0.5 μL/side) or muscimol (25 ng/0.5 μL/side). Activity was then measured in the presence of the cue (CS+) or the absence of the cue (CS-).
Results: Cocaine-paired, saline-infused rats exhibited greater activity in the presence of the cue (CS+) than in the absence of the cue (CS-). Inactivation of the BLA reversed this effect. Activity of saline-paired rats was unaffected by either the presence of the cue or by intra-BLA muscimol infusions.
Implications: These data support the idea that the BLA is important for the expression of cue-induced activity, supporting previous findings using other models of cue-induced reinstatement. Additionally, these data support the use of locomotor activity monitoring in further study of the neural mechanisms underlying drug-associated cues.

Disclosures: G.W. Diehl: None. **T.A. Paine:** None.

Poster

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NSF GRFP (KKH)

Title: Neural mechanisms of methylphenidate's dose-dependent behavioral effects

Authors: *S. A. CARMACK, K. K. HOWELL, S. G. ANAGNOSTARAS
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Abstract: Prescription psychostimulants containing methylphenidate (e.g. Concerta, Focalin, & Ritalin) are the mainstay of treatment for attention-deficit hyperactivity disorder (ADHD). In affected individuals, low doses reportedly reduce activity, improve attention, and reduce impulsivity. These low, therapeutic doses can also enhance learning in control populations, which has led to controversial off-label use of methylphenidate as a cognitive enhancer. We have recently developed a model using standard Pavlovian fear conditioning where we can consistently detect methylphenidate-induced memory enhancements at a low, clinically relevant dose and memory deficits at a high dose. These effects are dissociable from methylphenidate's other behavioral effects, including the induction of behavioral sensitization and conditioned

place preference. It is well established that methylphenidate inhibits both the dopamine and norepinephrine transporters, but the mechanisms underlying its cognitive enhancing and locomotor activating effects remain unknown. The present experiments investigated the neural mechanisms underlying methylphenidate's dose-dependent behavioral effects.

Disclosures: S.A. Carmack: None. **K.K. Howell:** None. **S.G. Anagnostaras:** None.

Poster

874. Neural Mechanisms of Stimulant Drug Abuse

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:874.16/W6

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant DA016662

Title: Amphetamine-associated contextual learning produces a constellation of structural plastic changes of synapses in basolateral amygdala circuitry

Authors: *D. J. RADEMACHER[1], J. A. ROSENKRANZ[2], A. HETZEL[2], N. MENDOZA-ELIAS[2,3], G. E. MEREDITH[3]
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Abstract: Our earlier work (Rademacher et al., 2010) with amphetamine (AMPH) conditioned place preference (CPP), a widely used behavioral assay for context-induced drug-seeking, demonstrated that learning to associate a context with AMPH increased the number of asymmetric (excitatory) synapses and the excitatory drive in the basolateral amygdala (BLA). As it was not established how the increase in excitatory drive translated to the neural targets, immunohistochemistry with a cell specific marker and the stereological quantification of synapses was used in the present study to determine if context-drug learning alters the number of asymmetric and symmetric (inhibitory) synapses contacting gamma-aminobutyric acid (GABA) interneurons and/or pyramidal neurons in the synaptic circuit of the BLA. Rats were tested for place preference using a conditioning procedure that was performed in a three-chambered apparatus. One group of animals received injections of AMPH (1.0 mg/kg, i.p.) in one compartment on days 1, 3, and 5 and injections of saline in the other compartment on days 2 and 4. A second group, the delayed pairing control group, was subjected to the identical conditioning schedule with AMPH. However, these animals spent 4 hours in their home cages before being placed into the CPP apparatus. A third group received saline pairings only. Seventy-two hours

after place conditioning, animals were exposed to the CPP apparatus in a drug- and saline-free state and place preference was measured. After the test, animals were deeply anesthetized and transcardially perfused with fixative. Brain sections through the entirety of the BLA were processed for glutamate decarboxylase (GAD) immunohistochemistry to label all cellular structures of GABA interneurons. AMPH CPP increased the number of asymmetric synapses contacting spines and dendrites of BLA pyramidal neurons, multisynaptic boutons contacting BLA pyramidal neurons and interneurons, and symmetric synapses contacting the dendrites of interneurons. These data suggest that approach behavior to a context previously paired with drug is driven by a 'constellation' of structural plastic changes of synapses in BLA circuitry.

Disclosures: D.J. Rademacher: None. **J.A. Rosenkranz:** None. **A. Hetzel:** None. **N. Mendoza-Elias:** None. **G.E. Meredith:** None.

Poster

874. Neural Mechanisms of Stimulant Drug Abuse

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Topic: C.17. Drugs of Abuse and Addiction

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CU Boulder UROP-HHMI Grant

Title: Stimulation of adenosine A1 receptors in the nucleus accumbens reduces dopamine D1 receptor-induced cocaine seeking and D1-mediated phosphorylation of AMPA receptors

Authors: *B. HOBSON, C. E. O'NEILL, R. K. BACHTELL
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Abstract: Chronic cocaine use can lead to an addiction that is especially difficult to treat because of the continuous potential for relapse. Stimulation of either AMPA glutamate or dopamine D_1 receptors in the nucleus accumbens (NAc) is sufficient to induce reinstatement of cocaine seeking in rodents. Identifying strategies to reduce the activity of these systems may shed light on the mechanisms involved in relapse, revealing potential therapeutic targets. Adenosine A_1 receptors co-localize with D_1 receptors on postsynaptic terminals of medium spiny neurons where they counteract D_1 receptor activity through opposing intracellular signaling. We hypothesized that dopamine D_1 stimulation in the NAc facilitates AMPA receptor activity to induce cocaine seeking and that stimulation of adenosine A_1 receptors in the NAc reduces D_1-mediated cocaine seeking by offsetting D_1 facilitation of AMPA glutamate activity. Male

Sprague Dawley rats self-administered cocaine during daily sessions over 3 weeks. Cocaine seeking was extinguished in 6 daily extinction sessions and reinstatement of cocaine seeking was induced by micro-infusion of the D_1 agonist, SKF 81297. We investigated the role of AMPA receptors in D_1-induced reinstatement using the AMPA receptor antagonist, CNQX, to block AMPA receptors, and viral-mediated gene transfer to bi-directionally modulate AMPA receptor activity in the NAc. Results show that either AMPA receptor blockade or downregulation of the GluA1 AMPA subunit impairs D_1-induced reinstatement. We next sought to determine whether D_1 stimulation facilitated AMPA receptor activity by analyzing synaptic phosphorylation of the GluA1 subunit. We observed that D_1 stimulation enhanced PKA-mediated GluA1^{S845} phosphorylation, an effect reversed by adenosine A_1 stimulation in the NAc. Finally, we tested the ability of A_1 stimulation in the NAc to inhibit D_1-induced reinstatement. Our results demonstrate that stimulation of A_1 receptors decreases D_1-induced cocaine seeking. Together, these findings suggest that A_1 receptor stimulation reduces D_1 facilitation of AMPA receptor phosphorylation in the NAc that is critical for D_1 receptor-mediated reinstatement.

Disclosures: B. Hobson: None. **C.E. O'Neill:** None. **R.K. Bachtell:** None.

Poster

874. Neural Mechanisms of Stimulant Drug Abuse

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Support: NIH Grant 5 F31 DA029401-02

Chapman Fellowship, Salk Institute

NIH R01-DA029706

Title: Deletion of Sorting Nexin 27 from dopamine neurons alters GABABR-GIRK currents and behavioral response to novelty

Authors: *M. B. MUNOZ[1,2], P. A. SLESINGER[1,3]
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Abstract: Natural rewards and addictive drugs increase the release of dopamine (DA) in the brain's reward circuit by augmenting the activity of dopaminergic neurons in the ventral tegmental area (VTA). Activation of G protein-gated inwardly rectifying potassium (GIRK or

Kir3) channels provides an important inhibitory signal for regulating the excitability of DA neurons. Here, we examined the effect of 5 daily injections of methamphetamine (2mg/kg) on GIRK signaling in mice (P33-42 days). Whole-cell patch clamp electrophysiology from DA neurons in acutely prepared slices containing the VTA was used to assess GIRK channel activity. We bath applied GABAB or dopamine D2 receptor agonists, baclofen and quinpirole, respectively, and observed an ~50% reduction of GABAB-GIRK currents with no change in D2R-GIRK currents. Psychostimulant-sensitive sorting nexin 27 (SNX27) is known to regulate GIRK channel surface expression and inhibitory currents. Thus, drugs of abuse have the potential to increase SNX27 trafficking of GIRK channels, dis-inhibiting DA neurons and altering reward responding in vivo. To examine the impact of SNX27 on GIRK channel trafficking in the reward pathway, we generated mice lacking SNX27 selectively in DA neurons by crossing conditional SNX27 mice (SNX27lox/lox) with DAT-Cre (Slc6a3+/cre) transgenic mice. In contrast to whole animal knockouts of SNX27, which are postnatal lethal, DA-specific knockouts are indistinguishable from wild-type littermates. DA neurons in VTA-containing slices from knockout animals exhibited similar capacitance, resting potential, and action potential duration to wild-type neurons. However, the induced firing frequency was higher in DA neurons lacking SNX27, suggesting an increase in excitability. Furthermore, GABAB-GIRK currents in DA neurons were reduced by ~70% compared to controls (floxed- and cre+-only animals), similar to results observed with repeated methamphetamine in wild-type mice. D2R-GIRK currents in DA neurons from SNX27 knockout mice were indistinguishable from those in wild-type mice. Behaviorally, mice lacking SNX27 in DA neurons exhibited an enhanced response to novelty with normal basal activity levels. These data identify a novel pathway for reward processing involving SNX27 and GIRK channels in VTA DA neurons and highlight SNX27 as a promising therapeutic target for addiction and other complex psychiatric disorders.

Disclosures: M.B. Munoz: None. **P.A. Slesinger:** None.

Poster

875. Monoamines and Behavior: Serotonin and Histamine

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Topic: C.18. Behavioral Pharmacology

Support: R01MH082773 to CB

T32MH015174 to KMN

Title: Inducible and tissue specific knock-down of the serotonin 1B receptor

Authors: *K. M. NAUTIYAL[1], K. F. TANAKA[4], C. BLANCO[2], S. E. AHMARI[2], R. HEN[3]
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Abstract: The serotonin 1B receptor (5-HT1B R), a GPCR that inhibits neurotransmitter release in axon terminals of serotonergic and non-serotonergic neurons, has been implicated in aggressive behavior and impulsivity using pharmacology and constitutive knock-out mouse models. The broad localization of 5-HT1B R in the basal ganglia, hippocampus, thalamus, cerebellum and raphe leaves open the possibility for the modulation of various neurotransmitter systems involved in the regulation of aggressive and impulsive behavior. Given evidence of serotonergic modulation of brain development, an inducible and tissue specific knock-out is necessary to delineate the role of 5-HT1B R in aggression and impulsivity. We have therefore generated a mouse line that permits temporal and spatial regulation of 5-HT1B R. A tetracycline operator (tetO) has been inserted between the promoter and coding region of the 5-HT1B R (tetO1B). Receptor binding and in situ hybridization revealed that these tetO1B mice have normal basal 5-HT1B R expression in the brain compared to 129 WT mice. Crossing tetO1B mice to transgenic mice expressing the tetracycline-dependent transcriptional silencer (tTS) under the control of β-Actin, CamKII or Pet promoters yielded mouse lines with tissue specific and inducible knock-down of 5-HT1B R . Initial characterization with autoradiography and in situ hybridization reveals promoter-specific 5-HT1B R expression patterns. β-Actin -tTS/tetO1B mice have complete elimination of all brain 5-HT1B R. CamKII-tTS/tetO1B mice lack 5-HT1B heteroreceptors in forebrain regions including the striatum, cortex and hippocampus. Pet-tTS/tetO1B mice have reduced autoreceptor 5-HT1B mRNA in the raphe. Behavioral assays using these mouse lines will assess the relative contribution of hetero- and auto-receptors to aggression and impulsivity. The developmental impact of altered 5-HT1B R expression in the brain will also be examined.

Disclosures: K.M. Nautiyal: None. **K.F. Tanaka:** None. **C. Blanco:** None. **S.E. Ahmari:** None. **R. Hen:** None.

Poster

875. Monoamines and Behavior: Serotonin and Histamine

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Topic: C.18. Behavioral Pharmacology

Support: NARSAD Young Investigator Award

Vanderbilt Conte Center

RO1-MH086629

Title: Modulation of behavior by 5-HT6 receptors in mice

Authors: R. J. BLUETT[1], T. P. SABORIDO[1], M. A. BUENDIA[1], D. P. BERMINGHAM[2], C. E. KILLINGER[1], *G. D. STANWOOD[1]
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Abstract: Serotonin is a critical modulator of stress, anxiety, and depression. Variations in the 5-HT6 receptor gene, in particular, have been associated with the pathophysiology and pharmacological treatment of several neurological and neuropsychiatric diseases. It has been hypothesized that 5-HT6 receptors may be a useful therapeutic target in the regulation of mood and cognition. In the current studies, we first examined the behavioral effects of a specific 5-HT6 receptor antagonist (SB-399885) and agonist (EMD-386088) in mice. Acute SB-399885 produced a dose-dependent sedative effect, while EMD-386088 induced hyperactivity. SB-399885 also decreased immobility in the forced swim test, suggesting a potential antidepressant effect. EMD-386088 had no effect on behavioral despair at the doses tested. We also examined behavioral phenotypes of 5-HT6 receptor knockout mice. These mutants did not display phenotypic variations in body weight, body temperature, anxiety or simple learning tasks. However, 5-HT6 receptor null mice were mildly hyperactive in open field tests and also exhibited slight decreases in immobility in tests of behavioral despair. 5-HT6 receptors are expressed prominently in dopamine target regions such as the striatum and frontal cortex, suggesting that the 5-HT6 receptor may be involved in modulating dopaminergic signaling. Preliminary immunoblotting data suggests that dopamine D1 receptors are significantly upregulated in the striatum of 5-HT6 receptor knockout mice, and are modestly decreased in the medial frontal cortex. SB-399885 significantly decreased anxiety responses of D1 receptor null mice as demonstrated by increased time exploring the open sections of an elevated zero maze. Taken together, these data suggest that 5-HT6 receptors affect motor activity and emotional behaviors and may serve as a substrate for functional interactions between the serotonin and dopamine systems.

Disclosures: R.J. Bluett: None. **T.P. Saborido:** None. **M.A. Buendia:** None. **D.P. Bermingham:** None. **C.E. Killinger:** None. **G.D. Stanwood:** None.

Poster

875. Monoamines and Behavior: Serotonin and Histamine

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Topic: C.18. Behavioral Pharmacology

Support: NIMH Intramural Research Program

NIH, NIMH RO1-MH39085

Title: Behavioral and temperature effects of tramadol in mice lacking the genes for monoamine oxidase (MAO) A and B or the serotonin transporter (SERT)

Authors: *M. A. FOX[1], M. G. PANESSITI[1], K. CHEN[2], J. C. SHIH[2], D. L. MURPHY[1]
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Abstract: Tramadol is an atypical opioid, which in addition to relatively weak actions on opioid receptors affects the reuptake and release of serotonin and norepinephrine, resulting in increased extracellular fluid (ECF) serotonin and norepinephrine levels. In mice, drugs that increase ECF serotonin levels can have a number of behavioral and physiological effects. For example, a cluster of behaviors (the "serotonin syndrome," S/S) is induced by increased serotonin levels, behaviors which include head weaving, forepaw treading, backward movement, hind limb abduction, Straub tail, tremor and low body posture. In addition, serotonin-enhancing drugs have been shown to induce hypothermia in mice. These behavioral and temperature effects are enhanced in conditions of high levels of ECF serotonin, for example following administration of a serotonin-enhancing drug such as the serotonin precursor 5-hydroxy-L-tryptophan (5-HTP), a serotonin reuptake inhibitor (SRI) or a monoamine oxidase inhibitor (MAOI). In addition, several genetic manipulations lead to high baseline ECF serotonin levels in mice, for example mice lacking the serotonin transporter (SERT) or mice lacking MAO A and B. We have previously shown that administration of various serotonin-enhancing drugs induce enhanced S/S behavioral and hypothermic effects in mice lacking SERT. Here, we compare the behavioral (overall S/S behavioral score) and temperature effects of tramadol (60 mg/kg ip) in mice lacking MAOA and B or SERT. Tramadol induced the overall S/S cluster of behaviors in all wildtype (WT) and knockout (KO) mice compared to vehicle, and these behaviors were increased in both MAOA/B-KO mice (~2-fold increase) and SERT-KO mice (~1.5-fold increase) compared to their respective controls. Further, tramadol induced a hypothermic response in all WT and KO mice compared to vehicle, although this effect was decreased in both MAOA/B-KO and SERT-KO mice compared to their respective WT controls. Genetic manipulations such as these evaluated in mice may model and predict adverse effects of many drugs including tramadol, MAOIs and SRIs in humans, as polymorphisms in the SERT and MAO genes in humans are known to reduce gene function. These polymorphisms have begun to be studied in individuals who have been treated with such drugs, taken these drugs in overdose and in suicide attempts, with some similar S/S and physiological consequences.

Disclosures: M.A. Fox: None. **M.G. Panessiti:** None. **K. Chen:** None. **J.C. Shih:** None. **D.L. Murphy:** None.

Poster

875. Monoamines and Behavior: Serotonin and Histamine

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Topic: C.18. Behavioral Pharmacology

Support: Royal Society of New Zealand Marsden Fund

Title: Effects of repeated exposure to MDMA on $5HT_{1a}$ autoreceptor function: Behavioral and neurochemical responses to 8-OHDPAT

Authors: *D. ARONSEN[1,2], B. ABRAHAM[2], J. COLUSSI-MAS[2], J. DO[2], K. JONES[2], S. SCHENK*[2]
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Abstract: A consistent effect of repeated exposure to MDMA is a decrease in tissue levels of serotonin (5HT). A variety of behavioral and neurochemical tests were conducted to determine whether the tissue deficits could be explained by an increased sensitivity of the $5HT_{1a}$ autoreceptor. Tests were conducted two weeks following MDMA exposure (either 300 mg/kg self-administered during 25-40 daily 2 hr test sessions or 4 injections of 10.0 mg/kg, ip, administered at 2 hr intervals in a single day). The response to several doses of the $5HT_{1a}$ agonist, 8-OHDPAT (0.003-0.5 mg/kg, sc), was measured as lower lip retraction (LLR), hypoactivity, hypothermia and 5-hydroxytryptophan (5HTP) accumulation following decarboxylase inhibition. 8-OHDPAT produced a dose-dependent increase in LLR and hypothermia and a decrease in all other measures, including the accumulation of 5HTP following decarboxylase inhibition. MDMA decreased tissue levels of 5HT and the accumulation of 5HTP but these effects were not reflected in changes in autoreceptor sensitivity. The only effect of MDMA pretreatment was a leftward shift in the dose effect curve for 8-OHDPAT produced hypothermia. Because all of the effects except for hypothermia have been attributed to $5HT_{1a}$ autoreceptor activation, the data suggest that MDMA failed to alter the sensitivity of this receptor. Hypothermia, however, has been attributed to postsynaptic $5HT_{1a}$ activation and this response became supersensitive following MDMA exposure. The data suggest that the decrease in tissue levels of 5HT produced by MDMA is due to a decrease in tryptophan hydroxylase activity and is not related to supersensitivity of the $5HT_{1a}$ autoreceptor.

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Poster

875. Monoamines and Behavior: Serotonin and Histamine

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Topic: C.18. Behavioral Pharmacology

Support: NIH R01 HD042634

R01 MH086539

Title: Fibroblast growth factor 8 deficiency impacts anxiety-like behavior in response to restraint stress in mice

Authors: *L. R. BROOKS, C. L. ENIX, S. C. RICH, C. A. LOWRY, P.-S. TSAI
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Abstract: Vulnerability to a number of psychiatric illnesses, including anxiety, may be neurodevelopmental in origin. Animal models suggest that serotonin (5-HT) neurons in the dorsal raphe nucleus (DR) are needed to establish functional stress- and anxiety- related circuitries, and environmental stressors can affect these circuits to precipitate anxiety-like behaviors. Fibroblast growth factor 8 (Fgf8) is critical for the development of 5-HT neurons, and its deficiency has previously been shown to lead to increased trait anxiety-like behavior in mice. However, it is unclear if the development of 5-HT neurons underlying stress- induced anxiety is also dependent on Fgf8 signaling. Given that stress is an important factor in the onset and expression of anxiety in humans, this study examined both stress-induced anxiety and 5-HT cell numbers in relevant DR subdivisions (dorsal, ventral, ventrolateral, caudal, interfascicular) in wildtype (WT) and *Fgf8*-deficient mice. WT and *Fgf8*-deficient mice were assigned to either a non-stressed or stressed group. Following one hour of no stress or restraint stress, adult *Fgf8*-deficient and WT mice were immediately tested for anxiety-like behavior in the elevated plus-maze (EPM). As expected, non-stressed WT mice spent significantly more time exploring the open arms compared to non-stressed *Fgf8*-deficient mice and their WT stressed counterparts. Surprisingly, stressed Fgf8-deficient mice spent significantly more time in the open arms than their non-stressed controls, suggesting that the stressed Fgf8 Het mice are less anxious than their non-stressed counterparts. There was no genotype difference in the stressed group. These findings suggest that stress impacts anxiety-like behavior differentially in WT and *Fgf8*-deficient mice. Neuroanatomical data support these findings by demonstrating decreases in both the

number and density of neurons immunopositive for the serotonergic marker, tryptophan hydroxylase, in subregions of the DR associated with stress- and anxiety-like behaviors. These results expand our knowledge on the elements governing the development of specific subpopulations of 5-HT neurons, their functional integrity, and their relationship to stress-induced anxiety-related behavior.

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Poster

875. Monoamines and Behavior: Serotonin and Histamine

Location: Hall F-J

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Program#/Poster#:875.06/W14

Topic: C.18. Behavioral Pharmacology

Title: Characterization of the highly selective 5-HT2C receptor agonists lorcaserin and CP-809101 on food and nicotine motivated behaviors in the rat

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Abstract: The therapeutic potential of a drug is governed by both its clinical efficacy and side effect property. Experimental evidence supports 5-HT2C agonists as offering therapeutic potential in indications such as obesity and nicotine dependence although relatively few members of this class have been evaluated. Agonist specific features such as intrinsic efficacy, functional and target selectivity and pharmacokinetics property highlight the need to evaluate multiple 5-HT2C agonists in terms of behavioural profiles predictive of clinical efficacy and side effect liability. Therefore in this study, we have evaluated the highly selective and chemically diverse 5-HT2C agonists CP-809101 and lorcaserin, and the prototypic 5-HT2C agonist Ro 60-0175 on (1) behaviors motivated by either food or nicotine reinforcement, (2) a nicotine drug discrimination procedure, and (3) side effect profiles. Furthermore plasma levels of lorcaserin were determined at a dose (1 mg/kg SC) effective against food and nicotine motivated behavior. Adult, male Sprague-Dawley (SD) rats were used throughout, except for the nicotine self-administration experiments which utilized Long Evans (LE) rats. In rats trained to discriminate nicotine (0.3 mg/kg SC) from saline using standard operant procedures, neither CP-809101 (0.3-

6 mg/kg SC), lorcaserin (0.3-3 mg/kg SC), nor Ro 60-0175 (0.3-2 mg/kg SC) engendered reliable nicotine responding across a pharmacologically relevant dose range, however in combination tests each produced a rightward shift in the nicotine D/R implying a weakening of the discriminative property of nicotine. In separate studies, each drug similarly reduced responding for both intravenous nicotine (0.03 mg/kg/infusion) and food made available under an FR5TO20s schedule of reinforcement (e.g. CP-809101: nicotine (infusions earned): veh=26.5+2.0, 1 mg/kg=20.6+1.6, 3 mg/kg=18.3+1.5, $P<0.01$; food (pellets earned): veh=148+3, 1 mg/kg=135+4, 3 mg/kg=104+10, $P<0.01$). In tests of feeding induced by either palatability or 22 h deprivation, each drug similarly reduced overall intake although palatability-induced feeding seemed the more sensitive. At high doses, both CP-809101 and lorcaserin (3-6 mg/kg) produced behavioural signs reflective of malaise, a reported side-effect of lorcaserin at supratherapeutic doses (Schram et al, 2011, Clin. Pharmacol. Ther. 89: 683-692). At a dose of 1 mg/kg SC, plasma levels of lorcaserin in LE and SD rats were similar (LE: 118+14 ng/ml; SD: 163+21 ng/ml) further suggesting equivalent effects of 5-HT2C agonists against nicotine and food motivated behaviours.

Disclosures: G.A. Higgins: Employment; InterVivo Solutions Inc. **L.B. Silenieks:** Employment; InterVivo Solutions Inc. **W. Lau:** Employment; InterVivo Solutions Inc. **I.A.M. de Lannoy:** Employment; NoAb BioDiscoveries. **D.K.H. Lee:** Employment; NoAb BioDiscoveries. **A.D. Le:** None. **P.J. Fletcher:** None.

Poster

875. Monoamines and Behavior: Serotonin and Histamine

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Topic: C.18. Behavioral Pharmacology

Support: NIDA Grant DA019114

NIDA Grant 1F31DA032532

Title: Reduced 5-HT1A regulation of anxiety-like behavior in adolescent male rats

Authors: *A. E. ARRANT, C. M. KUHN
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Abstract: Adolescence is marked by normal changes in behavior such as peaks in novelty seeking and risk taking as well as the emergence of mood disorders. Ongoing development of neural circuits between the prefrontal cortex and limbic brain regions, as well as the

monoaminergic systems that modulate these pathways, may underlie these changes in behavior. Acute treatment with indirect serotonergic agonists typically produces increases in behavioral inhibition in rodent anxiety tests. Prior studies in our lab showed that adolescent rats are less sensitive to these anxiogenic effects, and that drug-stimulated increases in extracellular serotonin do not completely explain this insensitivity. The goal of this study was to investigate the role of serotonin receptors in adolescent insensitivity to serotonergic behavioral inhibition.
Adult (PN67-70) and adolescent (PN28-32) rats were tested in the light/dark (LD) test for anxiety-like behavior after administration of the mixed 5-HT2 agonist meta-chlorophenylpiperazine (mCPP, 0.5 or 1 mg/kg i.p.), the 5-HT1A agonist 8-hydroxy-2-(dipropylamino)tetralin (8-OH DPAT, 0.25 or 0.5 mg/kg i.p.), or vehicle. mCPP was similarly anxiogenic to each age group, but 8-OH DPAT produced greater increases in anxiety-like behavior in adults compared to adolescents. Treatment with 8-OH DPAT (0.5 mg/kg i.p.) also produced greater activation of brain regions associated with anxiety-like behavior in adult rats, as measured by c-Fos immunostaining. We then performed site-specific injection of 8-OH DPAT into the prelimbic region of the medial prefrontal cortex to determine if the prefrontal cortex could be involved in the reduced effects of systemic 8-OH DPAT in adolescents. Infusion of 8-OH DPAT (100 or 200 ng) into the prelimbic cortex produced anxiogenic effects in adults, but not adolescents.
These data indicate that 5-HT1A modulation of anxiety-like behavior is immature in male adolescent rats. Available data generally demonstrate mature levels of 5-HT1A expression by adolescence in rodents, but experiments in both rodents and humans show immature connectivity between the prefrontal cortex and limbic brain regions such as the amygdala. The lack of anxiogenic effects of 8-OH DPAT in adolescent rats may reflect this immature circuitry. Reduced serotonergic regulation of these neural pathways in adolescents could result in altered responses to drugs that target the serotonergic system, including therapeutic drugs such as SSRIs or drugs of abuse such as the psychostimulants cocaine and MDMA.

Disclosures: A.E. Arrant: None. **C.M. Kuhn:** None.

Poster

875. Monoamines and Behavior: Serotonin and Histamine

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The Diane Goldberg Foundation

Title: Correlation between 5-HT1A mRNA expression and number of mitotic and progenitor cells in the human hippocampus

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Abstract: The serotonin 1A receptor (5-HT1AR) is implicated in morphogenesis. Stimulation of the 5-HT1A receptor is necessary for the proliferative response of cells treated with serotonin: antagonists of 5-HT1A receptor block this effect; 5-HT1AR knock-out mice do not show increased hippocampal neurogenesis and behavioral changes in response to fluoxetine. In response to stress, 5-HT1AR density and mRNA levels decrease via adrenal axis stimulation and hippocampal neurogenesis is reduced. In subjects with major depression (MDD) treated with antidepressants we found more mitotic and neural progenitor cells (NPCs) than in untreated MDDs and controls, selectively in the anterior dentate gyrus (DG). We aimed to asses if more mitotic cells and NPCs were associated with more 5-HT1AR mRNA in the human DG. Psychological autopsy identified 10 untreated subjects with major depression (MDD), nine MDD treated with antidepressants, nine non-psychiatric controls, matched for age, sex and postmortem interval. At autopsy, brain pH, neuropathology, and toxicology exams were performed. Frozen whole hippocampi were fixed and sectioned at 50 μm, mitotic cells and NPCs labeled by immunocytochemistry on sections at 2-mm intervals thoughout the anterior, mid and posterior DG and their number estimated by stereology. We carried out 5-HT1AR in situ hybridization using an 35S-labeled 5-HT1AR riboprobe and quantified the density of mRNA in CA1, CA3, DG and white matter.
In MDDs treated with tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI) or lithium, 5-HT1AR mRNA density (μCi/g) in the anterior DG correlates positively with mitotic cell density ($r = .987$, $p = .002$). In untreated MDDs, density of NPCs in the mid DG

correlates positively with 5-HT1AR mRNA density in the mid CA1 (r =.766, p=.027) and number of NPCs in the mid DG correlates with 5-HT1AR mRNA density in CA3 (r =.782, p=.022). In controls there is a positive correlation between number of NPCs in the anterior DG and 5-HT1AR mRNA density in the CA3 (r =.782, p=.038).
Results suggest role of 5-HT1AR in DG cell replication in the human brain. A larger sample will allow differentiating the effect of different classes of antidepressants.

Disclosures: A.N. Santiago: None. **S.A. Kassir:** None. **S. Migliarini:** None. **M.J. Bakalian:** None. **K.M. Burke:** None. **G. Musolino:** None. **C.B. Palavicino-Maggio:** None. **G.B. Rosoklija:** None. **A.J. Dwork:** None. **J.J. Mann:** None. **M. Pasqualetti:** None. **R. Hen:** None. **V. Arango:** None. **M. Boldrini:** None.

Poster

875. Monoamines and Behavior: Serotonin and Histamine

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:875.09/W17

Topic: C.18. Behavioral Pharmacology

Support: R01-MH07083-5

NARSAD Young Investigator Award 2010

Title: Prenatal fluoxetine exposure: Mitigation of social, emotional, motor and cognitive abnormalities with neonatal tactile stimulation.

Authors: *J. A. MILSTEIN[1], K. PLACEK[2], W. EHRIG[3], C. M. BOUSLOG[2], L. M. SITZ[2], D. O. FROST[1,4], A. M. BAILEY[2]
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Abstract: The selective serotonin reuptake inhibitor (SSRI) fluoxetine (FLU) is the recommended antidepressant treatment for maternal depression during pregnancy, but studies remain inconclusive regarding the long-term effects of prenatal exposure on offspring. In rodent models, prenatal FLU exposure has caused long-term anomalies, including increased anxiety and depression-like behaviors, deficits in motor development, altered cognitive ability, and decreased complexity in frontal lobe neuronal structure. Tactile stimulation, a form of environmental enrichment, offers potential as an intervention to compromised neurodevelopment by increasing dendritic spine length and density in the rat frontal cortex following neonatal cortical injury,

reducing anxiety-like behaviors in adult rats subject to early-life emotional stress, and serving a neuroprotective mechanism to the hippocampus following induced neonatal hypoxia-ischemia in rats. We aimed here to test the long-lasting effects of neonatal tactile stimulation on subjects exposed to FLU prenatally. Female Long-Evans rats were administered FLU (10mg/kg/day) or a vehicle solution via the drinking water from gestational day 1 (GD1) though postnatal day 7 (PD7). One-half of the pups from each litter were given tactile stimulation from PD3-PD21. During the adolescent period, PD25-PD35, animals were measured for social play behavior. Males exposed to FLU and given tactile stimulation showed fewer defensive behaviors (p=.053) and more social interaction than non-stimulated FLU-exposed peers (p < .001). At PD 90 all animals were tested for motor ability in the rotarod test, for spatial learning and reversal in an operant box, and for emotionality in an elevated plus maze and forced swim test. Fluoxetine impaired motor performance in females on the rotarod task (p = .051). In the operant box, FLU-exposed females were no different in acquisition of a spatial rule but showed a significant increase in the number of perseverative errors (p = .013) when the spatial rule was reversed. In the elevated plus maze, males exposed to FLU but given tactile stimulation showed decreased anxiety-like behavior compared to non-stimulated FLU-exposed peers (p = .002). Finally, females exposed to FLU showed a significant increase in time spent immobile (p=.026) in the forced swim test. The interaction between prenatal exposure to FLU and neonatal tactile stimulation on behavioral outcomes will be discussed.

Disclosures: J.A. Milstein: None. **K. Placek:** None. **W. Ehrig:** None. **C.M. Bouslog:** None. **L.M. Sitz:** None. **D.O. Frost:** None. **A.M. Bailey:** None.

Poster

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Program#/Poster#:875.10/W18

Topic: C.18. Behavioral Pharmacology

Support: NIH

NIMH

Title: Fluoxetine exposure during adolescence disrupts spatial memory performance in adulthood.

Authors: *S. D. INIGUEZ[1], M. J. STONE[2], R. A. RODRIGUEZ[3], G. DAYRIT[3], L. F. ALCANTARA[4], B. L. WARREN[4], E. M. PARISE[4], K. N. WRIGHT[4], C. A. BOLAÑOS-GUZMAN[4]

[1]Psychology, Cal State San Bernardino, San Bernardino, CA; [2]Psychology, [3]California State Univ., San Bernardino, CA; [4]Psychology, Florida State Univ., Tallahassee, FL

Abstract: Epidemiological reports indicate that major depressive disorders (MDD) are common in children and adolescents. The prevalence of adolescent MDD has resulted in parallel increases in the prescription of fluoxetine (FLX) and related serotonin reuptake inhibitor antidepressants within this population. Although such treatments can last for years, very little is known about the long-term consequences of antidepressant exposure during developmental periods prior to adulthood on memory performance later in life. Thus, we exposed adolescent male c57BL/6 mice (postnatal day [PD] 35) to FLX (10 mg/kg, twice daily) for 15 consecutive days. We then assessed animals' behavioral performance on the Morris Water Maze spatial memory task when they reached adulthood (PD 70+). Specifically, mice were trained to find the location of a submerged escape platform on a single day task (8 training trials), and memory for the platform location was re-tested after a 24 hr delay (distance traveled and velocity). To increase the demands of the spatial task, the mice returned to the spatial task once again, 24hr later, and completed a probe trial (escape platform absent), and the time to reach the quadrant of the target platform location, as well as total time spent in the quadrant were recorded. We found that FLX exposure during adolescence did not influence spatial memory acquisition on the training day. In addition, no differences between the groups were observed when spatial memory was examined 24 hr after training. On the other hand, mice exposed to FLX during adolescence swam longer distances to reach the location of the missing platform, when tested 48 hr after training (probe trial). Our results suggest that as the demands of the spatial memory task increase, spatial memory deficiencies become apparent in adult mice exposed to FLX during adolescence. Overall, these results underscore the need for further research for a clearer understanding of the long-term functional effects of FLX exposure on the developing nervous system.

Disclosures: S.D. Iniguez: None. **M.J. Stone:** None. **R.A. Rodriguez:** None. **G. Dayrit:** None. **L.F. Alcantara:** None. **B.L. Warren:** None. **E.M. Parise:** None. **K.N. Wright:** None. **C.A. Bolaños-Guzman:** None.

Poster

875. Monoamines and Behavior: Serotonin and Histamine

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:875.11/W19

Topic: C.18. Behavioral Pharmacology

Title: SB 399885: 5-HT_6 receptor antagonist is efficacious in animal models of food addiction and craving

Authors: *D. SHANMUGANATHAN, B. KANCHARLA, P. JAYARAJAN, R. NIROGI
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Abstract: Extensive research is being focused on the development of new pharmacological agents to control the food addiction/binge eating behaviour, which is commonly associated with bulimia nervosa, binge eating disorder and obesity. Hedonic properties of palatable food and addictive drugs are regulated by common brain substrates. 5-HT_6 receptor is involved in the central regulation of feeding and highly expressed in dopamine rich areas. In this study, the effect of 5-HT_6 antagonist on food addiction and craving was evaluated using palatable food induced binge eating and conditioned place preference task respectively. To investigate the role of 5-HT_6 receptor on glutamate mediated feeding control, effect of SB 399885 on dizocilpine induced hyperphagia was conducted. To rule out the nonspecific effects, behaviour satiety sequence was performed. SB 399885 significantly reduced the palatable diet consumption in binge eating rats and effectively blocked the expression of palatable food induced place preference. It significantly overturned the dizocilpine induced hyperphagia. SB 399885 reduced the frequency of eating behaviour without changing the other behavioural components of satiety sequence in rats. These results demonstrate that SB 399885 has potential to reduce the over-responsiveness to palatability through the enhancement of satiety signals and inhibition of palatable food induced craving. In conclusion, 5-HT_6 antagonism appears to be a useful pharmacological approach for the treatment of food addiction and associated disorders.

Disclosures: D. Shanmuganathan: Employment; Suven Life sciences. **B. Kancharla:** Employment; Suven Life sciences. **P. Jayarajan:** Employment; Suven Life sciences. **R. Nirogi:** Employment; Suven Life sciences.

Poster

875. Monoamines and Behavior: Serotonin and Histamine

Location: Hall F-J

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Program#/Poster#:875.12/W20

Topic: C.18. Behavioral Pharmacology

Support: GM38931

Title: A novel polymorphism (a378t) in the coding region of htph2 with unique effects on enzyme properties

Authors: *I. TEKIN[1], N. CARKACI-SALLI[1], M. C. BEWLEY[2], S.-S. SUNG[1], K. E. VRANA[1]
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Abstract: Tryptophan hydroxylase (TPH) catalyzes conversion of the aromatic amino acid tryptophan to 5-hydroxytryptophan, which is then converted to serotonin. Conversion of tryptophan to 5-hydroxytryptophan is the rate-limiting step in the biosynthesis of this neurotransmitter. The enzyme requires binding to iron in its ferrous form in order to be active. Recently, a single nucleotide polymorphism (rs78369038) in TPH2 coding region has been reported. Molecular modeling of the variant protein, A378T, suggests that replacement of the original alanine residue at position 378 with threonine prevents iron binding to the active site of the enzyme. Characterization of the effects of this SNP on TPH2 has been performed *in vitro* using recombinant protein. Radioenzyme activity assays showed that, this SNP causes disruption of activity of the enzyme. Disruption of iron binding seems to be due to occupation of the iron binding site by the relatively large side chain of threonine. This hypothesis has been tested via targeted mutagenesis of alanine at 378 into other amino acids with different structural properties (e.g. valine, glycine, methionine, and serine). Characterization of the protein bearing each of these mutations provides insight into the structural effects of these amino acid changes at the corresponding residue. The extent of iron binding for the different mutations remains to be determined.

Disclosures: I. Tekin: None. **N. Carkaci-Salli:** None. **M.C. Bewley:** None. **S. Sung:** None. **K.E. Vrana:** None.

Poster

875. Monoamines and Behavior: Serotonin and Histamine

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Topic: C.18. Behavioral Pharmacology

Support: NIH 5T32-DA-015040-09

H. Lundbeck, A/S

Burroughs Wellcome Fund

National Primate Research Center base grant #2P51RR000165-51

Title: Variations in escitalopram's behavioral effects due to the presence or absence of an allosteric site on the serotonin transporter

Authors: *K. E. MURRAY[1], K. J. RESSLER[1,2], M. J. OWENS[1]
[1]Psychiatry and Behavioral Sci., Emory Univ., Atlanta, GA; [2]HHMI, Atlanta, GA

Abstract: Escitalopram, the S-enantiomer of citalopram, is a selective serotonin reuptake inhibitor (SSRI) that binds to the serotonin transporter (SERT) to inhibit serotonin uptake. In addition to its classic SSRI mechanism it has been shown that a second escitalopram molecule is also able to bind allosterically to a distinct site on the SERT. The effects of this allosteric site are not fully understood. It is hypothesized that this allosteric site may contribute to the efficacy of escitalopram, therefore the site is thought to be a potential site for future drug development to enhance SSRI action and to understand SERT function. This study uses novel transgenic mice with either a wildtype human SERT (hSERT-wt) transgene or an allosteric-null hSERT (hSERT-mut) to examine behavioral outputs of the presence or absence of the allosteric site. Previously wildtype C56Bl/6J were shown to have decreased time immobile in the tail suspension test (TST) with increasing escitalopram doses (> 10 mg/kg escitalopram oxalate) but this same effect was not observed in the transgenic lines on a 129Sv (Taconic) strain background. This study aims to identify if the loss of escitalopram's effects is due to the strain background or if a difference in the SSRI's interaction with the human transgenes rather than the wildtype mouse SERT. Transgenic mice of both hSERT-wt and hSERT-mut strains on a C57Bl/6J background are submitted to a 6 minute TST at weekly intervals for 6 weeks to complete a dose-response curve over 6 doses (0-32 mg/kg escitalopram oxalate, i.p.). A shift in the dose-response curve for behavioral outcome between the hSERT-wt and the hSERT-mut lines would indicate of a role for the allosteric site in the escitalopram-hSERT interaction. In particular, a rightward shift in the hSERT-mut mouse dose-response curve compared to the hSERT-wt mouse curve would indicate that the allosteric site enhances escitalopram's actions.

Disclosures: K.E. Murray: None. **K.J. Ressler:** None. **M.J. Owens:** Research Grant; NIH, Lundbeck A/S, Cyberonics, Ortho-McNeil Janssen, AstraZeneca, Dainippon Sumitomo Pharma, SK Life Sciences, Sunovion Pharmaceuticals. Consultant/Advisory Board; H. Lundbeck A/S, R.J. Reynolds. Other; Patent: Method of assessing antidepressant drug therapy via transport inhibition of monoamine neurotransmitters (US 7,148,027 B2).

Poster

875. Monoamines and Behavior: Serotonin and Histamine

Location: Hall F-J

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Topic: C.18. Behavioral Pharmacology

Support: NIH Grant R01MH39085

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Boyd and Elsie Welin Professorship

Title: Postnatal blockade of the 5-HT2A decreases aggression in monoamine oxidase A-deficient mice

Authors: S. C. GODAR, M. BORTOLATO, S. TAMBARO, F. LI, K. CHEN, *J. C. SHIH
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Abstract: Monoamine oxidase (MAO) A is the primary enzyme responsible for the metabolism of serotonin (5-HT) and norepinephrine (NE) in the brain. Although the role of MAO A in aggression and emotional reactivity is well-established, the molecular underpinnings of this link remain elusive. MAO A knockout (KO) mice exhibit overt aggressiveness and social deficits, as well as elevated levels of brain 5-HT, especially during early developmental stages. Previous data have shown that 5-HT2A receptors play a key role in the control of aggressive responses. In order to examine the role of early postnatal 5-HT2A activation in the aggressive responses exhibited by MAO A KO mice, we studied the changes in resident-intruder aggression in these animals after treatment with the 5-HT2 receptor antagonist ketanserin (3 mg/kg/day) during the first two weeks of postnatal life. MAO A KO mice treated with ketanserin during the first postnatal week exhibited a robust reduction of fighting duration and number of aggressive bouts, as well as a marked increase in the latency to the first attack. In view of the lack of specificity of ketanserin for 5-HT2A receptors, we tested our hypothesis with a parallel set of experiments, using a transgenic model of MAO A/ 5-HT2A double KO mice. Similarly to ketanserin-treated mice, 5-HT2A/MAO-A double KO mice exhibited a marked decrease in fighting behavior. These data collectively suggest that 5-HT2A hyperactivation in early stages are critical in the aggressive responsiveness and antisocial trait resulting from low or no MAO A activity in early developmental stages.

Disclosures: S.C. Godar: None. **M. Bortolato:** None. **S. Tambaro:** None. **F. Li:** None. **K. Chen:** None. **J.C. Shih:** None.

Poster

876. High Throughput and Therapies

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:876.01/X3

Topic: C.19. Drug Discovery and Development

Support: Kunshan City, Jiangsu, China

KSDN, Jiangsu, China

Title: Innovations on drug discovery of Alzheimer's and other CNS diseases: Phenotypic screening with *Drosophila* disease models

Authors: *I.-F. PENG[1], Y. ZHONG[2,3]
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Abstract: In current drug discovery pipeline, in vitro biochemical and cell-based screening assays have been widely used before the pharmacological validation tests in in vivo animal models. Nevertheless, the low translation rate of drug candidates entering into in vivo systems impairs the pipeline progress in most CNS diseases with uncertain and/or complicated mechanisms. In parallel to those in vitro assays, we have developed several Drosophila CNS disease models as supplementary approaches. These animal models preserve behavioral deficits and pathological hallmarks similar to those observed in human patients, yet are low in running cost and are suitable for high-throughput screening expansions.
We have generated the fly Alzheimer's disease (AD) model through targeted expression of a transgene encoding a secretive form of human A-betaβ42 peptides in the Drosophila brain. Such expression leads to neurodegeneration with accumulation of A-beta 42, fibril deposits in the brain area, shortened life span, and severe age-dependent memory loss, which highly mimicking neuronal death and dementia syndromes in AD human patients. A computer-controlled massive screening system for olfactory learning assay has been developed on site, enabling high throughput screening studies for industrial needs. In our internal screening study of 5,000 compounds, we have identified >20 potential drug candidates for AD treatments. Interestingly, >50% of these candidates show potency on rescuing the memory loss in transgenic mice models. The toxicity and safety studies are carrying on to support further clinical studies in humans.
In addition to AD, we have also developed several other Drosophila disease models (Parkinson's disease, Schizophrenia, autism, Noonan syndrome, and neurofibromatosis type 1/von Recklinghausen disease) for drug discovery services.

Disclosures: I. Peng: None. **Y. Zhong:** None.

Poster

876. High Throughput and Therapies

Location: Hall F-J

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Program#/Poster#:876.02/X4

Topic: C.19. Drug Discovery and Development

Support: CIHR Grant

Title: Two-photon microscopy reveals that astrocytes and microglia contribute to blood-brain barrier disruption by focused ultrasound

Authors: *A. BURGESS[1], T. NHAN[1,2], A. KEATING[3], K. HYNYNEN[1,2]
[1]Imaging Res., Sunnybrook Res. Inst., Toronto, ON, Canada; [2]Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada; [3]Cell Therapy Program, Princess Margaret Hosp., Toronto, ON, Canada

Abstract: Focused ultrasound (FUS) has been used to successfully disrupt the blood-brain barrier (BBB), aiding in the delivery of therapeutic agents to the brain and leading to improvements in disease treatment. However, the mechanisms of BBB disruption and the microscopic effects of this disruption on resident brain cells are not well understood. In this study, we combine a custom-built ultrasound transducer with two-photon microscopy to conduct real time monitoring of BBB disruption in vivo. We have manufactured and tested a single element piezoelectric transducer with frequencies ranging from 1.15 to 1.30 MHz. Transgenic Wistar rats, expressing enhanced green fluorescent protein (EGFP), were anesthetized and a cranial window was made in the skull. The transducer, attached to a coverslip, was glued to the skull, covering the cranial window. Dextran-conjugated Texas Red (70 kDa) was injected intravenously. FUS (0.2-0.3 MPa estimated in situ pressure, 10 ms pulses, 1Hz pulse repetition frequency for a total duration of 120 s) was applied in the presence of preformed microbubbles. In vivo, we observed the retraction of astrocytic endfeet from the vessel wall at the onset of BBB disruption. Follow-up imaging (4-6 hours after initial BBB disruption) suggests that the endfeet are reattaching to the vessels and likely contributing to the functional recovery of the BBB. We also observed migration of EGFP-expressing glial cells towards disrupted vessels. Post-mortem immunohistochemistry was used to confirm these results. Real time, in vivo monitoring of FUS-induced BBBD will significantly enhance the understanding of the fundamental mechanisms and biological effects of this promising drug delivery technique.

Disclosures: A. Burgess: None. **T. Nhan:** None. **A. Keating:** None. **K. Hynynen:** None.

Poster

876. High Throughput and Therapies

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Topic: C.19. Drug Discovery and Development

Support: OT10/48

IOF.KP 08/007

KU Leuven - R&D

Title: Efficacy and safety of novel liposome-based vaccines against Tau pathology.

Authors: *C. THEUNIS[1], N. CRESPO-BIEL[1], V. GAFNER[2], O. ADOLFSSON[2], P. BORGHGRAEF[1], H. DEVIJVER[1], D. T. HICKMAN[2], N. CHUARD[2], M. P. LOPEZ DEBER[2], P. REIS[2], A. L. BUCCARELLO[2], M. PIHLGREN[2], A. PFEIFER[2], F. VAN LEUVEN[1], A. MUHS[2]
[1]LEGTEGG, KU Leuven, Leuven, Belgium; [2]AC Immune, Lausanne, Switzerland

Abstract: Primary tauopathies cause neurodegeneration, thought by misfolding and subsequent aggregation of protein Tau into oligomers and fibrils of unknown structure. Further aggregation yields neurofibrillary tangles in soma and neuropil threads in processes, as the argyrophilic markers for post-mortem pathological diagnosis. In Alzheimer's disease (AD), tauopathy is proposed to be secondary to, but co-diagnostic with amyloid pathology, while respective contributions to clinical course and final dementia remains open for analysis and debate. Therapeutic approaches have yet to prove specificity and efficacy, emphasizing the need for additional treatment options. Immunotherapy is emerging in pre-clinical studies as a promising approach for clearing misfolded proteins, despite the fact that protein Tau is mainly an intra-cellular target. Consequently, thorough preclinical assessment of Tau-based immunotherapy is instrumental to prove potential, but above all safety.
We analyzed efficacy and safety of newly generated phospho-peptides embedded in synthetic liposomes as Tau-targeted vaccines in Tau.P301L and bigenic Tau.P301LxGSK3b mice (biGT) mice, validated pre-clinical models (Terwel et al, 2005, 2008). Liposome-based vaccines were essentially prepared as for amyloid peptides (Muhs et al, 2007) to display synthetic phospho-peptides that cover important "patho-topes" of Tau. Injection into wild-type and transgenic mice elicited robust and stable antisera responses against phosphorylated peptides already after 2 immunizations. The polyclonal antisera specifically detected tauopathy in brain sections of biGT mice with proven tauopathy (Terwel et al., 2008), labeling neurofibrillary tangles and neuropil threads in hippocampus and cortex, and corroborated biochemically by western blotting. Specific ELISA demonstrated high and stable antibody titers against synthetic phospho-Tau-peptides as opposed to negative reaction with non-phosphorylated Tau-peptides. Neuro-inflammatory reactions or other negative side-effects were not observed in brain of transgenic mice after long-term regimes of vaccination. Re-stimulation of splenocytes from immunized mice with corresponding phospho-Tau peptides did not trigger more secretion of IFN-g or IL-4 than from naive splenocytes. After 3 months, immunized Tau.P301L mice presented with improved survival, delayed motor-impairment, reduced loss of body-weight and decreased phospho-Tau isoforms in brain.
The data corroborate the premise that liposome based phospho-Tau peptide vaccines have considerable potential as safe treatment option for Tau-mediated neurodegenerative diseases.

Disclosures: **C. Theunis:** Other Research Support; AC Immune. **N. Crespo-Biel:** None. **V. Gafner:** None. **O. Adolfsson:** None. **P. Borghgraef:** None. **H. Devijver:** None. **D.T. Hickman:** None. **N. Chuard:** None. **M.P. Lopez Deber:** None. **P. Reis:** None. **A.L. Buccarello:** None. **M. Pihlgren:** None. **A. Pfeifer:** None. **F. Van Leuven:** Consultant/Advisory Board; AC Immune. **A. Muhs:** None.

Poster

876. High Throughput and Therapies

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Program#/Poster#:876.04/X6

Topic: C.19. Drug Discovery and Development

Support: ADDF #291208

Title: Small molecules can rescue progranulin haploinsufficiency underlying frontotemporal lobar degeneration caused by GRN mutations

Authors: L. DE MUYNCK[1,2], S. RAITANO[3], M. GERAERTS[3], R. VANDENBERGHE[4], L. VAN DEN BOSCH[1,2], M. M. MAXWELL[5], A. G. KAZANTSEV[5], R. H. BROWN JR[6], W. ROBBERECHT[4,2,1], C. VERFAILLIE[3], *P. VAN DAMME[7,2]
[1]Lab. of Neurobiology, Vesalius Res. Ctr., VIB, Leuven, Belgium; [2]Exptl. Neurol. and Leuven Res. Inst. for Neurodegenerative Dis. (LIND), [3]Interdepartmental Stem Cell Inst., Univ. of Leuven, Leuven, Belgium; [4]Dept. of Neurol., Univ. Hosp. Leuven, Leuven, Belgium; [5]MassGeneral Inst. for Neurodegenerative Disease, Massachusetts Gen. Hosp., Harvard Med. Sch., Boston, MA; [6]Dept. of Neurol., Univ. of Massachusetts Med. Ctr., Worcester, MA; [7]Dept. of Neurol., Neurol. Department, UZ Leuven, Leuven, Belgium

Abstract: Frontotemporal lobar degeneration (FTLD) is a dementia-type as frequent as Alzheimer's disease in patients under 65. FTLD encompasses a range of neurodegenerative disorders with predominant neuronal loss in the frontal and temporal lobes. The most common cause of familial FTLD are mutations in the progranulin gene (GRN). Patients with GRN mutations have reduced GRN levels in cerebrospinal fluid and blood and GRN haploinsufficiency is thought to be the underlying pathogenic mechanism. In line with the neurotrophic properties of progranulin, pharmacological enhancement of GRN transcription holds a promising therapeutic strategy for FTLD caused by GRN mutations.
An unbiased high-throughput wet-lab screen using a neuro2a cell line in which luciferase expression is driven by a GRN promoter fragment found several compounds that induce an upregulation of luciferase signal. After initial hit confirmation, an in silico analogue search was

performed to identify additional small molecules structurally similar to the hits of the wet-lab screen. Compounds with confirmed GRN-upregulating capacity were further tested in fibroblasts from patients with a GRNIVS1+5G>C mutation and controls. Several small molecules were confirmed to increase GRN production and secretion by transcriptional enhancement in primary human fibroblasts. The reduced GRN mRNA and protein levels in primary cells of patients carrying a GRN loss-of-function mutation were completely restored to normal levels by these compounds.
Our data show that small molecules are capable to overcome GRN-deficiency caused by heterozygous loss of function mutations in GRN.

Disclosures: L. De Muynck: None. **S. Raitano:** None. **M. Geraerts:** None. **R. Vandenberghe:** None. **L. Van Den Bosch:** None. **M.M. Maxwell:** None. **A.G. Kazantsev:** None. **R.H. Brown Jr:** None. **W. Robberecht:** None. **C. Verfaillie:** None. **P. Van Damme:** None.

Poster

876. High Throughput and Therapies

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Program#/Poster#:876.05/X7

Topic: C.19. Drug Discovery and Development

Title: Cellular characterization of histone deacetylase (HDAC) inhibitors

Authors: *R. MÜLLER, H. MACK, M. VAN GAALEN, V. LAKICS
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Abstract: Members of the histone deacetylase (HDAC) family of proteins are widely known for their epigenetic function controlling gene transcription, and they are also reported to be involved in the regulation of the tubulin cytoskeleton and macromolecular breakdown. These processes have important physiological roles and their perturbation has also been linked to cancer and Alzheimer’s disease. In the current study, the effects of selective and pan-HDAC inhibitors were investigated in various pre-clinical model systems.
To study two different functions of HDACs in a cellular environment, we have measured tubulin acetylation by HDAC6 and the acetylation of histones by class I HDACs (e.g. HDAC1/2) in the human cell line NTera2 and also in rat primary neurons. Antibodies against acetylated histone 3 and 4 as well as acetylated tubulin were used in Western blot and immunofluorescence-based high-content analysis. First, the cellular potency and selectivity of a pan-HDAC inhibitor (SAHA), as well as inhibitors reported to be HDAC1/2 specific (CI-994, BRD-6929) or HDAC6 specific (tubastatin A, tubacin) were established. In addition, the effect of tubastatin A and

tubacin on tubulin acetylation and cell death were investigated in primary neurons challenged with different excitotoxins.
SAHA efficiently increased H3 and H4 acetylation both in NTera2 cells and in primary neurons reaching statistically significant effects at a concentration of 1µM. In NTera2 cells, SAHA also increased levels of acetylated tubulin with a similar potency. CI-994 and BRD-6929 affected histone but not tubulin acetylation confirming their selectivity against HDAC6. They were slightly less potent compared to SAHA. Tubastatin A and tubacin mainly affected tubulin acetylation inducing statistically significant changes at 0.1µM and 1µM, respectively. Interestingly, at high concentrations (10µM) these agents also strongly enhanced histone acetylation albeit the considerable cell loss induced likely confounds interpretation of these effects. Treatment of primary neurons with different excitotoxins resulted in cell death and a marked reduction of tubulin acetylation indicative of cytoskeletal damage. SAHA, tubastatin A, and tubacin reversed the de-acetylation, but were unable to protect against the neurotoxicity.
In summary, using Western blot and high-content analysis we have characterised the potency and selectivity of HDAC inhibitors in different cellular environments. These values were very similar in the neuronal cell line NTera2 and in rat primary neurons. Reported neuroprotective effects of HDAC inhibitors against oxidative damage could not be extended to excitotoxic injury.

Disclosures: R. Müller: Employment; Abbott GmbH Co. KG. **H. Mack:** Employment; Abbott GmbHCo. KG. **M. van Gaalen:** Employment; Abbott GmbH Co. KG. **V. Lakics:** Employment; Abbott GmbH & Co. KG.

Poster

876. High Throughput and Therapies

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:876.06/X8

Topic: C.19. Drug Discovery and Development

Support: The Open University

TAP Biosystems

Title: Stabilisation of hydrogels containing neural cells to form CNS tissue models for monitoring neuronal and glial cell behaviour *in vitro*

Authors: C. O'ROURKE[1], J. LOUGHLIN[1], R. DRAKE[2], *J. B. PHILLIPS[1]
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Abstract: Recreating the 3D environment of the CNS using hydrogel matrices allows neurons and glial cells in vitro to behave similarly to their counterparts in vivo, providing a relevant tool for neurobiological studies. The overall aim is to develop engineered neural tissue models to resemble functional CNS tissue, with anisotropic tracts of neurons and glia arranged within a hydrogel, at a scale suitable for drug screening. Stability of cellular hydrogels is important for the generation of engineered tissue models and our approach employs RAFT™ technology (TAP Biosystems) that removes some interstitial fluid, increasing matrix density to stabilise the architecture of the hydrogels. In order to develop a robust CNS tissue model using this method, it was necessary to assess the sensitivity of neural cells to the stabilisation process. Initial experiments used C6 glioma cells seeded within collagen gels which were then subjected to RAFT for 15 min. Cell viability was assessed by propidium iodide staining in control constructs with no treatment and in RAFT-treated constructs at time points of 24 and 72 hours. Results demonstrated that the RAFT process did not reduce cell viability when compared to controls. After 24 hours, 0.58 ± 0.14 % cell death was seen in the treated constructs and 1.38 ± 0.26 % was viewed in controls. After 72 hours, a similar pattern was observed with cell death of 0.33 ± 0.06 % in treated and 1.80 ± 0.27 % in controls. Following initial tests with C6 glioma cells, subsequent experiments used the same approach to assess the viability of a range of primary rat CNS neural cells and other relevant cell lines including astrocytes, oligodendrocytes, microglia and neurons. CNS co-culture systems will incorporate various combinations of cells that can be tailored to suit specific neuroscience research requirements and assessing the viability of cells is a critical step in the development of culture models. Stabilisation of the cellular hydrogels through increasing the cell and matrix density in this way will be used to establish versatile engineered neural tissues. A consistent model system will provide experimental platforms to monitor neural cell morphology, proliferation, differentiation and sensitivity, as well as neuron-glial interactions to generate rapid, reliable and reproducible results.

Disclosures: C. O'Rourke: None. **J. Loughlin:** None. **R. Drake:** Employment; TAP Biosystems. **J.B. Phillips:** None.

Poster

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Topic: C.19. Drug Discovery and Development

Support: Michael J Fox Foundation

Title: Autophagy enhancers as potential therapeutics for neurodegenerative diseases: using a flow cytometry based assay to monitor autophagy flux

Authors: *W. SHEN, S. HALLOWELL, K. L. PAUMIER, L. LI, P. LOOS, D. GEBHARD, R. DOYONNAS, A. F. STEPAN, T. T. WAGER, J. DUNLOP, W. D. HIRST, Z. BERGER
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Abstract: Macroautophagy (referred to here as autophagy) is a lysosome-dependent degradation mechanism. Although autophagy was initially identified as a starvation response, it plays critical roles in many biological processes, including degradation of damaged organelles, misfolded proteins and protein aggregates. As the aggregate-prone mutant proteins, such as mutant α-synuclein and huntingtin, play a prominent role in the pathogenesis of various neurodegenerative disorders, it has been hypothesized that an increase in autophagy could represent a potential therapeutic strategy for these diseases. Thus, it is of increasing interest to identify novel mechanisms that enhance autophagy as therapeutic targets for neurodegenerative diseases. Many conventional assays, such as GFP-LC3 based image analysis do not distinguish autophagy enhancers from blockers, as the accumulation of autophagosomes can result either from an increase in the formation or a decrease in the clearance of autophagosomes. Therefore it is critical to develop a reliable assay that measures autophagy flux.
Here we describe a flow cytometry based assay to monitor autophagy flux in vitro. We generated a monoclonal, inducible cell line that expresses GFP-SQSTM1/p62 fusion protein upon addition of doxycycline. SQSTM1/p62 (referred to as p62) is a ubiquitin-binding protein that targets ubiquitinated proteins for degradation by autophagy; p62 itself is also degraded in this process. We examined this degradation of GFP-p62 in response to known small molecule autophagy modulators and found that autophagy enhancers such as rapamycin and PI-103 increase the rate of degradation of GFP-p62, whereas the autophagy inhibitor Bafilomycin A1 had the opposite effect. We also tested the effect of siRNA knock-down of several autophagy regulatory genes on the degradation of GFP-p62. siRNA knock down of mTOR decreased the accumulation of GFP-p62, while knock down of ULK1 increased the GFP-p62 abundance. Our data suggested that this GFP-p62 based assay measures the autophagy flux and discriminates autophagy enhancers from blockers. In summary, these results, suggest that this GFP-p62 based flow cytometry assay can be adapted for moderate to high throughput compound or genome-wide siRNA screen for autophagy enhancers and inhibitors.

Disclosures: W. Shen: Employment; Pfizer. **S. Hallowell:** Employment; pfizer. **K.L. Paumier:** Employment; pfizer. **L. Li:** Employment; Pfizer. **P. Loos:** Employment; Pfizer. **D. Gebhard:** Employment; pfizer. **R. Doyonnas:** Employment; pfizer. **A.F. Stepan:** Employment; Pfizer. **T.T. Wager:** Employment; pfizer. **J. Dunlop:** Employment; Pfizer. **W.D. Hirst:** Employment; Pfizer. **Z. Berger:** Employment; Pfizer.

Poster

876. High Throughput and Therapies

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Program#/Poster#:876.08/X10

Topic: C.19. Drug Discovery and Development

Support: Discretionary funding to GMP

Title: The role of histone deacetylase inhibitors in the mitigation of type 2 diabetes-mediated cognitive deterioration: Implications for alzheimer's disease therapy

Authors: *G. M. PASINETTI
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Abstract: Our laboratory recently demonstrated that epigenetic chromatin modifications associated with type-2 diabetes (T2DM) may play a role in brain pathophysiology in neurodegenerative disorders. We previously discovered that there are significant changes in the expression of select chromatin modification enzymes, such as histone deacetylases (HDACs), in the brains of T2DM subjects compared to control subjects, and that these changes coincide with altered expression of proteins involved in synaptic function, such as PSD95 and synaptophysin. We hypothesized that T2DM may induce epigenetic modifications associated with increased susceptibility to Alzheimer's disease (AD)-type neurodegeneration. Using a mouse model of diet-induced T2DM, we found that, similar to humans, T2DM mice showed differential expression of epigenetic-modifying enzymes in the brain compared to controls. In particular, we found significant up-regulation of HDAC class IIa, including HDACs 4, 5, and 9, in the brains of diabetic mice. These alterations coincided with increased susceptibility to oligomeric Aβ (oAβ) induced synaptic toxicity and oAβ-induced synaptic dysfunction, as assessed by long term potentiation (LTP). Most interestingly, we found that inhibition of class IIa HDACs using an HDAC IIa-specific inhibitor, MC1568, increased transcription levels of PSD95 and synaptophysin in primary neuron cultures from C57Bl6/J mice and prevented LTP deficits found in old-T2DM mouse ex vivo hippocampal slices. The studies provide much-needed information regarding mechanisms underlying potential risks posed by diabetes in terms of diminishing cognitive function and may provide the basis for strategies aimed at early primary prevention against key risk factors for AD. This work was supported by discretionary funding to GMP.

Disclosures: G.M. Pasinetti: None.

Poster

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Montel Williams Multiple Sclerosis Foundation

Nancy Davis Multiple Sclerosis Foundation

Transverse Myelitis Association

Johns Hopkins Brain Science Institute

Title: Inhibition of GCPII for the treatment of cognitive impairment in multiple sclerosis

Authors: *K. RAHN[1], C. WATKINS[1], J. ALT[2], R. RAIS[1], M. STATHIS[2], I. GRISHKAN[1], C. CRAINICEAU[1], M. POMPER[1], C. ROJAS[2], P. CALABRESI[1], J. BRANDT[1], P. BARKER[1], B. SLUSHER[2], A. KAPLIN[1]
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Abstract: Cognitive impairment is a frequent comorbidity of multiple sclerosis (MS), but no treatments exist to target this aspect of disease. Human magnetic resonance spectroscopy data collected from MS patients demonstrated significant positive correlations between hippocampal N-acetyl aspartyl glutamate (NAAG) levels and performance on a battery of cognitive tests. Therefore, we hypothesized that pharmacological upregulation of NAAG, a peptide that is converted to N-acetyl aspartate and glutamate by the enzyme glutamate carboxypeptidase II (GCPII), would be an efficacious method of reversing cognitive impairment in MS. Using an animal model of MS (experimental autoimmune encephalomyelitis, EAE) and Barnes maze and fear conditioning behavior tests, we measured cognitive performance following chronic treatment of the GCPII inhibitor 2-PMPA. While disability levels (i.e. severity of paralysis) were unaffected due to the 2-PMPA treatment, two independent experiments confirmed that 2-PMPA-treated mice exhibited a significant improvement in Barnes maze and fear conditioning performance compared to vehicle-treated EAE controls. Post-mortem analysis revealed that the 2-PMPA can penetrate the blood brain barrier and that treatment significantly increases NAAG levels in the brains of these mice. In summary, these data support inhibition of GCPII for the treatment of cognitive impairment in MS.

Disclosures: K. Rahn: None. **C. Watkins:** None. **J. Alt:** None. **R. Rais:** None. **M. Stathis:** None. **I. Grishkan:** None. **C. Crainiceau:** None. **M. Pomper:** None. **C. Rojas:** None. **P. Calabresi:** None. **J. Brandt:** None. **P. Barker:** None. **B. Slusher:** None. **A. Kaplin:** None.

Poster

876. High Throughput and Therapies

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Topic: C.19. Drug Discovery and Development

Title: Ginsenoside Rg1 increase neurogenesis and synaptogenesis *in vivo* and *in vitro*

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Abstract: Basic and clinical study of Alzhemier's disease showed that senile plaques and Neurofibrillary tangles are not related to the degree of cognitive impairment. Neuro-synapse loss suspended signal delivery and signal transduction leading to neuropsychological dysfunction and memory disruption which was considered as the main pathological feature of Alzheimer disease. Thus the new strategies for prevention and treatment of dementia were increasing neurogenesis and synaptogenesis. Ginsenoside Rg1 isolated from ginseng was shown to improve learning and memory in 10 models of memory impairment. In anesthetized and freely moving rats, Rg1 could increase synaptic plasticity in both efficacy and structure. The latter is mainly to increase synapse density and dentritic spine density significantly. Their mechanism maybe due to the increase of intracellular calcium concentration and polymerization of F-actin. Furthermore, Rg1 increased hippocampal neurogenesis in vitro and in vivo, under physiological and pathological circumstances. NMDA receptor and iNOS are involved in the effect of Rg1 on increasing neurogenesis. Above mentioned findings suggest that ginsenoside Rg1 can be used for treatment of dementia and many kinds of memory impairment.

Disclosures: J. Zhang: None.

Poster

877. Taste: Sensing, Perception and Network

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Topic: D.01. Chemical Senses

Support: NIH Grant DC0047854

FSU WIMSE

Title: Water restriction enhances chorda tympani responses to NaCl and KCl in rats

Authors: *T. G. MAST, J. A. KOSTANSEK, S. M. KELLY, R. J. CONTRERAS
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Abstract: Our overall goal is to uncover salt-taste coding mechanisms. Here we investigated the effect of water restriction on the two different salt detection mechanisms present in the rat chorda tympani (CT) nerve. The first mechanism is dependent upon the epithelial sodium channel (ENaC), is blocked by benzamil, and is specific to the cation sodium. The second mechanism, while undefined, is independent of ENaC, and detects multiple cations. In nature, water is present as a low-salt solution, thus we hypothesized that water restriction would differentially modulate the two salt-sensing mechanisms in the CT to maximize detection of low-salt solutions. Specifically, the benzamil-insensitive CT fibers would have enhanced responses to hypertonic and reduced responses to hypotonic solutions; while benzamil-sensitive fibers would have enhanced responses to sodium solutions due to mechanistically increasing the benzamil-sensitive ENaC. We recorded CT whole nerve electrophysiological activity in response to lingual application of NaCl (30, 75, 150, 300, 500, and 600 mM), of KCl (30, 75, 150, 300, 500, and 600 mM) and of artificial rain in control and 24hr water-restricted male rats (n = 7, each). All stimuli were presented in artificial saliva and before and after lingual application of 5μM benzamil. The low-salt stimulus, artificial rain, did not excite the CT. Water restriction significantly enhanced the integrated CT responses to NaCl, $F(1,42)=10.8$, $p=0.002$, but not to KCl $F(1,42)=1.9$, $p=0.18$. The two salt-sensitive mechanisms each had enhanced responses to NaCl; benzamil-sensitive, $F(1,42)=14.9$, $p=0.0004$, and the benzamil-insensitive, $F(1,42)=4.6$, $p=0.04$, respectively. Surprisingly, the sodium-specific benzamil-sensitive pathway_and not the generalist benzamil-insensitive pathway_had enhanced KCl responses, $F(1,42)=15.8$, $p=0.0003$. Normalizing the CT responses to ammonium chloride slightly altered these effects. Thus, deprivation significantly alters CT responses to NaCl and KCl salts and the magnitude of this effect can be lost when the integrated signal is normalized to a third cation, ammonium chloride. Future experiments will investigate the roles, if any, that osmotic and volemic thirsts play in modulating CT responses to salts.

Disclosures: T.G. Mast: None. **J.A. Kostansek:** None. **S.M. Kelly:** None. **R.J. Contreras:** None.

Poster

877. Taste: Sensing, Perception and Network

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Topic: D.01. Chemical Senses

Title: Differences between cerebral activation of analgesia by sucrose and an artificial sweetener in humans

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Abstract: Intraoral infusion of sweet taste produces analgesia in adult humans (sweet taste-induced analgesia; SIA) (Kakeda 2010). However, few studies have investigated brain activation associated with SIA (Kakeda et al. 2010). To our knowledge, there is no information on how artificial sweeteners induce SIA in human brain. The aim of this study was to examine the differences between cerebral activation of SIA by sucrose and that by an artificial sweetener, using 3-T functional magnetic resonance imaging (fMRI).
The Human Experimentation Committee of Kyushu University approved all experimental procedures. Seven males and seven females (aged 22-33 years) participated in this study. Sucrose solution [24% (w/v)] and the solution of an artificial sweetener (Palsweet^{TM}) [8% (w/v)] were used for generating the sweet condition, while artificial saliva (25 mM KCl with 2.5 mM $NaHCO_3$) was used as the control solution. Sweet and control solutions were alternately applied to the participant's mouth for 20 sec. The participants were subjected to the cold pressure test which involved applying an ice pack onto the participant's dorsum manus for 20 sec. During fMRI scanning, participants underwent 5 events: "Sucrose", "Sucrose + Pain", "Palsweet", "Palsweet + Pain" and "Pain + control." Each event was repeated 12 times randomly. The ice pack was applied to alternate hands in each pain event. Participants pressed a button with their non-stimulated hand when they began to feel the pain. Cerebral activation of SIA by sucrose and Palsweet was compared in four contrasts: "Sucrose + Pain" - "Pain + control", "Palsweet + Pain" - "Pain + control", "Sucrose + Pain" - "Palsweet + Pain" and "Palsweet + Pain" - "Sucrose + Pain". An anatomical mask was applied on the statistical maps involving the taste and pain pathways.
Cerebral activation of SIA by sucrose was observed in the right putamen [$P < 0.05$ $FWE_{\text{-corrected}}$ with small volume correction (SVC)]. Cerebral activation of SIA by Palsweet was observed in the left postcentral gyrus, the left ACC, and the right rolandic operculum ($P < 0.05$ $FWE_{\text{-corrected}}$ with SVC). No significant neural activation was observed in "Sucrose + Pain" - "Palsweet +

Pain" contrast, whereas neural activation in the right rolandic operculum was detected in "Palsweet + Pain" - "Sucrose + Pain" contrast ($P < 0.05$ $FWE_{-corrected}$ with SVC).
Cerebral activation of SIA by sucrose was observed in the putamen, which is a dopaminergic area related to the behavioral pleasantness response, whereas in "Palsweet + Pain" - "Sucrose + Pain" contrast, neural activation was observed in the pain pathways, possibly suggesting that the effect of SIA by sucrose is more significant than that by Palsweet.

Disclosures: Y. Nakamura: None. **T.K. Goto:** None. **K. Tokumori:** None. **K. Kobayashi:** None. **K. Yoshiura:** None.

Poster

877. Taste: Sensing, Perception and Network

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Topic: D.01. Chemical Senses

Support: Oklahoma Center for the Advancement of Science and Technology HR10-141

The William K. Warren Foundation

Title: A common interoceptive and gustatory representation in the human insula.

Authors: *J. AVERY, K. L. KERR, J. C. BARCALOW, J. BODURKA, W. K. SIMMONS
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Abstract: The dorsal mid-insula is the location of primary gustatory cortex in the human brain. It receives direct afferent input from the gustatory nucleus of the thalamus, and numerous neuroimaging studies implicate this region in gustatory processing. The dorsal mid-insula also underlies interoceptive awareness (i.e., the awareness of internal body sensations). Visceral afferents are relayed through the nucleus of the solitary track, to the ventroposteromedial nucleus of the thalamus, before terminating in this region of the insula, and neuroimaging studies demonstrate activation of this region during tasks involving interoceptive attention. Two separate studies by our lab, one requiring focal interoceptive attention, and the other requiring passive gustatory stimulation, have found overlapping areas of activation in the insula during these disparate tasks. The apparent overlap could result from differences in insula organization among the two groups of subjects. It could also, however, reflect a common neural representation in the insula shared by both interoception and gustation, and idea that finds support in rodent electrophysiological studies that have identified insular neurons that respond to both taste and interoceptive stimulation. To date, however, there is no evidence for a common interoceptive-

gustatory representation in human insula. To assess this possibility, we asked healthy volunteers between the ages of 18 and 40 years to undergo fMRI while perform tasks involving interoceptive attention to visceral sensations as well as a “Gustatory Mapping” task using an MR compatible tastant delivery system. Imaging was conducted with a 3T MRI scanner and a 32-element receive-only brain surface coils array which granted superior image quality at high spatial resolution. Group conjunction analyses revealed overlapping patterns of activation for both tasks in bilateral regions of mid to posterior insular cortex. The identified regions were selectively responsive to visceral interoceptive attention and also exhibited strong responses to sweet versus neutral tastants. This observed overlap within the insula suggest the possibility that either gustation and interoception share some functional property to which the neurons within these regions are sensitive, or that neurons selective for gustation and interoception are intermingled in this region of the human insula and do not share a distinct topographic organization.

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Poster

877. Taste: Sensing, Perception and Network

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Topic: D.01. Chemical Senses

Support: T32 DC-000044

Title: Differences in the motivational efficacy of sucrose in sucralose preferring and avoiding rats

Authors: *G. C. LONEY, C. LOPEZ, C. LAMBRIGHT, L. A. ECKEL
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Abstract: Individual variation in the preference for, and consumption of, artificial sweeteners is often indicative of differential responding aimed at obtaining motivational stimuli. For instance, rats bred for a phenotypic difference in their baseline saccharin intake display similar differences in self-administration of sucrose, morphine and cocaine. Rats also display an innate phenotypic difference in their preference for sucralose. We have previously shown that rats can be classified as sucralose preferrers (SP) or sucralose avoiders (SA) based on their consumptions of sucralose and water during 2-bottle preference tests. This avoidance has been attributed to the fact that SA and SP perceive the taste of sucralose as qualitatively different. While SA generalize the taste of

sucralose to that of both sucrose and quinine, SP generalize the taste of sucralose solely to that of sucrose. This suggests that SA perceive a bitter side-taste in sucralose to which SP are relatively insensitive. This behavioral avoidance generalizes to saccharin as well as sucrose solutions adulterated with increasing concentrations of quinine. Here we investigated whether SA and SP differ in their operant responding for sucrose pellets as measured by differences in breakpoint responding in a progressive-ratio (PR) lever-pressing task. SA and SP were autoshaped to lever press for 45 mg sucrose pellets and advanced through a fixed-ratio (FR) 1, 3, 5 and 8 schedule of reinforcement before continuing to a PR schedule where the rats received daily saline injections. As the dopaminergic system has been implicated in the perceived rewarding efficacy of sucrose, after completion of the baseline PR schedule, rats were injected with 100, 200 and 300 μg/kg of the selective D2 receptor antagonist raclopride followed by 25, 50 and 100 μg/kg of the selective D1 receptor antagonist SCH 23390 (both presented in ascending order). Each drug concentration was administered at least 48 h after the previous concentration. On average, SP showed a higher breakpoint for sucrose responding under baseline saline conditions relative to SA (43.2 ± 9.6 vs. 29.3 ± 7.6, respectively). Statistical analyses revealed that both drugs except the lowest concentration of SCH 23390 were efficacious in reducing breakpoint responding for sucrose pellets (Ps < 0.01). No group differences in response to either drug were obtained using these concentrations. These findings demonstrate that rats differing in their taste perception of sucralose also differ in their operant responding for sucrose pellets under baseline conditions suggesting a difference in the motivational saliency of sucrose in these same rats.

Disclosures: G.C. Loney: None. **C. Lopez:** None. **C. Lambright:** None. **L.A. Eckel:** None.

Poster

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Title: CALHM1 constitutes a conduit for cellular ATP release and mediates sweet and bitter taste perception

Authors: A. TARUNO[1], A. LI[1], Z. MA[1], P. MARAMBAUD[2], I. MATSUMOTO[3], M. M. CIVAN[1], M. G. TORDOFF[3], *K. FOSKETT[1]
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Abstract: Calcium homeostasis modulator 1 (CALHM1) was discovered as a gene expressed throughout the brain and linked to Alzheimer's disease. Subsequent studies demonstrated that CALHM1 was a pore-forming subunit of a novel ion channel regulated by membrane voltage and extracellular Ca^{2+}, with a pore diameter large enough to allow permeation of small molecules up to 1 kD. Here, we have found that expression of CALHM1 induces a novel ATP permeability that releases ATP into the extracellular medium in response to maneuvers that activate the CALHM1 ion channel. CALHM1-mediated ATP release is insensitive to blockers of other putative ATP release channels and to inhibition of vesicular trafficking. CALHM1 is expressed in type II taste receptor cells that respond to sweet and bitter tastes by non-vesicular release of ATP as an essential neurotransmitter to afferent gustatory nerve fibers. Voltage-gated CALHM1 ion currents were recorded specifically in type II cells. *CALHM1* knockout mice lack the ion currents, do not release ATP from taste receptor cells in response to taste solutions, and are indifferent to bitter and sweet tastes. Thus, CALHM1 provides a missing link in taste perception, connecting signal transduction at the taste receptors and generation of action potentials to activation of afferent gustatory neural pathways. Other CALHM isoforms might also be involved in taste. They and CALHM1 may participate in physiologically important ATP release in other cell types. CALHM1 is a voltage-gated ion channel that mediates ATP release in taste buds as an essential mechanism of sweet and bitter taste transduction. Accordingly, CALHM1 is an appealing target for studying taste physiology.

Disclosures: A. Taruno: None. **A. Li:** None. **Z. Ma:** None. **P. Marambaud:** None. **I. Matsumoto:** None. **M.M. Civan:** None. **M.G. Tordoff:** None. **K. Foskett:** None.

Poster

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Support: NIH Grant R01DC000288

NIH Grant T32DC000011

Title: Characterization of rostral nucleus of the solitary tract neuron populations involved in oromotor reflexive circuitry

Authors: *J. A. CORSON[1], R. M. BRADLEY[2]
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Abstract: The efferent circuitry of the first-order gustatory nucleus, the rostral nucleus of the solitary tract (rNST), arises from two major pathways. Although much is known regarding the characteristics of ascending projections to the pons, the biophysical, morphological, and synaptic properties of the intramedullary descending projection neurons remains unstudied. To identify rNST descending projection neurons, iontophoretic injections of DiI were made into the reticular formation (the major target of this neuron population). One to three days post-injection, 300-400μm coronal or parasagittal (parallel to the solitary tract) brain slices containing the rNST were prepared for whole cell patch clamp recording. Most neurons (62%) responded to depolarizing current injection with a tonic firing pattern, while approximately 23% responded with an initial burst of action potentials followed by a steady-state depolarized plateau. Descending projection neurons also exhibited a number of different voltage activated currents, with some neurons expressing post-inhibitory rebound spikes, Ih currents, and A-type potassium currents. Synaptic connectivity with primary afferents was tested in a parasagittal plane of section developed to include the solitary tract, primary afferent terminal field, and projection neurons within a single 400μm slice. Small current pulses delivered to the rostral tip of the solitary tract with a bipolar stimulating electrode evoked monosynaptic excitatory post-synaptic currents in approximately half of the neurons. The remainder responded with postsynaptic currents with variable latency and reliability, indicative of polysynaptic connections. Pharmacological blockade of glutamate receptors revealed that monosynaptic responses were largely AMPAergic with only a minor NMDA component. The majority of neurons recorded were multipolar with dendrites extending into several rNST subdivisions. In contrast to other cell populations in rNST, descending projection neuron dendrites were often covered in spines, suggesting synaptic plasticity in the gustatory brainstem reflex circuitry. Individual axons were also visualized, constituting the first examination of single cell axonal morphology in the rNST for any cell population. This examination revealed that descending projection neurons often exhibit intra-rNST axon collaterals and boutons, suggesting a contribution of these neurons in intra-rNST circuits.

Disclosures: J.A. Corson: None. **R.M. Bradley:** None.

Poster

877. Taste: Sensing, Perception and Network

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:877.07/Y1

Topic: D.01. Chemical Senses

Support: NIH DK079182

NIH DC005435

Title: Glossopharyngeal transection alters oil intake in food or water deprived rats.

Authors: *H. FOO, E. HANDLY, N. HORVATH, R. NORGREN
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Abstract: Recent evidence suggests that taste buds within the circumvallate papillae are responsible for the detection of fat. Taste buds in the posterior lingual papillae (circumvallate and foliate) are innervated by the glossopharyngeal (GL) nerve. We tested the effect of bilateral transection of GL (GLX) on intake of 0.3 M sucrose, 4.8%, 16%, and 100% oil emulsions during *ad libitum* food and water, food restriction, or water deprivation. The stimuli were available semi-randomly interspersed with water trials. All trials were 10 sec with an average 5 sec intertrial interval. Under *ad libitum* conditions, Sham and GLX rats licked sucrose the most, followed by oil emulsions in a concentration-dependent manner (100% > 16% > 4.8%). Transection of the GL nerves did not affect the amount of sucrose or oil consumed. When food deprived, Sham rats lost their concentration-dependent intake of 16% and 100% oil. They consumed the same amount of 16% and 100% oil as 0.3 M sucrose. In contrast, the intake of the GLX rats did not change from the *ad libitum* condition, i.e. sucrose > 100% > 16% > 4.8% oil emulsions. When water deprived, Sham and GLX rats lost their concentration dependent intake of oil. Nevertheless, the GLX rats consumed less 100% oil than Sham rats. Thus, denervating the gustatory and somatosensory receptors of the posterior oral cavity does not eliminate oil intake but under some circumstances flattens the concentration response function.

Disclosures: H. Foo: None. **E. Handly:** None. **N. Horvath:** None. **R. Norgren:** None.

Poster

877. Taste: Sensing, Perception and Network

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

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Topic: D.01. Chemical Senses

Support: CRC Award, College of Veterinary Medicine and Biomedical Sciences, CSU

Title: Differential distribution of AgRP and POMC in taste buds of mice

Authors: S. H. MONAHAN, S. T. HENTGES, *L. M. STONE
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Abstract: Circuits in the oral cavity, digestive system and central nervous system work together to maintain homeostatic balance between nutrient intake and energy expenditure; a key component of these circuits is the ability to determine the nutritional status of the body. Disruption of the circuits can lead to obesity and associated diseases. In taste buds, changes in the palatability of foods due to changes in taste receptor responses may contribute to obesity. Recent studies indicate that hormone and peptide receptors are present in subpopulations of taste cells and activation of these receptors leads to changes in taste sensitivity (for review see Niki et al., 2010). These proteins are also found in the arcuate nucleus of the hypothalamus, in two main opposing circuits that regulate energy balance. Activation of the first circuit results in an increase in feeding. Cells within this orexigenic circuit express neuropeptide Y (NPY) and agouti gene-related peptide (AgRP). The opposing circuit is anorexigenic and contains cells that express proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). Nutritional signals such as glucose, fatty acids and hormones influence these neurons and lead to changes in feeding behavior and energy balance. Previous studies indicate that taste receptor cells express NPY (Zhao et al., 2005) and receptors for endocannabinoids and leptin receptors (for review see Niki et al., 2010). In the current study, we show that taste cells express AgRP and POMC. Subpopulations of taste cells that exhibit immunoreactivity (ir) for AgRP are present in circumvallate and foliate taste buds, but not in fungiform taste buds. In addition, although AgRP and the G protein α-gustducin are co-expressed in some taste cells, T1R3 positive taste cells do not appear to express AgRP-ir. This suggests that AgRP expression is associated with bitter sensitive taste cells rather than cells sensitive to sweet and umami stimuli. Taste buds from POMC-GFP mice exhibit GFP-ir indicating that POMC peptides may also be expressed in taste buds. POMC-GFP is present in both gustducin-ir cells and in cells that lack gustducin. In summary, taste receptor cells express peptides that are involved with maintaining energy homeostasis in the hypothalamus. Taste cells expressing these peptides also may have roles in regulating energy balance by affecting food palatability. Future experiments will investigate the relationship between taste cells expressing POMC and AgRP to determine whether these relationships are similar to, or different from, those between POMC and AgRP expressing cells in the hypothalamus.

Disclosures: S.H. Monahan: None. **S.T. Hentges:** None. **L.M. Stone:** None.

Poster

877. Taste: Sensing, Perception and Network

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Topic: D.01. Chemical Senses

Support: NIH grant DC007854

NSF grant IOS1145132

Title: Differential effects of electrical stimulation of the central amygdala and lateral hypothalamus on taste reactivity behaviors in conscious rats

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Abstract: Taste reactivity (TR) behaviors are controlled by brainstem gustatory structures including the rostral nucleus of the solitary tract (rNST), parabrachial nucleus (PBN), and medullary reticular formation (RT). Several forebrain structures including the central amydgala (CeA) and lateral hypothalamus (LH) are inter-connected with brainstem gustatory nuclei and have been shown to modulate the electrophysiological responses to taste inputs within the rNST and PBN. The current study investigated the behavioral effects of electrical stimulation of the CeA and LH in conscious rats. Stainless steel electrodes were implanted into the CeA or LH of 60 male Wistar rats using a stereotaxic device. During the same surgical session intra-oral cannulas were implanted. After a one-week recovery/adaptation period the CeA or LH was electrically stimulated (0.4 ms duration, 50 Hz, 0.1-0.2 mA) for 5 min while TR behavioral responses were videotaped. During the stimulation period, 4 rats in each group received an intra-oral infusion (0.233 ml/min) of either NaCl (0.1M), sucrose (0.1 M), HCl (0.03 M), quinine HCl (0.003 M) or nothing. In the absence of taste stimuli, both CeA and LH stimulation elicited ingestive, but not aversive, TR behaviors. LH stimulation decreased ingestive TR behaviors caused by NaCl (by 72%), sucrose (by 62%), HCl (by 62%), and quinine HCl (by 79%), and aversive responses to quinine HCl (by 80%). On the other hand, CeA stimulation did not alter ingestive behaviors to any tastant tested but increased the aversive responses to NaCl (by 24-fold) and HCl (by 3.5 fold). To investigate potential neural mechanisms underlying these behavioral effects, the numbers of Fos-immunoreactive (Fos-IR) neurons in the rNST, PBN, and RT were counted. One hour after TR behaviors were videotaped, the rats were sacrificed, perfused, and their brains processed for Fos-IR. CeA and LH stimulation differentially affected

neuronal activity as indicated by the numbers of Fos-IR neurons in brainstem gustatory structures. CeA stimulation increased Fos-IR neurons within the rNST, PBN, and parvocellular RT during NaCl infusion while LH stimulation increased Fos-IR neurons in the rNST (particularly the ventral and lateral subdivisions) or the RT to each tastant tested. In sum, electrical stimulation of either the CeA or LH in conscious rats elicited ingestive TR behaviors but LH stimulation generally decreased TR responses to oral infusion of all tastants while CeA stimulation increased aversive responses to NaCl and HCl. These behavioral responses may be explained in part by the differential effects of CeA and LH stimulation on neural activity in gustatory brainstem nuclei.

Disclosures: M.S. King: None. **C.A. Riley:** None. **N.K. Nagrani:** None.

Poster

878. Auditory System: Learning and Plasticity

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Topic: D.02. Auditory

Support: NIH NIDCD R01 DC009607

NIH NIDCD T32 DC000046

Sloan Foundation

Title: Intra-laminar connections in supragranular mouse core auditory cortex show functional anisotropy

Authors: *P. V. WATKINS[1], S. BANDYOPADHYAY[2], P. O. KANOLD[2]
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Abstract: The functional responses of cortical neurons are sculpted by cortical microcircuits. Auditory cortex neurons show a tonotopic gradient of frequency selectivity based on an orderly projections from the thalamus. Intracortical excitatory and inhibitory connections from neurons in specific locations relative to each cell and to the tonotopic gradient can form modules to combine features to create complex receptive fields. However, the intralaminar micro-circuit organization of the supragranular layer of auditory cortex is unknown. Previous studies involving anatomy or simultaneous patch recordings demonstrated that intralaminar connection probability decreases with distance. Recent application of quantitative optical techniques to neurophysiology has allowed for micro-circuitries of cortex to be measured much more precisely. Here, we

characterize the inhibitory and excitatory micro-circuits in supragranular layers of mouse auditory cortex in 2D by a combination of laser scanning photostimulation, whole cell patch recordings and 2-photon Ca2+-imaging. We find that both inhibitory and excitatory connections within auditory cortex show a spatial anisotropy with regards to the tonotopic axis on different spatial scales. Such anisotropy was absent in the visual cortex. These results suggest a stereotypical integration of stimulus feature information across the supragranular layers of auditory cortex.

Disclosures: P.V. Watkins: None. **S. Bandyopadhyay:** None. **P.O. Kanold:** None.

Poster

878. Auditory System: Learning and Plasticity

Location: Hall F-J

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Program#/Poster#:878.02/Y5

Topic: D.02. Auditory

Support: NIH Grant HD 42527

Title: Perception of phasing, and motor response to subliminal phase shifts

Authors: *J. WHITALL[1], P. VISWANATHAN[1], J. L. CONTRERAS-VIDAL[2], F. A. KAGERER[3]
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Abstract: Unilateral tapping studies have shown that adults adjust to both perceptible and subliminal changes in phasing or frequency. This study focuses on the phasing responses to abrupt/perceptible and gradual/subliminal changes in auditory-motor relations during bilateral tapping in response to stimuli presented alternating between the two ears.
We investigated these responses in 18 healthy adults (mean age: 24.5 yrs) with and without a high perceptual acuity relating to phase shifts of acoustical stimuli as determined by an auditory threshold determination (threshold<20°: 'high' acuity). After two baseline trials of 180° phase difference (=beats regularly alternating between the two earphone speakers), the phase relationship was changed over the next eight trials either abruptly (20° or 45° shift), or gradually, increasing in 5° steps, with two repetitions per step, amounting to a total phase shift of 20°. Adults in both groups alternately tapped their index fingers in synchrony with auditory cues set at a frequency of 1.4 Hz. Both groups modulated their responses to perceptible and to subliminal changes as low as 1.4% (a 5º change) in phasing. The high threshold participants were more variable than those with low threshold in their responses in the gradual condition. Both groups

showed a synchronization asymmetry between dominant and non-dominant hands associated with the abrupt condition, as well as the later blocks of gradual condition.
Our findings suggest the presence of a subliminal phasing response mechanism to auditory cues that appears to be relatively independent of the ability to consciously perceive the auditory cues.

Disclosures: J. Whitall: None. **P. Viswanathan:** None. **J.L. Contreras-Vidal:** None. **F.A. Kagerer:** None.

Poster

878. Auditory System: Learning and Plasticity

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Topic: D.02. Auditory

Support: NIH Grant R01DC07657

Title: Stimulus temporal coherence strongly influences rapid plasticity in primary auditory cortex

Authors: Y. XU, P. YIN, *S. A. SHAMMA
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Abstract: Temporal coherence of stimulus features is a key property of sounds that emanate from single source. Consequently, it is important to understand how it may influence the direction and extent of the rapid plasticity postulated to occur during the streaming of concurrent sounds. We postulated that when animals listen attentively to coherent and or incoherent stimuli, responses would adapt to effectively encode the correlational structure of the stimuli. Specifically, that correlated responses would promote mutually excitatory interactions among the neurons, and hence would rapidly enhance the responses. The opposite would occur between uncorrelated neurons, causing rapid response depression. Ferrets were trained to attend globally to two-tone sequences that were played randomly either to be synchronous (SYNC) or alternate (ALT) on a trial-by-trial basis, and to detect a transition to a random cloud of tones by licking a waterspout for reward. Neuronal activities were collected in the primary auditory cortex (A1) during performance of the task compared to passively listening to the same stimuli. In general, neuronal responses to the two-tone sequences (in both SYNC and ALT) adapted rapidly to a steady-state after 3-4 repeats of the tone burst. However, in accord with the hypothesis, a large difference in the steady-state responses was observed for ALT and SYNC responses. Specifically, SYNC (ALT) trial responses were enhanced (suppressed). Analysis of the response time-course indicated that response changes were largely due to a late response component (40-

70 ms time window from the tone onset). These changes were also consistent with receptive field changes measured immediately following the end of tone sequences using the random tone clouds. Specifically, we observed strongly suppressed receptive-fields following ALT sequences, but no changes or slightly enhanced receptive-field following SYNC sequence. These results provide neuronal evidence for the role of stimulus temporal coherence in modulating responses during attentive listening to complex sounds.

Disclosures: Y. Xu: None. **P. Yin:** None. **S.A. Shamma:** None.

Poster

878. Auditory System: Learning and Plasticity

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Topic: D.02. Auditory

Support: NIH DC009836

NIH DC5029

Title: Closing the loop on auditory learning in mice: Behavioral characterization and neurophysiologic correlates of learning to hear weak signals in noise

Authors: J. P. WHITTON[1,2], K. E. HANCOCK[2], *D. B. POLLEY[1,2,3]
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Abstract: Mice are a powerful model system for studies of auditory circuit function and plasticity but, because of their general reluctance to remain still, are difficult subjects to train in conventional auditory behavioral tasks. Given the importance of linking perceptual changes to the development and plasticity of the auditory system, we have tailored a “closed-loop” auditory discrimination task to the mouse, which we believe is better suited to their cognitive and motoric proclivities. In the task, mice were trained to use continuous auditory feedback to locate a randomly placed invisible target in a training arena. In order to provide these cues, the mouse’s position in the behavioral arena was coupled to changes in stimulus features in real time. In this way, the mice were essentially performing a “hot-cold” task, where closer proximity to the target elicited stimulus features similar to the target and greater distance from the target translated to larger deviations from the target stimulus features. In this task, mice were trained to discriminate signal-to-noise ratio by modulating the signal amplitude in a fixed noise background according

to the mouse's Euclidian distance from the target location. The least favorable SNR in the acoustic gradient served as the target auditory cue. Mice learned the procedure within 20 daily training sessions. Over approximately 60 additional training sessions, some mice made modest improvements in their performance accuracy on the task, while reducing the amount of time needed to reach the randomly placed target by 20-30%. Additionally, the animals more efficiently used the auditory gradient to navigate the training arena evidenced by an increase in their ratio of movement trajectories that were directed toward the target. Extracellular recordings from the primary auditory cortex of trained animals suggest that the representation of tonal signals presented in background noise is enhanced relative to passively exposed control mice.

Disclosures: J.P. Whitton: None. **K.E. Hancock:** None. **D.B. Polley:** None.

Poster

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Topic: D.02. Auditory

Support: GACR P304/12/G069

GACR P303/11/J005

Title: Reaction of neuronal networks in the murine auditory cortex to loud noise exposure

Authors: O. NOVAK, *J. M. SYKA
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Abstract: It is well known that exposure to a very loud sound produces deteriorated hearing function in mammals. However, the details of this auditory deterioration are unknown to a large extent. Using in vivo two-photon calcium imaging, we investigated the reaction of the neuronal networks in the supragranular layers of the murine (C57BL/6J) auditory cortex to noise exposure.
In mice anaesthetized with ketamine/xylazine, we recorded calcium traces (after the injection of OGB1 and sulforhodamine101) as the responses of individual neurons to pure tone amplitude-modulated stimuli. Tonal receptive fields and post-stimulation time histograms (PSTH) of several neurons recorded at the same time were constructed from the responses. In addition, a z-scan of the investigated area up to a depth of 350 μm was obtained. The animals were then exposed to a loud noise (white noise, 120 dB SPL, 30 min). Following the noise exposure, the previously recorded neurons were identified and their responses to tonal stimuli were recorded

again. Finally, the z-scan was repeated as well.
There were no identifiable changes in the morphology of neurons and glial cells throughout the investigated cortical layers when compared before and after exposure to noise. As regards the calcium traces, there were two types of reaction of neurons to noise exposure observed. Some of the neurons preserved their acoustical stimulus-locked firing pattern, but their tonal receptive fields were changed with an increased threshold at the best frequency. The second population of neurons was affected more seriously. The neurons did not maintain their stimulus-locked firing pattern and exhibited increased spontaneous activity.
Our data offer an insight into the changes that occur in the supragranular layers of the auditory cortex in mice after exposure to a loud sound.

Disclosures: O. Novak: None. **J.M. Syka:** None.

Poster

878. Auditory System: Learning and Plasticity

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Topic: D.02. Auditory

Support: NIH R03-DC010475

Title: Sensory-motor classifications of speech: Selectivity at the interface between speech perception and production

Authors: *G. B. COGAN[1], T. THESEN[2], C. CARLSON[3], W. K. DOYLE[4], O. DEVINSKY[3], B. PESARAN[1]
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Abstract: Neural recordings obtained directly from the surface of the human brain offer the possibility of high fidelity recordings during the performance of uniquely human faculties such as speech and language, faculties for which animal models offer limited opportunities. Here we build on previous results that sensory motor transformations between speech perceptual auditory representations and production motor representations occur bilaterally and throughout the dorsal stream, and examine the selectivity of sensory-motor representations for spoken words.
Ten patients with pharmacologically resistant epilepsy were sub-durally implanted with an 8 x 8 electrode array (1 cm spacing; Adtech) in the temporal-frontal region. Patients were presented with one of 7 auditory CVC (consonant-vowel-consonant) syllables that differed solely on the vowel (hat, het, heat, hoot, hut, hot, hit). Subjects had to either overtly repeat these syllables after

a short delay of 2 seconds (listen-speak), or repeat after a delay without any overt sound (listen-mime). For the present analysis, these two conditions were collapsed together. A control passive listening condition was also present but not analyzed in the current study. We performed a binary classification using a multivariate linear classifier on each combination of tokens presented on electrodes that display sensory motor activity (active during both the presentation of the token as well as the repetition) using the high gamma response (70-160 Hz) on each trial. Out of 36 measured sensory motor sites, 31 displayed a significant number of binary classifications (31/36, 86.11 %). For sites that displayed significant selectivity, classification scores ranged from 0.61 to 0.90 (mean+/1sd = 0.68 +/- 0.06) (chance = 0.50). The average number of significant classifications per significant electrode was 7.16 (out of a maximum of possibility of 21). Furthermore, classifications at the sensory-motor sites during either the auditory epoch or the production epoch alone displayed different selectivity patterns: 72 % of significant binary contrasts were selective in the auditory epoch or the production epoch but not both. This suggests different forms of representation during each task epoch. Taken together, these results suggest that the sensory-motor system that links speech perception and production is selective for individual word tokens and that this selectivity gives further evidence for a transformation from sensory to motor based representation at the interface of the speech perceptual and production systems.

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Poster

878. Auditory System: Learning and Plasticity

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Topic: D.02. Auditory

Support: NIH Grant

Title: Information flow in the ascending auditory system

Authors: *M. J. SCHACHTER[1], F. E. THEUNISSEN[2]
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Abstract: The neural representation of complex sounds has been characterized at different levels of the avian auditory system, and one of the conclusions of this body of work is that neurons in higher auditory areas, such as NCM (Nidopallum caudal medial) and CM (caudal Mesopallium) can have selective responses for conspecific songs or, more generally, familiar stimuli. However,

the role of the ascending circuitry in generating these complex responses is not well understood. To better study how the information flow in the ascending auditory system and, in particular, the role of both the local and global circuit architecture, we examined the population response of 192 cells recorded in the auditory midbrain, thalamus and pallium to a set of 18 different songs. As in previous work, we characterized the spectro-temporal receptive field (STRF) and the information both of single neurons and of neuronal assemblies to study changes in response properties. We further characterized non-linear response properties by fitting STRFs with different input and output non-linearities. Nonparametric output nonlinearities enhanced model performance primarily through a threshold-like mechanism.
In parallel, we modeled a cell response from the linear combination of the responses of other neurons to obtain what we call a neural receptive field (NRF). We used these NRFs to find neural assemblies with similar response properties as well as putative functional connectivity patterns within and across regions. Functional connectivity appears recurrent and on average hierarchical. CM cells prefer input from Field L (primary auditory cortex) and other CM cells. Field L cells prefer input from CM, Field L, and OV (auditory thalamus). Interestingly, both Field L and CM responses share some precise spike-timing properties of MLd cells, indicating a preservation of midbrain response properties up the hierarchy to cortex. The preservation of responses with high temporal precision appears to be a distinctive feature of auditory systems; we postulate that it is so because temporal information remains critical for high-level tasks such as separating song from noise in auditory scene analysis.

Disclosures: M.J. Schachter: None. **F.E. Theunissen:** None.

Poster

878. Auditory System: Learning and Plasticity

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Topic: D.02. Auditory

Support: BMBF #01GQ0811

Title: Encoding of complex sound in inferior colliculus

Authors: *D. LYZWA[1], M. J. HERRMANN[2], *F. WÖRGÖTTER[3]
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Abstract: Spectro-temporal response properties along different spatial axis within the inferior colliculus to complex sound are studied. Vocalizations, being natural stimuli and showing a variety of spectral and temporal modulations are particularly interesting and can capture evoked responses which are neglected by artificial simplified sounds.
We study responses from guinea pigs to acoustically presented species-specific vocalizations. The multi-unit recordings were taken simultaneously from 32 positions in the central nucleus of the inferior colliculus of Dunkin Hartley guinea pigs using a double shank electrode.
The vocalizations differ in spectral content and envelope shape, ranging from low to broad spectral distributions and from a highly periodic to complex envelope, and can be ordered accordingly.
Response preferences to features of the complex sounds depend largely on the characteristic frequency of the neural population, showing e.g. enhanced response for low frequency content and periodic envelopes at low CF.
Post-stimulus time histograms of the neural populations can be partly predicted by taking into account cochlear frequency band filtering, and their measured tuning curves in the ICC.
Neural discrimination between different vocalizations using linear discriminant analysis yields time windows of 0.5 ms as sufficient to distinguish features of these complex sounds.
Pooling responses from recording sites with fairly different response properties leads to improved discrimination, and is not decreased when lifting temporal correlations by shuffling across trials, which indicates independent encoding schemes. Only directly neighboring (100 μm) neural populations respond in a similar manner and have highly correlated PSTHs.
This suggests that for electrical stimulation in the IC several contacts are necessary in order to best approximate the evoked response to complex sounds.

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Poster

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Program#/Poster#:878.09/Y12

Topic: D.02. Auditory

Support: NIDCD R01DC006397

Title: A temporal integration mechanism for enhancing frequency tuning between brainstem inputs and the inferior colliculus

Authors: *C. CHEN, H. L. READ, M. A. ESCABI
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Abstract: Sound information is processed through parallel pathways that synapse within the inferior colliculus (IC) and yet there is little direct evidence for how these inputs serve to create or preserve neural representations of sound. Using tetrode arrays we provide evidence for the presence of pre-synaptic action potentials ("prepotentials") within the cat IC and demonstrate a dramatic enhancement in spectral selectivity between putative input (PIN) and target IC neurons (ICN). Two pathways of PINs are identified that reflect the presence of functionally distinct parallel pathways to the IC. PINs were matched in best frequency to ICN yet exhibited broadly tuned and temporally delayed patterns of excitation and inhibition. A subset of PINs had temporally precise and spectrally interleaved peaks that that resemble a comb-filter. A neuron model that takes into account the synaptic activity of PINS and the subsequent membrane integration in the IC accounts for the radical sharpening of tuning observed in ICN responses. Sharpening of tuning is accomplished by temporal integration of the converging PIN activity where spectral excitation and inhibition are out of phase and summate at the BF but are overlapped and cancel away from BF. Thus spectral selectivity can be newly generated through constructive and destructive interferences by a temporal integration mechanism not requiring local surround inhibition. (Supported by NIDCD R01DC006397).

Disclosures: C. Chen: None. **H.L. Read:** None. **M.A. Escabi:** None.

Poster

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Support: NIH-NIDCD 04845

Schmitt Foundation for Brain Research

Center for Visual Science

Title: Inactivation of ventral prefrontal cortex impairs audiovisual working memory.

Authors: *B. PLAKKE[1], J. HWANG[2], M. D. DILTZ[1], L. M. ROMANSKI[1]
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Abstract: The ventral frontal lobe is well known for its role in language processing and social communication. Previous work from this lab has demonstrated that individual neurons within ventrolateral prefrontal cortex (VLPFC) process and integrate face and vocal information (Romanski et al., 2005; Sugihara et al., 2006). We have also recently shown that VLPFC neurons are active during audiovisual working memory (Hwang and Romanski, 2010). The effect of VLPFC lesions on tasks which assess learning or memory has been equivocal, with some studies demonstrating an impairment on visual learning and memory, auditory discrimination, rule learning, and decision making, while other studies have failed to demonstrate any deficits during working memory tasks. In the current study we asked whether VLPFC was essential in an audiovisual non-match-to-sample (NMTS) task designed to assess auditory and visual working memory. During the task, monkeys attended the audiovisual sample and were required to press a button when a non-matching stimulus, i.e. one whose auditory or visual track differed from the sample audiovisual movie, occurred. Our subjects, rhesus macaques, detected the non-match with a button press in order to receive a juice reward. We inactivated VLPFC while subjects performed the task by cooling the cortical surface of VLPFC below 20° C. During each testing session, 100 baseline trials were completed (WARM trials) and then the cortex was cooled below 20° C, and another 100 trials were completed (COLD trials). We assessed performance accuracy and reaction time in the auditory and visual non-match trials of the audiovisual NMTS task during WARM and COLD trials. Performance accuracy was significantly decreased during COLD trials compared to performance during WARM trials. Interestingly, performance accuracy was significantly worse on auditory mismatch trials compared to visual mismatch trials during the COLD trials. Reaction time was not significantly different between warm and cold trials. This data confirms that VLPFC plays a role in audiovisual processing and that VLPFC may be essential in auditory working memory.

Disclosures: B. Plakke: None. **J. Hwang:** None. **M.D. Diltz:** None. **L.M. Romanski:** None.

Poster

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Support: NIDCD Grant DCO2514

Title: Macaque sensitivity to compound amplitude modulation: Effects of component frequency and phase

Authors: *K. N. O'CONNOR, Z. CLINE-EGRI, J. S. JOHNSON, M. L. SUTTER
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Abstract: Most of the variation in the temporal envelope of natural sounds occurs at relatively low frequencies (Voss and Clark 1975; Singh and Theunissen 2003). Slow amplitude modulations (AM) are particularly prominent in human and animal vocalizations, and it is evident that this envelope variation carries important communicative information. For example, comprehension of human speech is possible with as few as three modulated noise bands (Shannon et.al. 1995). How the brain extracts this information is currently of much interest in both psychophysical and physiological acoustics. Several adaptation and masking studies support the notion that temporal envelope analysis is effectively based on a "modulation filterbank", a bank of independent bandpass filters each sensitive to a narrow range of AM frequencies (e.g., Kay and Matthews 1972; Bacon and Grantham 1989; Ewart and Dau 2000). To test this model, and examine its possible physiological bases, we have begun training rhesus macaques to detect envelope modulation in compound AM (cAM) stimuli, consisting of broadband noise carriers where the modulation function is the sum of two sinusoids. The frequencies of these sinusoids are either quite close (e.g., 5 and 6.25 Hz), designed to lie within a modulation filter, or farther apart and so not within the same filter (5 and 15 Hz). Additionally, the phases of the two sinusoids are either aligned at 0 deg or 180 deg apart. This phase manipulation produces two signals with identical modulation spectra, but different waveforms and envelopes. If cAM sensitivity is based upon a modulation filterbank we would expect summation effects for detection of the signal components lying within a filter, but little or no summation for those not within. We would also expect phase difference to have no effect on summation. To date, we have obtained preliminary results from one macaque. Comparison of thresholds for detection of the pure sine component AM, to those for detection of compound AM, suggests that summation is greater in the case of 5 + 6.25 Hz cAM, but that it also occurs to some degree for the 5 + 15 Hz cAM. It appears that phase also has an effect, with greater summation for cAM with non-aligned phase. Thus, our results at this point are not strongly supportive of a modulation filterbank model in the macaque comprising narrow independent filters, though they may be more congruent with a model in which the filters share a common noise source.

Disclosures: K.N. O'Connor: None. **Z. Cline-Egri:** None. **J.S. Johnson:** None. **M.L. Sutter:** None.

Poster

878. Auditory System: Learning and Plasticity

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:878.12/Y15

Topic: D.02. Auditory

Support: NIH Grant DC006877

Title: Activity-dependent intrinsic plasticity of principal cells of the medial superior olive near the onset of hearing

Authors: T. BABOLA, S. CHERRY, *N. L. GOLDING
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Abstract: Within days after hearing onset, principal neurons of the medial superior olive (MSO) transform from slow, electrically compact neurons into fast, leaky neurons capable of detecting sub-millisecond differences in the timing of binaural excitatory and inhibitory inputs, cues used for encoding the location of sounds along the horizontal plane. While the electrical maturation of intrinsic properties has been characterized previously, little is known about how this transformation occurs developmentally or what mechanisms are involved. To address these questions, we delivered repetitive synchronous activation of excitatory inputs to MSO neurons in brainstem slices from gerbils just prior to hearing onset (P9-P11). Subthreshold synaptic stimulation (trains of 10 monolateral stimuli at 100 Hz, repeated 20 times once every 5 s) produced an average decrease in input resistance (R_N) of 44±11% (n=8), the equivalent of ~2 days of development after hearing onset. The resting potential (V_m) also hyperpolarized by ~17 mV (from -54±1 mV to -71±3 mV, n=8). These changes typically occurred 5-10 minutes after stimulation and persisted for the duration of recordings (>30 min. post-stimulus). When subthreshold synaptic stimuli (4-10 mV amplitude) were paired with action potentials triggered at the peak of each EPSP by depolarizing current pulses, a similar decrease in R_N was observed (54±9%) along with a 10±4 mV hyperpolarization of the resting membrane potential (n=5). However, action potential trains alone did not elicit these changes. Activity-dependent changes in R_N were attenuated by blocking NMDA receptor-mediated calcium influx with 50 µM AP-5 (17±11% decrease in R_N, 8±2 mV hyperpolarization of V_m, n=4) and completely blocked by depleting intracellular calcium stores with 100 µM cyclopiazonic acid (2±7% decrease in R_N, 0±2 mV change in V_m, n=7). In addition, LY379268 (100 nM), a group II metabotropic glutamate receptor (mGluR) agonist, decreased R_N by 40±5% and hyperpolarized the membrane potential by 9±2 mV (n=8). The similarity between the effects of the group II mGluR agonist to those induced by synaptic stimulation suggest that these receptors may play an important role in the maturation of intrinsic electrical properties. Based on our results, we propose a model for the development of temporal precision in MSO principal cells in which the increase in temporal correlations across excitatory inputs during early auditory experience activate mGluRs and NMDARs, which then cooperatively trigger calcium-induced calcium release and subsequent changes in intrinsic ion channels, improving the speed and precision of binaural cue processing.

Disclosures: T. Babola: None. **S. Cherry:** None. **N.L. Golding:** Research Grant; NIH grant DC006877.

Poster

879. Retina: Circuits and Synapses

Location: Hall F-J

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Program#/Poster#:879.01/Y16

Topic: D.04. Vision

Support: NIH Grant EY15573

NSERC Discovery Award

NB is a VA Senior Career Scientist Award (NB)

UCLA Oppenheimer Seed Grant

Title: Depletion of vesicular GABA in horizontal cells eliminates inhibitory feedback to photoreceptors in mammalian retina

Authors: *A. A. HIRANO[1,2], X. LIU[3], L. PÉREZ DE SEVILLA MÜLLER[3], J. BOULTER[4], S. BARNES[6,3,2], N. C. BRECHA[5,2]
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Abstract: While it is accepted that horizontal cells provide inhibitory feedback to photoreceptors, the cellular mechanisms and transmitter(s) underlying this inhibition in mammalian retina are less well understood. The localization of vesicular GABA transporter (VGAT) and other SNARE and synaptic proteins to horizontal cell processes and endings indicate the vesicular release of GABA may in part participate in horizontal cell function. The goal of this study was to determine whether vesicular GABA in horizontal cells participates in inhibitory feedback to photoreceptors. We generated a Cx57-iCre mouse line and crossed it with a transgenic line containing LoxP-flanked VGAT genes to eliminate VGAT selectively in horizontal cells (Cx57-VGAT null). Immunohistochemistry for VGAT on retinal sections confirmed that VGAT was eliminated from horizontal cell processes and endings in the outer retina. Analysis of iCre expression in whole-mounts of Cx57-iCre retinas indicated that iCre was expressed in all horizontal cells, identified by calbindin immunolabeling, in all regions of the retina. Moreover, Cx57- iCre crossed with a cre reporter line, to generate the Cx57-tdTomato (Cx57-Tom) line, showed fluorescent protein expression in horizontal cells distributed to all regions of the retina. Homologous coupling of horizontal cells in heterozygous Cx57-iCre-

VGAT null mice is intact. To assess inhibitory feedback to photoreceptors, retinal slices from Cx57-VGAT null and control mice loaded with Fluo-4 were imaged on a Zeiss Pascal confocal microscope. Brief superfusion with 30 mM K^+ activated photoreceptor L-type Ca channels. 50 µM Kainate (KA) and 50 µM NBQX were used to alter the feedback signal from horizontal cells. In slices from control mice, KA inhibited high-K^+-evoked Ca^{2+} signals in photoreceptors by 22% and NBQX enhanced signals by 23%, consistent with previous reports. In contrast, in retinal slices from Cx57-VGAT null mice, KA failed to inhibit high-K^+-evoked Ca^{2+} signals and NBQX failed to enhance these signals in photoreceptors, suggesting that inhibitory feedback from horizontal cells was inactive in Cx57-VGAT null mice. Taken together, the results indicate that vesicular GABA from horizontal cells is critical for feedback inhibition onto photoreceptors.

Disclosures: A.A. Hirano: None. **X. Liu:** None. **L. Pérez de Sevilla Müller:** None. **J. Boulter:** None. **S. Barnes:** None. **N.C. Brecha:** None.

Poster

879. Retina: Circuits and Synapses

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:879.02/Y17

Topic: D.04. Vision

Support: NINDS intramural research program (NS002986)

Title: Feedback inhibition extends the dynamic range of luminance and contrast coding

Authors: *N. W. OESCH, J. S. DIAMOND
NINDS/NIH, BETHESDA, MD

Abstract: The nervous system often encodes changes in signal level, rather than absolute level allowing for efficient coding of inputs over a large range. In the visual system, where this challenge is particularly acute, the measure of difference between luminance values is referred to as contrast. Recently, we demonstrated that the rod bipolar cell (RBC) synapse in the retina both computes contrast and relays luminance information in its synaptic output, and we demonstrated that contrast is calculated by a mechanism intrinsic to the synapse. This mechanism, in isolation, accurately computes contrast over only a restricted range of background luminance values, yet our perception of contrast remains constant across the entire range of vision. This argues that additional mechanisms must shape the computation of contrast. The rod bipolar cell synapse receives local reciprocal feedback inhibition, yet the role of local feedback inhibition in visual function is currently unknown. To examine the role of feedback inhibition in the computation of contrast we made whole-cell current and voltage-clamp recordings from the post-synaptic

partner of the RBC, the AII amacrine cell, and recorded post-synaptic potentials and excitatory post-synaptic currents in response to visual stimulation, while pharmacologically manipulating feedback inhibition. Here we demonstrate that feedback inhibition extends the range over which both contrast and luminance can be coded by the RBC synapse.

Disclosures: N.W. Oesch: None. **J.S. Diamond:** None.

Poster

879. Retina: Circuits and Synapses

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Program#/Poster#:879.03/Y18

Topic: D.04. Vision

Support: Intramural Research Programs of NINDS and NIAAA, National Institutes of Health

NIH Grant EY018814

Title: A forward transgenic approach to zebrafish retinal circuitry

Authors: P. M. COHEN[1], F. ONO[2], S. E. BROCKERHOFF[3], *R. F. NELSON[1]
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Abstract: Zebrafish (Danio rerio) are a genetic model for vertebrate color vision with retinas that are rich in color processing neural circuitry. A large facet of this circuitry is the lateral inhibitory network of horizontal cells that regulate cones, and which have different spectral responses to light of different wavelengths and irradiances. In this study, we used a forward transgenic ‘enhancer trap’ approach to produce a transgenic line that selectively labeled a subset of horizontal cells with eGFP. We then crossed this line with a TαCP-tdTomato line that labeled all cones with tandem dimer (td) Tomato in order to determine how these horizontal cells connect to cones of different spectral types. These connections were examined in flatmount and 25 µm frozen sections. Horizontal cells were imaged by confocal microscopy and z-stacks were taken at intervals of 1.1 µm (×40 water immersion objective) and 0.71 µm (×63 water immersion objective). The results demonstrate that the forward transgenic-labeled horizontal cells contact at least one ultraviolet cone and one double cone, indicating that they are H2 cells. The double cone contacts were confirmed in zpr-1 labeled retinas. Other studies have shown that this pattern of connections arises in spectrally biphasic types. We found that the transposon gene insertion was located in a non-coding DNA region on chromosome 14. We suspect that this insertion is within a regulatory sequence, and may report on the expression of a distant conserved gene. Our results

show that the enhancer trap method, a forward genetic approach, can selectively label chromatic horizontal cells, enabling visualization of their specific contacts with cones.

Disclosures: P.M. Cohen: None. **F. Ono:** None. **S.E. Brockerhoff:** None. **R.F. Nelson:** None.

Poster

879. Retina: Circuits and Synapses

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Topic: D.04. Vision

Support: ANR-10-BLAN-141-01

Fovea Pharmaceuticals

Global Research Laboratory (KAIST, South Korea)

Title: The structure of the adult mouse retina inner nuclear layer is severely affected by Otx2 haplodeficiency.

Authors: R. TORERO-IBAD[1], H.-T. KIM[2], J. RHEEY[1], D. ACAMPORA[3], A. SIMEONE[3], *K. L. MOYA[4], A. PROCHIANTZ[1], J. KIM[2]
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Abstract: Otx2 homeoprotein transcription factor is expressed in a variety of neural tissues including the retina where its messenger RNA can be detected in photoreceptors and bipolar cells. We analyzed the retina of mice with constitutive haplodeficiency in which one Otx2 allele was replaced by GFP (Otx2 +/GFP mouse). The expression of marker genes for different cell types was analyzed by RT qPCR in retina from 3 month-old Otx2+/GFP mice and compared with that of their wild-type littermates. No difference was observed in the expression of markers for retinal ganglion cells (RGCs), horizontal cells, amacrine cells or photoreceptors whereas the expression of PKCα mRNA - a marker of some bipolar cells - was reduced in the retina of the mutant mice. Histological analyses confirmed no cell loss in the ganglion cell layer and showed no change in the thickness of the outer nuclear layer suggesting no reduction in the number of RGCs, displaced amacrine cells or photoreceptors. However, the inner nuclear layer (INL) was significantly thinner in mutant mice and the number of cells in this layer was reduced by about 23%. Chx10/Vxx2 immunofluorescence showed that a reduced number of bipolar cells explained

the cell loss in the INL. The number of PKCα-positive cells was reduced in the INL corroborating the RT qPCR results indicating a loss of rod ON bipolar. Interestingly, no recoverin-labeled cell bodies could be observed in the INL of 3 month-old Otx2 +/GFP mouse retina reflecting a total absence of type 2 OFF bipolar cells. In conclusion, our results show that RGCs and photoreceptors are not sensitive to Otx2 haplodeficiency, whereas INL integrity, notably its bipolar cell content, depends on full Otx2 gene expression.

Disclosures: R. Torero-Ibad: None. **H. Kim:** None. **J. Rheey:** Research Grant; Fovea Pharmaceuticals. **D. Acampora:** None. **A. Simeone:** None. **K.L. Moya:** Research Grant; Fovea Pharmaceuticals. **A. Prochiantz:** Research Grant; Fovea Pharmaceuticals. **J. Kim:** None.

Poster

879. Retina: Circuits and Synapses

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:879.05/Y20

Topic: D.04. Vision

Title: Computational model for the formation of asymmetric connections from starburst amacrine cells to ganglion cells underlying direction selectivity in the developing retina

Authors: *S. TANAKA[1], K. YANAGAWA[1], M. MIYASHITA[2], M. YAMADA[3], S. USUI[3]
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Abstract: To date, numerous studies have been attempted to elucidate how direction selectivity is formed in some types of retinal ganglion cells (GCs) during early development. Recent reports from Feller's group have clarified that the conductance of $GABA_A$Rs at postsynaptic sites from starburst amacrine cells (SACs) to a direction selective ganglion cell (DSGC) in a null side markedly increased between postnatal 7 and 14 days (P7-P14). Since blockade of neural activity during this period did not affect the formation of direction selectivity, the mechanism underlying the formation DSGCs is suspected to be activity independent. Nevertheless, it seems hard to consider that some innately expressed molecules govern the connectivity from a large number of SACs to each GC. In this study, we propose a two-step mechanism for DSGC formation, where two steps I and II are divided by the timing of GABA switch around P8. (I) Cholinergic connections from SACs to GCs are transiently formed depending on neural activity before P8. (II) $GABA_A$Rs are gradually aggregated in an activity-independent manner by taking over postsynaptic positions of nAChRs. That is, the early formed cholinergic connections scaffold the

asymmetric GABAergic connections from SACs to GCs. If this scenario holds, we need to focus on step (I) and examine whether asymmetric cholinergic connections can be formed from SACs to GCs. To build a model, we took into account Ca^{2+}-permeable nAChRs composed of α7 subunits, $GABA_A$Rs depolarizing membrane potential of GCs, Ca^{2+} influx from extracellular space through voltage gated calcium channels, Ca^{2+}-induced Ca^{2+} release from intracellular calcium stores, lateral diffusion of nAChRs and $GABA_A$Rs on the membrane of dendrites, and Ca^{2+}-dependent interaction between nAChRs and postsynaptic membrane-binding proteins. We derived a nonlinear synapse formation model based on the above observations. Computer simulations showed that asymmetric connections from SACs to a GC are formed if and only if synaptic outputs from SACs are limited to the 1/3 of distal dendrites of SACs, as consistent with the functional morphology of SACs.

Disclosures: S. Tanaka: None. **K. Yanagawa:** None. **M. Miyashita:** None. **M. Yamada:** None. **S. Usui:** None.

Poster

879. Retina: Circuits and Synapses

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Program#/Poster#:879.06/Z1

Topic: D.04. Vision

Support: Tulane Research Funds

Title: Cannabinoid receptor 1 agonists differentially modulate light-evoked OFF responses in mouse retinal ganglion cells under light adaptation

Authors: *J. N. PAHNG, J. YANG, G.-Y. WANG
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Abstract: Endocannabinoids (eCBs) are retrograde neurotransmitters that are released from postsynaptic cells, bind to presynaptic cannabinoid receptor 1 (CB1R), and prevent release of transmitter from the presynapse. Studies in brain have shown that CB1R activation may inhibit postsynaptic neurons by preventing glutamate release from the presynapse, or it may excite postsynaptic neurons by preventing GABA release from the presynapse. In retina, CB1Rs have been localized to both glutamatergic and GABAergic presynaptic sites suggesting that retinal eCBs have a role in modulating both excitatory and inhibitory neurotransmission. Indeed, synthetic cannabinoid WIN 55,212-2 (WIN) application has been shown to decrease spontaneous glutamate and GABA currents recorded in mouse retinal ganglion cells (RGCs). However, it is currently unknown how eCBs modulate light-evoked responses in RGCs. To understand the role

of eCBs in visual signal processing in the retina, we performed whole-cell voltage clamp recordings of light-evoked OFF responses in mouse RGCs before and after bath application WIN under light adaptation. We found that WIN decreased the amplitude of light-evoked OFF responses in one population of retinal ganglion cells, but increased the amplitude of light-evoked OFF responses in another population of retinal ganglion cells. $GABA_C$- specific antagonist TPMPA prevented the WIN-induced increase in the amplitude of light-evoked OFF responses. Similar results were observed in experiments where WIN was replaced with the eCB 2-arachidonoylglycerol (2-AG). The results, in conjunction with previous CB1R localization studies, indicate that WIN decreases light-evoked OFF amplitudes in one population of RGCs by primarily reducing glutamate release, but WIN increases light-evoked OFF responses in another population of RGCs by reducing GABA release onto bipolar cell axon terminals.

Disclosures: J.N. Pahng: None. **J. Yang:** None. **G. Wang:** None.

Poster

879. Retina: Circuits and Synapses

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Program#/Poster#:879.07/Z2

Topic: D.04. Vision

Support: NSF Grant 0924383

NSF Grant 0924372

Title: Distinctive patterns of alterations in proton efflux from goldfish retinal horizontal cells monitored with self-referencing H+-selective electrodes.

Authors: J. JACOBY[1], E. NAYLOR[2], A. BAKER[2], E. TRAN[3], T. GRABLE[2], H. QIAN[4], R. P. MALCHOW[1], *M. A. KREITZER[2]
[1]Biol. Sci. & Ophthalmology & Visual Sci., Univ. of Illinois at Chicago, Chicago, IL; [2]Biol., Indiana Wesleyan Univ., MARION, IN; [3]Univ. of Texas, Austin, TX; [4]Natl. Eye Inst., Bethesda, MD

Abstract: The H+-hypothesis of lateral feedback inhibition in the outer retina predicts that depolarizing agents should increase H+ release from horizontal cells. To test this hypothesis, self-referencing H+-selective microelectrodes were used to measure extracellular H+ fluxes from isolated goldfish horizontal cells. We found a more complex pattern of cellular responses than previously observed from horizontal cells of other species examined using this technique. One class of cells had an initial standing signal indicative of high extracellular H+ adjacent to the cell

membrane; challenge with glutamate, kainate or high extracellular potassium induced an extracellular alkalinization. This alkalinization was reduced by the calcium channel blockers nifedipine and cobalt. A second class of cells displayed spontaneous oscillations in extracellular H+ that were abolished by cobalt, nifedipine and low extracellular calcium. A strong correlation between changes in intracellular calcium and extracellular proton flux was detected in experiments simultaneously monitoring intracellular calcium and extracellular H+. A third set of cells was characterized by a standing extracellular alkalinization which was turned into an acidic signal by cobalt. In this last set of cells, addition of glutamate or high extracellular potassium did not significantly alter the proton signal. Taken together, the response characteristics of all three sets of neurons are most parsimoniously explained by activation of a PMCA pump, with an extracellular alkalinization resulting from exchange of intracellular calcium for extracellular H+. These findings argue strongly against the H+ hypothesis of lateral inhibition.

Disclosures: J. Jacoby: None. **E. Naylor:** None. **A. Baker:** None. **E. Tran:** None. **T. Grable:** None. **H. Qian:** None. **R.P. Malchow:** None. **M.A. Kreitzer:** None.

Poster

879. Retina: Circuits and Synapses

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Program#/Poster#:879.08/Z3

Topic: D.04. Vision

Support: NIH Grant EY09256

Title: The influence of dopamine on the spiking activity of dopaminergic amacrine cells

Authors: *M. L. RISNER, D. G. MCMAHON
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Abstract: Dopamine is a major neurotransmitter that regulates learning and memory, mood, motor movement, sleep and waking, and vision. Dopaminergic neurons contributing to these processes are present in several areas of the central nervous system, including the substania nigra, ventral tegmental area, and retina. Dysfunction of dopaminergic neurons in these areas is related to Parkinson's disease, mood disorders, schizophrenia, addiction, and low vision.
We have recently generated a mouse line with a conditional allele of tyrosine hydroxylase (*TH*KO), the rate-limiting enzyme in dopamine production, and this conditional tyrosine hydroxylase allele is subject to deletion by a Cre/lox recombination system. To investigate the spiking properties of dopaminergic amacrine (DA) cells in *TH*KO animals, loose-patching recordings were made from whole-mount retinas. In addition, we examined if the D2 dopamine

receptor, native to wild-type DA cells, is present in *TH*KO retinas by applying exogenous dopamine to the bath solution.
Preliminary data suggests that the baseline spike dynamics of DA cells in *TH*KO retinas are similar to those observed in wild-type animals. We have also found that DA cells in THKO retinas are sensitive to exogenous dopamine. We will further investigate the role of dopamine in light adaptation in this mouse model at the retinal ganglion cell level.

Disclosures: M.L. Risner: None. **D.G. McMahon:** None.

Poster

879. Retina: Circuits and Synapses

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:879.09/Z4

Topic: D.04. Vision

Title: Ganglion cell morphology is altered in late stages of Retinitis Pigmentosa

Authors: *E. E. O'BRIEN[1,2,3], U. GREFERATH[2,3], E. L. FLETCHER[2,3]
[1]Dept. of Electrical & Electronic Engin., [2]Dept. of Anat. and Neurosci., Univ. of Melbourne, Melbourne, Australia; [3]Bionic Vision Australia, Melbourne, Australia

Abstract: Retinitis Pigmentosa (RP) refers to a family of inherited photoreceptor degenerations resulting in blindness. Our research focuses on further understanding the disease process of RP particularly during mid to late stages when total loss of photoreceptors has occurred and significant remodelling of inner retinal neurons has taken place. Triple mutant transgenic mice, rd1-FTL-Thy1, contain a mutation in the β subunit of phosphodiesterase 6 leading to RP, an axon-targeted β-galactosidase (β-gal) reporter system which is under the regulation of the c-fos gene as well as a fluorescent protein that labels a subset of ganglion cells in the retina. Retinae from mice between P90 to P365 were prepared as wholemounts and processed using immunohistochemistry. In rd1-FTL-Thy1 mice we have previously observed an increase in β-gal expression during photoreceptor degeneration (P10-26) followed by a complete loss of expression (P90). At P120 strong upregulation of β-gal reoccurred in the central retina, despite the loss of all photoreceptors and this expression remained until P365. Interestingly, co-localisation with glial cell markers revealed a glial dysfunction in this area. To investigate whether this β-gal expression was also associated with any morphological change in ganglion cells, we classified ganglion cells in the wild-type and rd1-FTL-Thy1 retina (cells=1065, n=28) based on their soma area, dendritic field size and stratification of dendrites into the inner plexiform layer. The dendritic fields of some ganglion cells (cells=605, n=28) were also further

analysed for their total length, total area and quantity of branching points. Interestingly, there was a decrease in size and complexity of the largest ganglion cell type, A, in the degenerated retina from as early as P90. In contrast, the smaller cell types, B and C, remained unchanged throughout degeneration. However, at P330 reductions in the coverage and complexity of these smaller cells types was observed only in regions that also expressed β-gal. This indicates that the integrity of neighbouring neurons and glia may impact surviving ganglion cells at later stages of degeneration. At P330 bi-stratified ganglion cells also showed a reduction in their coverage across the retina. Overall, there were significant changes in many types of ganglion cells at late stages of degeneration. We propose that these changes in ganglion cell morphology will most likely impact the function of individual cells as well as the retinal circuitry in the degenerated retina.

Disclosures: E.E. O'Brien: None. **U. Greferath:** None. **E.L. Fletcher:** None.

Poster

879. Retina: Circuits and Synapses

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Topic: D.04. Vision

Support: NIH Grant R01EY015514

Title: A novel transgenic approach to study the function of horizontal cells on color and contrast vision

Authors: *L. C. HOLZHAUSEN, R. H. KRAMER
MCB, Univ. of California, Berkeley, CA

Abstract: Color vision allows us to appreciate the world in all its detail and beauty. The neural processing that generates color vision begins at the first synapse of the retina, where cone photoreceptors provide input to bipolar and horizontal cells (HCs). HCs project laterally in the retina, integrate inputs from many photoreceptors, and send feedback signals to cones establishing the center-surround receptive field, which is important for contrast vision. HCs also mediate feedback between cones with different spectral sensitivities and contribute to color opponency, providing the basis for color vision. Here, we use a transgenic approach in zebrafish to study the role of HCs in color and contrast vision.
In order to selectively depolarize or hyperpolarize HCs pharmacologically, we use two transgenic zebrafish lines that express exogenous neurotransmitter receptors in HCs. To enable HC-depolarization, we express a FMRF-amide sodium channel (FaNaC) from Helix aspersa.

FaNaC is a sodium selective channel that is activated by the peptide FMRF-amide. To hyperpolarize HCs, we express the newly developed chimeric channel PSAM-GlyR (Magnus et al. (2011), Science). PSAM-GlyR possesses a pharmacologically selective actuator molecule (PSAM), which binds to a chemically unique agonist. Its ion pore domain is from a glycine receptor. Together, both transgenic lines allow us to specifically activate or inhibit HCs pharmacologically, independent from any light-mediated cone signals.
We have successfully generated transgenic zebrafish lines that exhibit germline transmission of the FaNaC and PSAM-GlyR genes. Using electroretinogram (ERG) recordings on zf-larvae, we have found that expression of FaNaC in HCs does not affect retinal light responses in the absence of FMRF-amide. After the FMRF-amide addition we have detected a change in the b-wave amplitude in ERGs. The ERG b-wave amplitude represents synaptic transmission from cones to on-bipolar cells. Our results suggest that depolarization of HCs via FaNaC causes a change in cone synaptic transmission to bipolar cells, presumably through HC-mediated lateral inhibition of cone synapses. Thus, our transgenic approach provides a unique way to regulate HC activity in vivo and to investigate the role of HCs on the visual response in general as well as contrast and color vision in particular.

Disclosures: L.C. Holzhausen: None. **R.H. Kramer:** None.

Poster

879. Retina: Circuits and Synapses

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:879.11/Z6

Topic: D.04. Vision

Support: NIH Grant EY012204

Title: Disruption of internal pH gradients alters cytosolic Cl- in retinal amacrine cells

Authors: *V. S. KRISHNAN, E. GLEASON
Louisiana State Univ., Baton Rouge, LA

Abstract: We have previously demonstrated that nitric oxide (NO) can release Cl- from an internal store and that this release is partially dependent upon an NO-dependent acidification of the cytosol (Hoffpauir 2006; McMains and Gleason 2011). We continue to pursue the mechanisms underlying this release of internal Cl- in amacrine cells because the cytosolic Cl- concentration determines the effect of incoming GABAergic and glycinergic synaptic inputs on amacrine cell voltage responses. Our current hypothesis is that endosomal compartments constitute the store because they are known to contain 40-60 mM Cl- and they sustain substantial

proton gradients due to the inward pumping of protons on the V-type proton pumps. Here, we examine the effects of weak bases (methylamine (MA) and chloroquine (CQ)) on the proton distribution in amacrine cells loaded with the acidic compartment label LysoSensor. Additionally, using whole cell voltage clamp recordings of GABA-gated Cl- currents, we explore the effects of methylamine and chloroquine on cytosolic Cl-.
Cultured amacrine cells were loaded with 100nM LysoSensor Green for 20 min, at RT, in the dark. The typical pattern of LysoSensor fluorescence in amacrine cells under control conditions was highly non-uniform with dense patches of fluorescence eccentric to nuclei and in discrete bright spots distributed unevenly throughout neuronal processes. Exposure to either MA (10mM) or CQ (100µM) caused a reversible reduction (mean percent reduction, MA 15%; CQ 23%) of fluorescence intensity in these regions presumably due to the buffering of compartmentalized protons. To test the effects of these reagents on the Cl- store, amacrine cells were voltage clamped in zero Cl- internal and external solutions to isolate the store. Cells were recorded either under control conditions or with internal solution containing either 10 mM MA or 100µM CQ. Cells were held at -70 mV and GABA (20 µM) was applied in 400 msec pulses. Under control conditions, the mean amplitude of the inward GABA-gated current was (3.9±1.7pA) reflecting very low cytosolic Cl- due to dialysis from the 0Cl- solution in the recording electrode. With either MA or CQ in the pipet, GABA-gated currents were significantly larger (MA, 79.7±15.9pA, $p<0.001$; CQ, 26.1±5.0pA, $p<0.0001$) implying that with the reduction in the proton gradient across internal membranes, additional Cl- is being added to the cytosol from an internal compartment. Together, these results suggest that a compartment (such as endosomes) that normally sustains a large proton gradient functions as the Cl- store.

Disclosures: V.S. Krishnan: None. **E. Gleason:** None.

Poster

879. Retina: Circuits and Synapses

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Topic: D.04. Vision

Support: Grant-in-Aid for JSPS Fellows

KAKENHI21300148

Title: Independent modulation of bipolar cell outputs by reciprocal and lateral inhibition in the inner retina

Authors: *M. TANAKA, M. TACHIBANA
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Abstract: Bipolar cells (BCs) send excitatory outputs to amacrine cells (ACs) and ganglion cells, and receive reciprocal and lateral inhibitory inputs from ACs in the inner retina. However, it is not yet known how each inhibitory input modulates BC excitatory outputs and contributes to visual signal processing. Here, we performed whole-cell recordings from the giant axon terminal of Mb1 (ON-type) BCs in goldfish retinal slices and examined the properties of reciprocal and lateral inhibition. Depolarization of an Mb1-BC terminal under voltage-clamp induced reciprocal inhibition in itself and lateral inhibition in neighboring Mb1-BC terminals. Both inhibitory inputs were GABAergic but effects of GABA antagonists were different between two inputs, suggesting that each input may be mediated by a different pathway. After blockade of electrical coupling among dendrites of Mb1-BCs, lateral inhibition was abolished but reciprocal inhibition remained intact. Thus, reciprocal and lateral inhibition may be induced by activation of a single Mb1-BC and multiple Mb1-BCs, respectively. Light stimulation induced reciprocal and lateral inhibition in Mb1-BC terminals. Lateral inhibition became prominent as the area of light stimulation was increased up to approximately 1 mm. Lateral inhibition reduced the light-evoked depolarization of Mb1-BCs at weak light intensity whereas reciprocal inhibition reduced it at high light intensity. Thus, reciprocal and lateral inhibition operates at different light-intensity ranges. We constructed a model of an electrically coupled Mb1-BC array with reciprocal and lateral inhibition based on the experimental results, and we monitored the outputs of the Mb1-BC array to simulated visual inputs and endogenous random noise. We found that the different activation properties of reciprocal and lateral inhibition were effective in noise reduction and improvement of the signal-to-noise ratio of Mb1-BC outputs. Therefore, independent modulation of Mb1-BC outputs by reciprocal and lateral inhibition seems to play important roles in visual signal processing.

Disclosures: M. Tanaka: None. **M. Tachibana:** None.

Poster

879. Retina: Circuits and Synapses

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Topic: D.04. Vision

Support: NIH Grant EY06472

Title: Amacrine cells containing vGluT3 in baboon retina

Authors: *D. W. MARSHAK[1,3,3], S. L. MILLS[2], J. M. SUH[1], D. M. DOLINO[1], W. S. LIU[1], R. A. JACOBY[3]
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Abstract: The goal of these experiments was to describe the morphology and synaptic connections of amacrine cells in the baboon retina that contain immunoreactive vesicular glutamate transporter 3 (vGluT3). Retinas were labeled with anti-vGluT3 using an immunoperoxidase method and studied by electron microscopy. There were two types of immunofluorescence experiments, in which the double- or triple-labeled material was analyzed by confocal microscopy. Either vGlut 3 cells and other populations of neurons were labeled using antibodies from two different species, or else retinal ganglion cells were filled with Neurobiotin by intracellular injection, labeled with fluorescent avidin and the retinas labeled with anti-vGluT3. Antibodies from a third species and directed against synaptic markers gephyrin or ribeye were also included in some experiments. The labeled amacrine cells had the morphology characteristic of A8 or knotty type 1 cells described previously, and their dendrites formed two plexuses on either side of the center of the inner plexiform layer. The primary dendrites received synapses from a large GABA-ergic amacrine cell, and the higher order dendrites were both pre- and post-synaptic to other amacrine cells. Based on the light microscopic results, these likely include AII cells, starburst cells, knotty type 2 cells and unidentified glycinergic amacrine cells, but not the polyaxonal amacrine cells coupled to ON parasol cells. The vGluT3 cells received input from ON bipolar cells and made synapses that were not associated with ribbons onto OFF bipolar cells, likely including the DB3 diffuse type. Many synapses from vGluT3 cells onto retinal ganglion cells were observed in both plexuses. In the light microscopic experiments, vGluT3 cells made approximately equal numbers of contacts with midget and parasol ganglion cells, including both ON and OFF types. This was surprising because the vGluT3 cells had been expected to contribute preferentially to the pathway providing input to parasol cells based on their stratification pattern. Puncta containing immunoreactive gephyrin, an inhibitory synapse marker, were found at some of the appositions between vGluT3 cells and each of the four types of labeled ganglion cells; this finding is consistent with a previous report that puncta containing glycine receptors are found in neurons postsynaptic to vGluT3 cells. However, as had also been reported previously, vGluT3 cells did not contain immunoreactive glycine transporter 1. Thus, the vGluT3 cells would be expected to have ON responses to light and make synapses onto neurons in both ON and OFF pathways, but the identity of their neurotransmitter is still uncertain.

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Poster

879. Retina: Circuits and Synapses

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Support: NIH Grants EY005102 and EY014235 to S.C.M.

Title: GABA-mediated signaling from a retinal interneuron switches back and forth between cones at night and bipolar cells in the day due to dopamine-mediated GABAA receptor trafficking.

Authors: *S. C. MANGEL, A. J. CHAFFIOL, H. CHOI, M. ISHII, Y. CAO, A. ADELAJA, C. RIBELAYGA
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Abstract: **Purpose**: $GABA_A$ receptor ($GABA_AR$) expression and activity are increased when intracellular cAMP/PKA increases (Vithlani et al., 2011). Because cone-bipolar cell (cBC) dendrites express dopamine D_1, but not D_2Rs, and cone synaptic terminals express D_2, but not D_1Rs, cAMP/PKA activity is high in cBC dendrites in the day following prolonged (>30 min) light adaptation when their D_1Rs are activated, but high in cone terminals at night in the dark when their D_2Rs are not activated by the retinal circadian clock. We therefore determined whether 1) cones and cBCs express $GABA_A$Rs in the day and night and 2) the effects of GABA release from horizontal cells (HCs), an interneuron presynpatic to cones and cBCs, vary with day and night.
Methods. Rabbits and goldfish were light (photopic)-adapted for 1 h in the day or dark (low scotopic)-adapted for 1 h in the day or night. Retinal sections were processed in an identical manner for immunostaining with anti-$GABA_AR$ (β2/3) antibodies (rabbits: bd-17; fish: 62-3G1). Double labeling with cell type-specific antibodies determined whether $GABA_A$Rs are located on cone terminals (zpr-1) and ON-cBC dendrites (Goalpha). The effects of gabazine ($GABA_AR$ antagonist) on rabbit cBCs and goldfish cones were studied in the day and night using whole-cell patch-clamp recording.
Results. $GABA_AR$ antibody labeling 1) was observed on ON-cBC dendrites, but not cone terminals, following prolonged light adaptation in the day under control conditions but not in the presence of a D_1R antagonist, 2) was observed on cone terminals, but not on cBC dendrites, following prolonged darkness at night, and 3) was minimal following prolonged darkness in the day. In the day, following application of dopamine and APB (mGluR6 agonist that blocks cone to ON-BC signaling), rabbit ON-cBCs produced opposite polarity (i.e., hyperpolarizing) surround responses that were blocked by gabazine. Gabazine increased the input resistance of

goldfish cones at night, but had minimal effect in the day.
Conclusions. These results suggest that 1) prolonged light adaptation increases D_1R activation, which elevates cAMP/PKA, so that $GABA_A$R expression and activity of ON-cBC dendrites are enhanced, producing ON-cBC surrounds via direct GABA-mediated HC input to ON-cBC dendrites, and 2) when the retinal clock elevates cAMP/PKA activity in cones at night by decreasing activation of their D_2Rs, $GABA_A$Rs are expressed and active on cone terminals to a greater extent than during the day when the clock activates the D_2Rs. When bright illumination in the day activates D_1Rs on cBCs, HCs use GABA to signal cBCs, but at night when it is dark and the retinal clock does not activate D_2Rs, HCs use GABA to signal cones.

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Poster

879. Retina: Circuits and Synapses

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Topic: D.04. Vision

Support: ARC DP0988227

Title: Direct inhibition onto retinal ganglion cells plays a critical role in surround inhibition and direction selectivity

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Abstract: In the brain, the exquisite balance between excitation and inhibition is important in determining neuronal output. The response of the neuron is dependent on the integration of excitatory and inhibitory inputs and the level of activity of its surrounding networks. In this study, we examined the size tuning properties and direction selectivity of ganglion cells in whole-mount mouse retinae by doing dynamic-clamp recordings. Firstly, we assessed the effects of different levels of inhibition on spatial-tuning properties of ganglion cells. Light-evoked excitatory and inhibitory conductances recorded in response to different spot sizes were injected into 14 ganglion cells. The ratios of excitatory and inhibitory conductances injected varied between 1:0 and 1:2 (5 different combinations). Secondly, digitally synthesised directionally tuned conductances (modelled on physiological responses of ON-OFF direction-selective ganglion cells) were injected to elucidate the origin of direction selectivity in 32 cells. To test

whether or not direction selectivity can be achieved by varying the amplitude and timing of directional tuned excitatory and inhibitory conductance, we injected a family of excitatory and inhibitory conductance of different angles into 21 cells. After injection of light-evoked excitatory and inhibitory conductances of different stimulus sizes (ratio = 1:1), the neuronal responses generated by the onset of conductance exhibited characteristic area-response function displaying surround inhibition. Surround inhibition was present in all conditions of different ratios; with larger indices of surround inhibition as the level of inhibitory conductance injected increased. Comparison between responses to directionally tuned ON-OFF conductances with and without inhibition at the preferred direction showed stronger responses in the absence of inhibition. For conductances in the null direction, there was no/little response to the injection of control conductance but a much larger response after inhibition was removed. This clearly indicates that inhibition is vital in conferring direction selectivity. For the 21 cells tested with various amplitude and timing of directional tuned excitatory and inhibitory conductances, directional tuning of inhibitory conductances showed to be the critical factor determining direction selectivity in ganglion cells.

Disclosures: J.Y. Huang: None. **D.A. Protti:** None.

Poster

879. Retina: Circuits and Synapses

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Topic: D.04. Vision

Support: NIH Grant EY017353

Title: Two-photon imaging of light-evoked calcium responses in bipolar cell axon terminals of the wholemount mouse retina

Authors: *M. CHEN[1], S. PARK[1], B. BORGHUIS[1], L. LOOGER[2], J. ZHOU[1]
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Abstract: Bipolar cell (BC) axons release glutamate in the inner plexiform layer of the retina where they also receive inhibitory feedback input from amacrine cells. To make direct measurements of the receptive field properties of BC axon terminals, we used two-photon imaging to characterize light-evoked Ca responses at individual synaptic boutons of BCs in the mature mouse retina. The fluorescent calcium indicator protein GCaMP3 was expressed in On BCs following *in vivo* electroporation of a DNA construct encoding GCaMP3 under the mGluR6

promoter. In each retina, patches of On bipolar cells, including rod BCs and various subtypes of On cone BCs, were found to express GCaMP3. The morphology of individual GCaMP3-expressing BCs were characterized by reconstructing vertical stacks of two-photon images taken from the wholemount retina. These On BCs, especially rod BCs, often generated spontaneous Ca transients. They also gave robust responses to light flashes. The kinetics of the Ca responses to light flashes varied from subtype to subtype, with rod BCs showing a more sustained decay phase. The cone BCs showed either a sustained or a transient decay phase, depending on the cell type, and had a center-surround receptive structure. We also examined the light responses of BC terminal boutons that contacted the On starburst processes in ChATcre-td-tomato mice and of BC terminals that contact the dendrites of On-Off direction-selective ganglion cells (DSGCs) which were patch-clamped and filled with a red fluorescent dye. More than one type of On cone BC was found to make apparent contacts with the displaced starburst dendrites and gave robust responses to moving light-bar stimulation. The directional properties of the Ca responses at these BC axon terminals are currently under investigation, though preliminary experiments so far have not detected pronounced direction selectivity.

Disclosures: M. Chen: None. **S. Park:** None. **B. Borghuis:** None. **L. Looger:** None. **J. Zhou:** None.

Poster

879. Retina: Circuits and Synapses

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Support: Knights Templar Eye Foundation

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March of Dimes

Research to Prevent Blindness

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Title: The role of vGluT2 in non-image forming vision mediated by melanopsin-expressing retinal ganglion cells

Authors: *A. DELWIG, K. AHERN, M. M. LAVAIL, D. R. COPENHAGEN
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Abstract: Retinal ganglion cells in the eye rely on glutamatergic neurotransmission to relay visual signals to the brain. These cells use vesicular glutamate transporter 2 (vGluT2) to sequester glutamate into synaptic vesicles. In the present study, we investigated the role of vGluT2 in visual signaling from melanopsin-expressing retinal ganglion cells (mRGCs). mRGCs mediate non-image forming responses to light including avoidance of light and light-induced distress ultrasonic vocalization in neonatal mice and pupillary light reflex (PLR) in older animals. We used conditional knockout of VgluT2 from mRGCs to examine these responses. We found that in neonatal mice, avoidance of light and light-induced distress vocalizations are completely dependent on vGluT2. In adults, loss of VgluT2 results in inability to fully dilate pupils in darkness and to stabilize pupil size. Loss of VgluT2 also results in decreased PLR and in increased delay to maximum constriction. Image-forming vision, evaluated by optokinetic response, was not affected in conditional vGluT2 knockout mice. We conclude that non-image forming vision in young neonates depends exclusively on vGluT2-dependent glutamatergic transmission from mRGCs. In adults, vGluT2-dependent glutamatergic signaling from mRGCs contributes most but not all of the light signals for the control of pupil size.

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Poster

879. Retina: Circuits and Synapses

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Topic: D.04. Vision

Support: DARPA SyNAPSE HRL0011-09-C-001

Title: A spiking retina model with cone opponency

Authors: *S. E. CHELIAN, N. SRINIVASA
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Abstract: Retinas are believed to contain much more sophisticated processing than previously imagined. Their mixed analog-digital signaling, space variant sampling, and gain control are unmatched even by today's most advanced imaging sensors. There have been several attempts to model retinal processing in both hardware and software. However, these models often compromise biological fidelity in favor of performance requirements. Furthermore, a detailed simulation of retinal processing constrains subsequent modeling of visual processing and sensorimotor control. Here, a new model is presented that matches several key functionalities

including cone opponency in a bio-plausible way. In particular, relative proportions and spatial distributions of cone receptors were taken from retinal imaging datasets. Horizontal cells pool responses from several light receptors following a scaling function that controls both receptive field size and density. Bi-polar cell center surround receptive field dynamics were modeled with the shunting or membrane equation with inputs coming from single light receptors and horizontal cells. Finally, habituative dynamics at the ganglion cell layer use the depressing synapse model to differentiate between parvo- and magno- cellular cells. Ganglion cell activations are also converted into stochastic spike trains. Tests compare this model to the Virtual Retina, a recent monochrome spiking retina model, and show improved scaling capabilities. An 845 frame sequence with 50 ms. per frame was extracted from the CASTLE virtual environment with various resolutions. Processing times and number of neurons used for the current model and Virtual Retina were recorded with 3 runs per image resolution. In comparison to Virtual Retina, the current model is faster both in aggregate (35.0% for 64x64 and 5.36% for 128x128 images) and on a per neuron basis (69.5% for 64x64 and 81.2% for 256x256). Thus, while maintaining bio-plausibility, the current model exhibits several retinal functionalities including cone opponency along with greater scaling performance. Future applications will include modeling thalamocortical visual computations as well as the control of virtual agents.

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Poster

879. Retina: Circuits and Synapses

Location: Hall F-J

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Topic: D.04. Vision

Support: EY021831

Title: Evidence for functional role of CB1 cannabinoid receptors in the mammalian cone pathway

Authors: *A. STRAIKER[1], L. PARDON[2], J. PATEL[3], S. VISWANATHAN[2]
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Abstract: CB_1 cannabinoid receptor immunoreactivity has been documented in the vertebrate retina. We investigated the retinal function of transgenic mice lacking CB_1 receptors (CB_1-/-) or the enzyme monoacylglycerol lipase (MGL-/-) involved in the breakdown of 2-arachidonoyl glycerol (2AG), an endogenous cannabinoid.
Full-field photopic flash electroretinograms (ERG) were recorded with DTL electrodes, differentially between the two eyes from anesthetized (ketamine 80 mg/kg, xylazine 10 mg/kg) wild type (WT), CB_1-/- and MGL-/- mice using an ESPION system. Pupils were dilated with phenylephrine HCL (2.5%) and accommodation blocked with tropicamide (1%). The cornea was kept moist with methylcellulose (1%) under custom contact lenses. 0.6 to 171 (P) cd.s/m^2 test flashes were delivered on a 112 (P) cd/m^2 background using blue light (640 nm, 40 nm ½ band width).
ERG waveforms of WT, CB_1-/- and MGL-/- showed a- and b-waves that increased in amplitude with flash intensity. For the 171 (P) cd.s/m^2 test flash, the average a-wave implicit time of MGL-/- was 12.8 ms and shorter than those of WT (19 ms, $p<0.0013$) and CB_1-/- (21.8 ms, $p<0.00079$). The average b-wave implicit time of CB_1-/- was 87.3 ms and delayed relative to WT (54.3 ms, $p<0.00037$) and MGL-/- (32ms, $p<0.000072$). The b-wave of CB_1-/- also took a longer time to recover as indicated by the ½ band width of 129 ms relative to those of the WT (37 ms) and MGL -/- (27 ms). The b-wave of MGL-/- was followed by a large negative potential. The a- and b-wave amplitudes were not significantly different for the 3 groups. However, the intensity response function of the b-wave amplitude indicated a smaller semi-saturation constant [14 (P) cd.s/m^2] with a slope of 1.75 for CB1 -/- relative to WT [67 (P) cd.s/m^2, slope 0.83] and MGL -/- [20 (P) cd.s/m^2, slope 2.1].
The significant alteration in the photopic flash ERG responses of transgenic mice lacking CB_1 receptors or the enzyme MGL provide further evidence for a functional a role of CB_1 receptors and endogenous cannabinoids in retinal information processing.

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Poster

879. Retina: Circuits and Synapses

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Support: CIHR

NSERC

Title: Differential expression of cannabinoid receptors CB1 and CB2 within the vervet monkey retina

Authors: *J. M. BOUSKILA, P. JAVADI, C. CASANOVA, J.-F. BOUCHARD, M. PTITO
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Abstract: The presence of the cannabinoid receptor type 1 (CB1R) has been largely documented in the rodent and primate retinas in recent years. There is however some controversy concerning the presence of the CB2 receptor (CB2R) within the central nervous system. Only recently did CB2R has been found in the rodent retina. Certainly, the involvement of both cannabinoid receptors in cannabis-like effects seems to support the presence of CB2R in the retina. The aim of this study was twofold: 1) to characterize the distribution patterns of CB2R in the monkey retina and compare this distribution to that previously reported for CB1R and 2) to solve the controversy on the presence of CB2R in the neural component of the retina. We therefore thoroughly examined the cellular localization of CB2R in the vervet monkey (Chlorocebus sabeus) retina, using confocal microscopy. Our results demonstrate that CB2R, as CB1R, is present throughout the retinal layers with however striking dissimilarities. Double labelling of CB2R and glutamine synthetase shows that CB2R is restricted to Müller cell processes, extending from the internal limiting membrane with very low staining, to the external limiting membrane with heavy labelling. Furthermore, double labeling of CB2R and retinal markers allowed us to exclude possible CB2R expression in retinal neurons. We conclude that CB2R is indeed present in the retina exclusively in the retinal glia whereas CB1R is expressed mainly in the neuro-retina. These results extend our knowledge on the expression and distribution of cannabinoid receptors in the monkey retina, although further experiments are still needed in order to clarify their role in retinal functions.

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Poster

879. Retina: Circuits and Synapses

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Topic: D.04. Vision

Title: The influence of hepatocyte growth factor during phagocytosis of non-specific targets by retinal pigment epithelium

Authors: C. MARRA[1], J. BLAIZE[3], J. TACHJADI[3], *W. J. L'AMOREAUX[2]
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Abstract: Inhibition of outer segment (OS) processing by retinal pigment epithelium (RPE) has been linked to photoreceptor injury and retinopathy onset. Sub-retinal clearance by RPE is facilitated by specialized phagocytosis featuring both RPE-specific and traditional FCγγR mediated signaling cascades. As a result, RPE are capable of internalizing both specific and non-specific external targets. We believe that hepatocyte growth factor (HGF) activation of its cognate receptor (cMet) may play an integral role in RPE phagocytosis. Lack of cMet signaling results in impaired phagocytosis in macrophages. Phagosome formation triggered by Rac1 is as an essential contributor to RPE phagocytosis following activation by PI3K. Since PI3K phosphorylation occurs downstream of c-Met dimerization, we hypothesize that cMet activation is responsible for mediating both specific and non-specific phagocytic clearance by RPE.
To test our hypotheses, cultured ARPE-19 and RPE-J cells were grown to 70% confluence, prior to 24hr serum starvation. Post starvation, cells were exposed to various concentrations of HGF for 1, 20 or 60 min. or 24 hr before preparing for immunohistochemical investigation. Phosphorylated and total receptor (c-Met) and enzyme (focal adhesion kinase [FAK], PI3K) concentrations were targeted in an attempt to gauge the influence of exogenous HGF on expression levels. In a concurrent study, cells were challenged with fluorescently labeled E.coli or latex beads to evaluate binding and internalization immediately following exposure to exogenous HGF. Intensity values suggest that RPE respond maximally to concentrations of 25 ng/ml of HGF when compared to controls. Unsurprisingly, phosphorylated c-Met concentrations were not significantly altered after 24 hrs; however, immuno-detection data show a two-fold increase in c-Met phosphorylation after 20 min. of exogenous HGF exposure. Elevated HGF treatments also appear to increase binding of both fluorescently labeled challengers. Our findings suggest that RPE respond to increases of exogenous HGF concentrations by up-regulating its receptor and subsequent second messengers systems. In addition, our data show a significant increase of fluorescently labeled E. coli. Interestingly, when RPE are exposed to wortmannin (chemical antagonist of PI3K) prior to elevated HGF in vitro, biding and internalization of non-specific debris appear to be reduced. Taken together, these findings suggest that RTK cross-talk initiated by c-Met activation may be sufficient in mediating general uptake of external debris by RPE.

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Poster

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INNT/INCT/CNPQ

FAPERJ

PRONEX-MCT

UFF

Title: The selective activation of group III metabotropic glutamate receptors subtypes produces different patterns of Gaba immunoreactivity and glutamate release in the retina

Authors: *E. GUIMARÃES-SOUZA, K. C. CALAZA
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Abstract: Glutamate is the major excitatory neurotransmitter in the retina, being released by photoreceptors onto bipolar cells which, in turn, release glutamate onto ganglion cells. Glutamate can activate both ionotropic and metabotropic glutamate receptors, which are widely distributed in the retina. Group III metabotropic glutamate receptors, with the exception of mGluR6, are mostly found in sublayers of the inner plexiform layer. Thus, we decided to investigate the role of the activation of group III metabotropic glutamate receptors on neurotransmitter release from cells in the chicken retina. Non-selective group III agonist L-SOP promoted a decrease in the number of GABA-positive cells in relation to the control, and also in the immunoreactivity found in all the sublayers of the inner plexiform layer. This effect was prevented by the non-selective group III antagonist MAP-4, by GAT-1 inhibitor (NNC-711) and by antagonists of ionotropic glutamate receptors (MK801 and DNQX). Under the superfusing conditions used, L-SOP did not alter endogenous glutamate release from retinal cells. VU 0155041, an mGluR4 positive allosteric modulator, reduced GABA-immunoreactivity in amacrine cells of the inner nuclear layer and in sublayers 2 and 4 of the inner plexiform layer, and also evoked an increase in the release of glutamate from retinal cells, and this effect was inhibited by calcium absence. AMN 082, a selective mGluR7 positive allosteric modulator, also decreased GABA-immunoreactivity in both inner nuclear layer and sublayers 1, 2 and 3 (OFF sublayers), and increased glutamate release, effect that was also inhibited by calcium absence. DCPG, an mGluR8 selective agonist, did not significantly alter GABA-immunoreactivity in inner nuclear layer cells and it did not alter glutamate release. However, it did significantly increase GABA-immunoreactivity in sublayers 4 and 5 (ON sublayers). The results suggest that mGluRIII are involved in the

modulation of glutamate and GABA release in the retina, and that each subtype might be responsible for regulating neurotransmitter release in different regions, maybe participating in distinct visual pathways. mGluR4 might be involved with starburst amacrine cell circuitry, while mGluR7 and mGluR8 might participate, respectively, in the OFF and the ON pathways.

Disclosures: E. Guimarães-Souza: None. **K.C. Calaza:** None.

Poster

879. Retina: Circuits and Synapses

Location: Hall F-J

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Program#/Poster#:879.23/AA2

Topic: D.04. Vision

Support: MEXT Grant S1001034

Title: Morphology and distribution of tyrosine hydroxylase-immunoreactive cells in the developing gerbil retina.

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Abstract: The morphology and distribution of the catecholaminergic neurons in the vertebrate retina have been examined with immunohistochemical technique by many researchers. The catecholaminergic retinal neurons in various animals were first found during a few days after birth. We have examined the development of catecholaminergic neurons in the gerbil retina by using antibodies against tyrosine hydroxylase, the enzyme that controls dopamine synthesis. Gerbils were given a dose of Nembutal, and then perfused transcardially with 0.1M phosphate buffer (PB; pH 7.4) for wholemount preparations, and with 0.1M phosphate buffer added to 4% buffered paraformaldehyde for sections. After removed eyeballs were frozen with a rapid jet of liquid carbon dioxide, they were cut with section of 16μm using a cryostat. The sections and wholemount preparations were incubated in rabbit anti-tyrosine hydroxylase (TH) for 24h at 4°C. They were examined by ABC (avidin-biotin-peroxidase complex) staining method. In this study, TH-immunoreactive (TH-IR) cells were first detected on the first postnatal day in lightly labeled somata located in the inner part of the cytoblast layer (CBL), and in fine, poorly branching dendrites extending toward the middle of the thin and immature IPL. We have found two kinds of TH-IR amacrine cells. The two types were clearly different in somata and stratification of TH-IR dendrites in the inner plexiform layer (IPL) at P (postnatal day) 7. One type (type A) of TH-IR cells have monostratified dendrites extending in the outermost layer of

the IPL, while the other type (type B) cells have dendrites extending in the middle of IPL. The type A somata were larger and more densely stained than those of the type B cells. The type A somata showed no significant growth, while the type B somata showed significant growth between P7 and P14 days. After P 14 little change was seen in the sizes of the type B somata. After P28, the diameters of type A somata did not overlap with those of the type B somata. The type B mean density decreased drastically between P7 and P28 days, while little change was seen in the number of the type A cells. These results suggest that two kinds of dopaminergic amacrine cells have different developmental properties in the developing gerbil retina.

Disclosures: H. Imada: None. **K. Sakai:** None. **E. Miyachi:** None.

Poster

879. Retina: Circuits and Synapses

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Support: Eyesight Foundation of Alabama

NEI Grant P30EYE3039

NEI Grant R01 EY07845

Title: Muscarinic AChR activation affects sustained concentric ganglion cells' center-surround receptive field organization

Authors: *C. E. STRANG, Y. LONG, K. E. GAVRIKOV, K. T. KEYSER
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Abstract: Previous studies have demonstrated that muscarinic acetylcholine receptors (mAChRs) help to shape retinal ganglion cell response properties and that the reported effects of mAChR activation vary by ganglion cell type. Thus, the purpose of this study was to identify specific ganglion cell types whose response properties were affected by muscarinic activation. This portion of the study focused on the effects of m1-m4 mAChR activation on morphologically and physiologically identified ganglion cell subtypes. Rabbit eyecups were used for whole cell patch clamp recordings to test the effects of mAChR agents on ganglion cell light responses. Patch clamp recordings were used to identify brisk and sluggish sustained ON and OFF ganglion cells. Light responses were recorded before, during, and after the application of non-specific muscarinic agonist methacholine (Mcho). Muscarinic receptor antagonists, including pirenzepine

(PZP; m1), himbacine (m2/m4), and 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP; m3), were also applied to determine the contributions of specific mAChR subtype activation. Cell morphology was confirmed by injecting Lucifer Yellow. Retinas were further processed with antibodies against choline acetyltransferase to determine the stratification levels of the dendrites relative to the stratification of starburst amacrine cells.Muscarinic AChR activation and blockade affected the relative weighting of contributions to center and surround of ganglion cell receptive fields. Bath application of Mcho decreased the center On responses and increased surround inhibition of sustained ON cells. In contrast, Mcho application increased the center responses of sustained OFF cells and decreased surround inhibition. Specific mAChR subtype blockade had consistent effects on the light responses of sustained ON cells, for example Himbacine, PZP, and 4-DAMP all decreased the light responses suggesting that both excitatory and inhibitory mAChR subtypes contribute to shaping the center response. Differential effects of specific mAChR blockade on the light responses of sustained OFF cells suggesting that activation of excitatory and inhibitory mAChRs can change the relative weighting of center vs. surround components of the receptive field. Our data suggest that mAChR activation affects center responses through receptors expressed in the vertical pathway, and affects surround responses through receptors expressed on horizontal and/or amacrine cells. The activation of excitatory and inhibitory mAChR subtypes expressed in vertical and lateral pathways appeared to be essential for center-surround balance.

Disclosures: C.E. Strang: None. **Y. Long:** None. **K.E. Gavrikov:** None. **K.T. Keyser:** None.

Poster

879. Retina: Circuits and Synapses

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Program#/Poster#:879.25/AA4

Topic: D.04. Vision

Title: Prostaglandin (PGF2α) suppresses TEA -sensitive K currents by decreasing conductance without changing voltage-dependency in isolated rat retinal ganglion cells

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Abstract: The glaucoma is one of the most important disorders which lead to visual loss. It has been reported that Latanoprost, a prostaglandin F2α(PGF2α) analog, decreases intraocular pressure in glaucoma. It is also suggested that Latanoprost may protect retinal ganglion cells

(RGCs) against cell death in glaucoma. However, few studies have been reported on the effects of these drugs on the ionic currents in RGCs. We suppose that such protective effects of PGF2α-analog may be through a modulation of ionic channels in RGCs. In the present study, we identified RGCs by retrograde labeling with Fast Blue (FB). FB was injected into the superior colliculi in neonatal rats (3-8 days old) by a Hamilton syringe under cryoanesthesia. Two days after the operation,these rats were anesthetized with ether and decapitated. Enucleation of their eyes was performed and RGCs were enzymatically dissociated by trituration. Isolated RGCs were dispersed in the recording chamber and FB-labeled RGCs were identified under an epifluorescence microscope.
The effects of PGF2α on voltage-dependent and TEA-sensitive potassium currents (I_K) were analyzed by whole-cell patch clamp method. I_K was recorded in the Ca-free solution containing TTX(0.5μM) and 4-aminopyridine(4AP; 5mM). PGF2α suppressed TEA-sensitive K currents of the rat RGCs by 25~35 %. PGF2α also decreased conductance of TEA-sensitive K currents (control: 12.2±2.4nS; PGF2α: 8.0±1.9nS) without changing voltage-dependency of TEA-sensitive K currents.
The present study suggests the possibility that the modulatory action of PGF2α on RGC I_K may have some effect on the survival of RGCs under high ocular pressure.

Disclosures: T. Yamamoto: None. **M. Furuta:** None. **Y. Nishimura:** None. **M. Kondo:** None. **Y. Uji:** None.

Poster

879. Retina: Circuits and Synapses

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Program#/Poster#:879.26/AA5

Topic: D.04. Vision

Support: NSERC194670

Title: Origins of intrinsic signals in the retina of pigmented rabbits

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Abstract: Intrinsic optical imaging is a technique that measures neuronal activity indirectly through the coupling between metabolic activity of neuronal populations and the blood supply. Intrinsic responses have been observed in the retina, but the factors determining these signals and their exact anatomical origin have not been clearly identified. The purpose of this study was to

characterize the intrinsic retinal responses and determine the anatomic origin of these signals. Retinal intrinsic optical imaging is performed using a modified fundus camera that captures the reflected light from the retina, illuminated by near infrared light (≈ 750 nm). Stimulation of the retina is performed by a brief spot illumination of green light (≈ 550 nm). Experiments were performed on anesthetized and paralyzed pigmented rabbits. Concurrent with the imaging acquisition, electroretinograms were also recorded to have control values of retinal activity. The experiments were performed in scotopic conditions and started after 30 min of adaptation. Experimental trials consisted of 24s recordings (10 Hz frame rate) and after 3s of baseline measurements, retinal stimulations (200 ms) were given.
Initially, the relation between stimulus intensity and responses amplitude was tested. A non-linear proportional relationship was found between intrinsic signal amplitude and the stimulation intensity used (27% increase from 1.5 to 2.5 log cd.sm-2). To begin studying the contribution of different retinal cell types in these responses, we inhibited sequentially the activity of each of the retinal layers using intra vitreous injections of pharmacological agents: tetrodotoxin (TTX) to inhibit the innermost retinal layer (ganglion cells, amacrine cells), 2-amino-4 phosphonobutyrate (APB) for inhibiting the ON pathway and cis-2,3-piperidinedicarboxylic acid (PDA) for inhibiting the OFF pathway of the retina. Two sequences of injections were tested: 1) TTX, APB and PDA or 2) TTX, PDA, and APB.
Preliminary data show that TTX decreased intrinsic signals amplitude by 20% and suggests a minimal role of ganglion cells in the genesis of intrinsic responses. In the first sequence of injections the amplitude of intrinsic signals decreased by 40% after injection of APB and an additional 46% reduction was observed after the injection of PDA. For the second sequence of injection, intrinsic responses were reduced by 46 % after the injection of PDA, while subsequent injection of APB decreased responses amplitude by only 11%. Our results suggest that the activity from cells located in the outer and middle layers of the retina are responsible for the intrinsic signals observed in the retina.

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Poster

879. Retina: Circuits and Synapses

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Support: NIH Grant EY017428

Research to Prevent Blindness

Title: Pathway specific activation of AMPAR subtypes on retinal ganglion cells

Authors: *R. S. JONES, R. C. CARROLL, S. NAWY
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Abstract: In the retina, AMPA-type glutamate receptors (AMPARs) are expressed on a variety of cell types, including retinal ganglion cells (RGCs). Both the GluA2-containing, Ca^{2+}-impermeable AMPARs (CI-AMPARs) and the GluA2- lacking, Ca^{2+}-permeable AMPARs (CP-AMPARs) contribute to light-evoked responses in RGCs; however specific roles for each type are not well understood. Here, we investigated the conditions for activation of CI- and CP-AMPARs in the ON pathway by measuring the light-evoked IV relationship in mouse RGCs. We find that for dim light intensities CP-AMPARs are activated exclusively and as the light intensity is increased CI-AMPAR responses are recruited. Blocking reuptake of glutamate with TBOA during dim light IV measurements reveals a large CI-AMPAR component similar to what is observed with bright light without TBOA. These results suggest that CP-AMPARs are closest to presynaptic release sites and that CI-AMPARs are located father away in the synapse and can be activated by glutamate spillover, which may be generated in response to bright light. In addition, we wanted to determine whether the dim- versus bright- light dichotomy reflects a difference in rod versus cone AMPAR utilization. We found that in RGCs of mice lacking functional cones, rod pathway responses elicited over a range of light intensities carry a large CP-AMPAR component. However, in RGCs of mice lacking functional rods, cone pathway responses are almost exclusively driven by CI-AMPARs, save for the dimmest light intensities. This suggests that AMPAR subtypes are segregated in the postsynaptic membrane, which may enable rod and cone signaling pathways to activate distinct postsynaptic responses despite converging on the same presynaptic terminal.

Disclosures: R.S. Jones: None. **R.C. Carroll:** None. **S. Nawy:** None.

Poster

879. Retina: Circuits and Synapses

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Midwest Eye Banks

Research to Prevent Blindness Career Development Award

NIH Core Grant EY00700

Title: NMDA receptor expression and functional contributions to the extrinsic light response in intrinsically photosensitive retinal ganglion cells

Authors: *B. STAFFORD, K. Y. WONG
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Abstract: Purpose: There are at least five different types of intrinsically-photosensitive ganglion cell (ipGC; M1-5), each with its own morphology and melanopsin-mediated (intrinsic) light response. In addition, ipGCs also receive rod and cone inputs, similar to other ganglion cell (GC) types, and these inputs generates an extrinsic light response in these cells. However, relatively little is known about the composition of the receptors used by ipGCs to encode rod and cone inputs. Most GC types express NMDA receptors (NMDARs), and, in the case of some types, use them in a contrast-dependent fashion to encode light responses. Other GC types express functional NMDARs, but, apparently, do not use them to encode light responses. Whether ipGCs express and/or use NMDARs to encode their extrinsic light response is unknown. Here, we examined the subunit-specific expression patterns of functional NMDAR subunits as well the contribution NMDARs make to the extrinsic light response in the ipGCs.
Methods: Retinas were isolated from dark-adapted mine in which ipGCs were selectively labeled with EGFP (Ecker et al. 2010). EGFP-labeled cells were visualized with 950nm laser on a multiphoton microscope and targeted for whole-cell recording. Under voltage-clamp conditions, responses to exogenous application of NMDA were recorded at a series of holding potentials. To determine subunit specificity, responses were recorded under control conditions and in the presence of ifenprodil (10 µM), a GluN2B subunit specific NMDAR antagonist. To study the NMDA contribution to the extrinsic light response, light-evoked responses (500 micron flashes of white light) were recorded from GFP+ cells under current-clamp conditions with either control conditions or with MK-801 added to the intracellular solution.
Results: So far we have examined NMDA expression in M1 ipGCs. M1 cells express functional NMDARs and activation of these receptors is sufficient to induce spiking in these cells. NMDARs contribute to the extrinsic light response in M5 ipGCs. When NMDARs are blocked intracellularly in M5 cells, light-evoked spike output is blocked.
Conclusions: Our findings indicate that some ipGCs express functional NMDARs. Further, we find that at least one type uses NMDARs to generate its extrinsic light response. Knowledge about how the extrinsic light response is generated in ipGCs will help create a more complete understanding of how rod/cone signals might interact with and/or modify non-image forming visual function of ipGCs.

Disclosures: B. Stafford: None. **K.Y. Wong:** None.

Poster

879. Retina: Circuits and Synapses

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Support: NHMRC 632640

ARC Centre of Excellence in Vision

Title: Differences in the expression of GABA-A receptor subunits, GABA-C and glycine receptors on bipolar types in primate retina.

Authors: *U. GRUNERT[1], C. J. ABBOTT[2], K. A. PERCIVAL[1]
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Abstract: Background: GABA and glycine are the major inhibitory transmitters in the mammalian retina. Bipolar cells receive GABAergic and glycinergic synapses via multiple types of amacrine cells. Electrophysiological studies of rodent bipolar types suggested that the proportions of GABA and glycine receptors differ between different types of bipolar cells. Here we studied the distribution of $GABA_A$, $GABA_C$ and glycine receptors on different bipolar types in primate retina using immunohistochemistry.
Methods: Vertical cryostat sections through the retinas of macaque and marmoset monkeys were double labeled with antibodies against bipolar types and antibodies against $GABA_A$, $GABA_C$ and glycine receptor subunits. The density of immunoreactive puncta associated with bipolar cells was quantified using an image analysis program (Imaris). Five bipolar types (OFF-midget, DB3, DB4, DB6, and rod bipolar cells) were identified and the expression of the alpha1, alpha2, alpha 3 and gamma 2 subunits of the $GABA_A$ receptor, $GABA_C$ and the alpha 1 subunit of the glycine receptor was determined.
Results: $GABA_A$ and $GABA_C$ receptors were expressed by all bipolar types but at variable densities. The alpha 1 subunit of the $GABA_A$ receptor was most strongly expressed by OFF midget bipolar cells, whereas the alpha 3 subunit was most strongly expressed by the cone ON bipolar type DB4. $GABA_C$ receptors were most strongly expressed by rod bipolar cells and by the ON cone bipolar types DB4 and DB6. The alpha 1 subunit of the glycine receptor was mostly expressed by OFF midget bipolar and DB3 cells.
Conclusions: The differential expression of GABA and glycine receptors on bipolar types in primate retina is consistent with the idea that inhibition varies between different bipolar pathways.

Disclosures: U. Grunert: None. **C.J. Abbott:** None. **K.A. Percival:** None.

Poster

880. Vision: Retinal Circuits and Subcortical Pathways

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Topic: D.04. Vision

Support: NSF GRFP

Title: A ganglion cell in the mouse retina with nearly linear light response

Authors: *B. KRIEGER, J. SANES, M. MEISTER
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Abstract: The notion that the mammalian retina is a simple spatiotemporal pre-filter of the visual scene has started to be replaced with the idea that the retina performs preprocessing to extract specific image attributes. In recent years a number of retinal ganglion cell (RGC) types have been described as feature detectors that selectively respond to distinct visual aspects such as movement in a particular direction or looming objects. Here we report one RGC type that departs substantially from this theme.
The so-called W7 RGCs are labeled in a transgenic mouse line produced by random insertion of a gene for a fluorescent reporter. They represent a natural RGC type as assessed by their regular spacing in a mosaic covering the retina. In constant uniform light the W7 cells fire at an elevated baseline rate (10-20 Hz) with very regular inter-spike intervals, unlike many other mouse RGCs that require a threshold stimulus to fire at all. The W7 cell has a sustained Off response with a classic center-surround receptive field. It modulates the firing rate nearly linearly with the stimulus, except for short periods of silence in bright light. As a result it can report even weak stimuli, down to a few percent contrast. Further receptive field modeling suggests that the W7 cell pools input from Off bipolar cells, each of which has an antagonistic surround, with only mild rectification at the bipolar cell synapse.
These characteristics of the W7 cell suggest that it plays a complementary role to the more specialized types of feature detectors in the mouse retina. We will discuss in particular their function in encoding the natural stimuli that the retina encounters during locomotion.

Disclosures: B. Krieger: Research Grant; NSF GRFP. **J. Sanes:** None. **M. Meister:** None.

Poster

880. Vision: Retinal Circuits and Subcortical Pathways

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Topic: D.04. Vision

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Title: Learning invariances from retinal inputs

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Abstract: The visual cortex is known to develop representations which are invariant to certain stimulus features. These include low level features, for example the phase of an oriented grating, as well as higher level features such as gaze direction. Numerous studies have proposed that such representations are learned by exploiting differences in the timescales over which different features persist. In general, however, these studies use raw visual stimuli as input to cortical networks, and so ignore the effects of spatio-temporal signal processing in the retina. We investigate how retinal processing contributes to the learning of invariances in visual cortex, in particular the development of complex cell properties in V1, and image stabilization under fixational eye movements.

We present a model retina with different cell types that captures both the mostly linear behaviour of midget cells, as well as much of the non-linear behaviour of parasol cells. We then use this model retina as an input layer to a cortical invariance learning network that exploits a self-organised topographical structure to learn selectivity to slowly varying features (Michler et al, J Neurophysiol 2009).

Under continuously drifting stimuli this model learns complex cell-like representations. We find that cells receiving parasol cell input display a higher degree of phase invariance than those trained on midget cell input, while remaining orientation selective. When trained on a combination of midget and parasol inputs, the complex units tune preferentially to parasol cells. Furthermore, we show how, using a non-linear combination of midget and parasol inputs, a cortical network may learn to distinguish local and global image shifts, generating a gaze invariant representation.

Our results suggest that retinal non-linearities - such as those associated with parasol ganglion cells - facilitate the learning of complex cell properties and, together with the bifurcation of

retinal inputs into parvo-cellular and magno-cellular pathways, support image stabilisation under fixational eye movements.

Disclosures: G. Greene: None. **E. Ehrhardt:** None. **T. Gollisch:** None. **T. Wachtler:** None.

Poster

880. Vision: Retinal Circuits and Subcortical Pathways

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Yazdanbakhsh: NSF OMA-0835976

Title: Fixational eye movements increase acuity in a retinal Bipolar-Amacrine-Ganglion circuit model

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Abstract: The mammalian retina contains at least 11 Bipolar (B), 25 Amacrine (A), and 20 Ganglion (G) cell types, each with characteristic dendritic morphologies, stratification patterns, and physiological characteristics, all connected in complex, highly-structured networks with diverse electrical and chemical synapses. While the retina has been widely considered a simple interface between environment and brain with filters, relays, and image compression, the diversity of anatomical and physiological features present in the retina raises an important unanswered question: is retinal complexity an epiphenomenon of natural selection's lack of design or does it serve a purpose, endowing the retina with unforeseen computational capacities, previously masked by technological and experimental limitations.
To address this question, we developed a mathematical model of a B-A-G cell network with eccentricity-dependent receptive fields and focused on the poorly understood issue of how retinal processing differs in the presence and absence of involuntary fixational eye movements

(microsaccades and drift). We defined a spatio-temporal modulation index to characterize the ability of G-cells to resolve different spatial and temporal frequencies. Model parameter values were guided by data on anatomical and physiological properties of midget B-cells, parvocellular G-cells, and electrically-coupled, wide-field A-cells. The rates, extents, and directions of microsaccadic and slow drift movements were in the range of those reported for mammals. Our results indicate that there exists a biologically-motivated parameter set within which the model with fixational eye movement increases retinal sensitivity, lowering the detection threshold for sinusoidal gratings over a range of spatial frequencies (cycles per degree) and temporal flicker rates without the need for extra-retinal aid. Our future studies will investigate the mechanisms that underlie this potential image enhancing feature of fixational eye movements and the model retinal substrates on which it depends. Additionally, our current model has the capacity with parameter changes to implement symmetrical versus asymmetrical connectivity, the lack or presence of direction selectivity, and propagation delays. Simulating these conditions will enable us to further explore the computational implications of differences between mammalian retinas from rabbit to macaque and to offer predictions for future retinal experiments.

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Poster

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Topic: D.04. Vision

Support: NIH Grants EY014454 and EY021372 (YVW and JBD)

Title: Parallel nonlinear processing at retinal bipolar-ganglion cell synapses

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Abstract: Nonlinear processing in the retina has been implicated in a variety of response properties important for natural vision, including contrast adaptation and precise spike timing. Furthermore, nonlinear processing occurs simultaneously across parallel processing streams, which converge onto ~20 different types of output neurons, the retinal ganglion cells (RGCs). RGC types can be distinguished by both their morphology and response properties. To better understand the synaptic mechanisms driving parallel processing across RGC types, we recorded excitatory currents from four RGC types in the in vitro mouse retina during temporal white noise

stimulation of different contrasts. Standard linear-nonlinear (LN) characterizations of the currents revealed different levels of contrast adaptation across cell types. However, the LN model failed to capture transient components of the currents. Inspired by nonlinear modeling previously applied to explain temporal precision in spike train outputs, we constructed models structured to simulate potential physiological mechanisms such as presynaptic inhibition and synaptic depression, and fit them to the observed synaptic currents. We found that the currents recorded in most RGC types were well described by excitation combined with a delayed, multiplicative suppression, which explained the transient features not captured by the LN model. Furthermore, a single set of parameters that captured these transient features also explained the current responses across contrast, outperforming separate LN models fit at each contrast. There were also consistent differences in the nonlinear architecture across RGC types, potentially explaining the diversity in computations across parallel processing streams.

Disclosures: D.A. Butts: None. **Y.V. Wang:** None. **J.B. Demb:** None.

Poster

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Support: NSF CAREER CBET0547457

Title: Adaptation to stimulus mean and variance in the Limulus lateral eye

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Abstract: The vertebrate eye has been shown to dynamically adjust the sensitivity of its output neurons (retinal ganglion cells) to changes in luminance mean and variance though a collection of cellular and synaptic mechanisms distributed across the retinal network. Luminance mean adaptation has also been demonstrated in the output neurons of the invertebrate eye (eccentric cells), and the aim of this study was to investigate whether the homology in visual processing extends to luminance variance adaptation as well. Prior work on the lateral compound eyes of horseshoe crabs suggests that it does not but those studies did not employ a white noise stimulus, which has been effective at revealing contrast gain controls in the vertebrate eye. The spike trains of individual eccentric cells were thereby recorded from live horseshoe crabs to white noise stimuli of varying contrast delivered to optically-isolated ommatidial receptors. The stimulus was also input to a cell-based model of the Limulus lateral eye that can accurately simulate the eye's

response to behaviorally-relevant underwater movies. Linear-nonlinear models estimated from the spike output of model cells were unaffected by stimulus contrast, consistent with the longstanding idea that the retina is fairly linear at a given operating point. However, linear-nonlinear models estimated from the spike output of eccentric cells decreased in gain with increasing contrast of white noise, suggesting a heretofore unknown mechanism of contrast adaptation may operate in the retina. Given the simple organization of the horseshoe crab eye, determining whether this mechanism is distinct from those known to mediate light adaptation has broad implications to vision research.

Disclosures: C. Passaglia: None. **T. Valtcheva:** None. **D. Covert:** None.

Poster

880. Vision: Retinal Circuits and Subcortical Pathways

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Gatsby Charitable Foundation

Title: Parallel pathways for information processing in the retina

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Abstract: Over 20 different ganglion cell types have been identified in the mammalian retina that represent parallel channels of information transmission. Each population of ganglion cells covers the entire visual field and sends a different transformation of the observed image to higher brain areas. We want to understand the origins and functional implications of this diversity. Efficient coding theory postulates that the early visual system is organized so as to transmit image information to the brain most efficiently given the statistics of natural scenes, constraints from sensory noise, and metabolic limits on neural activity. Early applications of the theory treated the retina as a linear spatial filter and derived successful predictions for the center-surround receptive fields of retinal ganglion cells. However, this theory cannot account for the splitting of the visual signal into 20 parallel pathways. Furthermore, it is apparent that the computations performed by many ganglion cells are highly nonlinear. By incorporating this new knowledge into the efficient coding framework we are exploring the constraints and possible

benefits of the diversification among retinal pathways.
A prominent instance of nonlinear pathway splitting in the retina involves the ON/OFF dichotomy. The ON and OFF channels differ in their polarity of responses to light and dark increments in a visual scene. We quantified the information gain in a system with two cell types of opposite light responses, ON and OFF, and compared it to a system where both cells are ON type. Both systems are constrained by the total firing of the cells and by Poisson noise in their spike count. The system with ON and OFF cells is more efficient. However, the difference becomes notable only when the stimulus distribution is sparse, namely when strong light increments or decrements are rare.
We also examined the diversification of ON-OFF direction selective cells in the retina. These neurons respond to moving stimuli traveling in one of the four cardinal directions, but not in the opposite direction. First we considered the encoding of a single stimulus parameter, the direction of movement, by a population of cells constrained as before by the total firing rate. For an intermediate range of firing rates, the optimal distribution of direction tuning curves around the circle is not uniform, at the expense of biasing information transmission to selected stimuli. We also analyzed what solutions emerge when one considers encoding of a second stimulus component, the stimulus velocity. This work will help us understand the constraints and consequences that have shaped the evolution of multiple pathways in the retina.

Disclosures: J. Gjorgjieva: None. **H. Sompolinsky:** None. **M. Meister:** None.

Poster

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Topic: D.04. Vision

Title: Dendritic NMDA spikes in retinal direction selective ganglion cells

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Abstract: Direction selective ganglion cells (DSGCs) in the retina reliably detect direction of movement of visual stimuli over a wide range of stimulation conditions. Direction selectivity (DS) in DSGCs depends on directionality shaped synaptic input from bipolar and starburst amacrine cells. However, the mechanism by which synaptic information is processed in DSGCs to generate DS spike output is presently unclear. To examine this question we mimicked bipolar activation by focal electric stimulation near identified dendritic processes of DSGCs and

recorded EPSPs from the somas. We found that the response to stimulation was highly nonlinear - while low intensity stimuli evoked EPSPs which were less than 4mV in size and scaled linearly with stimulation intensity, stronger stimuli generated EPSPs with average amplitude of 7.9±0.4mV, which were 211±36% above linear prediction. Dendritic calcium imaging at the location of stimulation revealed that the supralinear responses were accompanied by an increase in the dendritic calcium signal which was restricted to ~30µm region around the stimulation site. Application of NMDAR blocker AP5 blocked the supralinear responses and significantly reduced dendritic calcium signals. Further addition of AMPAR blockers completely abolished recorded responses.
In agreement with synaptic stimulation experiments, we found that NMDARs have a significant role in shaping the light responses of DSGCs; AP5 reduced both the amplitudes of subthreshold EPSP responses and the number of action potentials by about 50% for visual stimulations with stationary spots and moving bars. However, the reduction in the number of action potentials was similar between the null and preferred directions, and therefore the DS index was not significantly affected by NMDAR blockers. We conclude that excitatory inputs to DSGCs can generate NMDAR dependent regenerative events (NMDA spikes) which in turn amplify firing in DSGCs.

Disclosures: A. Poleg-Polsky: None. **J.S. Diamond:** None.

Poster

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Topic: D.04. Vision

Title: Combining morphology and genetics towards a solution of the cell type identification problem

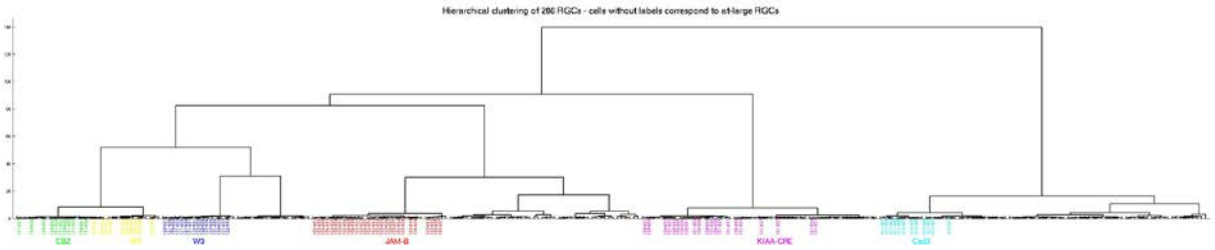
Authors: *U. SÜMBÜL[1], S. SONG[2], M. BECKER[3], K. MCCULLOCH[3], R. H. MASLAND[3], S. SEUNG[1]
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Abstract: Identifying the circuit elements is a crucial step towards mastery of neural circuits. Advances in genetic engineering have made it possible to image certain genetically defined components in some systems. Yet, the gene-to-cell type matching is not one-to-one in general and the cell type identification problem remains elusive for most meaningful circuits. The retina

has a well established morphological organization and a number of genetic labels have emerged recently. Two cells can belong to the same type only if they receive input from the same set of retinal cell types. For retinal ganglion cells(RGCs), this implies a close matching of stratification patterns. We characterized the stratification specificity of genetically defined RGC types at the resolution of imaging, with the starburst amacrine cell dendrites (ChAT bands) as fiducial marks. This information is used for classification. Genetic labels guide the inclusion of further morphological features, creating a hierarchy of such features, and ultimately a classification of cell types.

Convolutional neural networks are used to enhance the image quality. The arbors are traced semi-automatically using the Simple Neurite Tracer of the FIJI software package. The ChAT bands are detected automatically, quasi-conformally mapped to two parallel planes, and used to unwarp the RGC arbor trace in the wavy tissue. The stratification profiles of different RGCs are registered against each other using the flattened ChAT bands. Further morphological features are added by calculating those features within a moving thin in-plane slice, thus creating a continuum in depth for every feature.

We obtained the stratification profiles of more than 280 genetically defined RGCs including JAM-B, CB2, W3, W7, KIAA-CRE, Cad3 lines, and RGCs of unknown types from GFP-M animals. The stratification of known cells was remarkably precise, with submicron standard deviations of the peak plane. Hierarchical clustering was consistent with the available genetic labels, and suggests identities for the at-large RGCs, where there are no known genetic markers.



Disclosures: U. Sümbül: None. **S. Song:** None. **M. Becker:** None. **K. McCulloch:** None. **R.H. Masland:** None. **S. Seung:** None.

Poster

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CIHR to GBA

Title: A stationary spatial representation of moving edges in a population of motion sensing cells in the retina

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Abstract: Introduction
The point-by-point mapping of visual space on the retina is critical for creating a realistic representation of the world. However, the slow transduction of information by the early visual system poses a serious challenge for coding moving stimuli. During the 50-100 ms response latency of second order retinal neurons, moving objects can cover a substantial distance. The visual system must therefore compute delays and dynamically update the spatial map to accurately localize objects. Here we show that such a computation is made by a population of directionally selective ganglion cells (DSGCs) within the retina, providing insights into the biophysical mechanisms underlying this process.
Methods
We recorded responses to moving stimuli from electrically coupled and uncoupled ON-OFF DSGCs (cDSGCs and uDSGCs) and reconstructed their dendritic arbors using 2-photon techniques.
Results
In response to moving stimuli (~200-2000 um/s), the position where uDSGCs first detected the leading edge shifted in space in a velocity-dependent manner, consistent with a fixed transduction delay (i.e. the edges of faster moving stimuli initiated responses further in space compared to slower moving edges). Remarkably, cDSGCs registered the leading edge at nearly the *same* spatial location (~150 μm *before* the nearest dendrite) over the 10-fold range of velocities tested. The ability to code moving edges at a relatively stationary location was found to require lateral interactions. When stimuli were presented locally over the cDSGC (400 μm mask), these cells lost their ability to compensate for delays, and the location at which edges initiated responses drifted in space as a function of velocity, similar to uDSGCs. A similar trend was observed when gap junctions were pharmacologically blocked (18β-glycyrrhetinic acid), suggesting that these were required for mediating lateral interactions. Finally, we constructed a mathematical model to demonstrate that a velocity-dependent increase in gap junction-mediated signals from neighbouring cells can endow cDSGCs with the unique ability to encode a veridical spatial representation of moving edges in the retina.
Conclusions
A population of DSGCs in the retina dynamically corrects the representation of moving stimuli

online to remove processing latencies via a novel mechanism involving gap junctions. The new mechanism allows the retina to convey the actual spatial coordinates of moving objects, via retinotopic connections, to higher visual centres. This active updating of the spatial map at the level of retinal ganglion cells likely aids the accurate and rapid localization of objects moving in space.

Disclosures: S. Trenholm: None. **V. Balasubramanian:** None. **D. Schwab:** None. **G.B. Awatramani:** None.

Poster

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Title: Genetic analysis of visual circuits controlling image stabilization

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Abstract: As the head moves, retinal motion signals drive compensatory eye movements that stabilize the retinal image to optimize visual performance. A critical relay for such motion signals is the accessory optic system (AOS), a collection of midbrain nuclei that receive visual inputs from On-type direction selective retinal ganglion cells (ON-DSGCs). Through an anatomical screen, we discovered a transgenic mouse line with GFP expression largely confined to ganglion cells innervating the AOS, paving the way to detailed structure-function relationships

of AOS circuits. Retinofugal labeling in this mouse line is largely restricted to the medial terminal nucleus and the nucleus of the optic tract; except for sparse labeling in the lateral geniculate nucleus and superior colliculus, other retinal targets lack substantial labeling. Intracellular dye fills and patch recording showed many GFP cells were ON-DSGCs, with characteristic form, and with all three canonical directional preferences. However, bistratified cells resembling ON-OFF DSGCs as well as non-directional selective cells were also encountered. Our results suggest that AOS nuclei stabilize images based on inputs received from both canonical ON-type DSGCs, but that other RGC subtypes may also contribute. Genetic tools such as those described here should prove valuable for parsing the function, structure, development and plasticity of defined visual circuits.

Disclosures: O.S. Dhande: None. **M.E. Estevez:** None. **R.N. El Danaf:** None. **L. Quatrrochi:** None. **P.L. Nguyen:** None. **D.M. Berson:** None. **A.D. Huberman:** None.

Poster

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Title: Using segregated on and off leaflets in the ferret lateral geniculate nucleus to explore push-pull circuitry

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Abstract: En route to the primary visual cortex, visual information coming from the retina is influenced by local thalamic circuits in the lateral geniculate nucleus (LGN). Receptive fields of relay cells in the LGN are made up of concentrically arranged subregions that have opposite preference for luminance contrast, On or Off. Furthermore, within each subregion there is a ‘push-pull’ arrangement of excitation and inhibition for stimuli of reverse contrast. Push-pull has been implicated in linearizing the response profile of relay cells, playing an important role in promoting efficient detection of local contrasts and decreasing sensitivity to redundancies in the stimulus. Past studies have postulated that push-pull is generated by circuits that operate as follows: preferred stimuli evoke feed forward excitation from the retina whereas stimuli of the reverse contrast elicit feed forward inhibition generated by local interneurons having the opposite stimulus preference. In species commonly used to study visual processing, On and Off cells are

intermingled in the LGN, making it difficult to study On and Off circuitry. However in the ferret LGN, cells within the principal laminae are separated according to their preference for On or Off stimuli. This segregation into On and Off leaflets makes the ferret a promising model system to study the structure of On and Off circuits. Hence, we first investigated whether ferret relay cells show a push-pull organization of excitation and inhibition. Different hypothetical circuits that might underlie relay cell responses are listed: 1. Relay cells having dendrites spanning across leaflets could receive inhibition from interneurons residing in the sublamina selective for stimulus of the opposite sign. 2. Relay cells that do not cross leaflet boundaries could derive inhibition from the projections of interneurons in the adjoining leaflet. 3. Alternatively, there could be a mechanism by which interneurons within a leaflet provide inhibition to relay cells in its layer by converting the sign of its retinal input; for example, an interneuron receiving On retinal input would provide Off inhibition. We have begun to investigate these scenarios using dye-filled pipettes to make whole cell recordings from neurons in the ferret LGN. Our immediate goal was to map receptive fields there and explore spatial arrangements of excitation and inhibition within subregions. We have found that relay cells with dendrites that span leaflets have push-pull responses and are currently exploring responses in other morphological populations of cells.

Disclosures: V. Suresh: None. **J. Wong:** None. **F. Sommer:** None. **J. Hirsch:** None.

Poster

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Topic: D.04. Vision

Support: NSERC

The Dana Foundation

Title: Flicker modulation isolates the magnocellular layers of the human lateral geniculate nucleus

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Abstract: The lateral geniculate nucleus (LGN) is composed of multiple neural populations with distinct retinal inputs and thus stimulus response properties. The magnocellular (M) and parvocellular (P) layers receive differ in response properties on several stimulus dimensions,

including color, contrast, spatial frequency, and temporal frequency. We sought develop an experimental paradigm to robustly differentiate between these two cell types in the human LGN using functional magnetic resonance imaging (fMRI). Participants were scanned using a Siemens Trio 3T MRI scanner and 32-channel head coil in York University's Neuroimaging Laboratory. Anatomical regions of interest (ROIs) were manually generated for the LGN and surrounding thalamic nuclei by manually tracing 4 hours of interpolated proton density weighted images. fMRI scans utilized a EPI sequence with 19 coronal slices, 2 mm thick, with a 128 matrix and 192 mm field of view resulting in an in-plane resolution of 1.5 mm2 [TR = 2 s, TE = 42 ms, flip angle = 90^0]. Ten scans were acquired per participant, who fixated on a contrast-reversing rotating hemi-field. Contrast reversal modulated between 2 Hz - 30 Hz at a frequency independent from rotation. Scans were co-registered, linear detrended, averaged and analyzed with a Fourier transform. The flicker modulation activated only a portion of the LGN that was activated by the retinotopy stimulus. By coregistering the functional data to the high-resolution anatomical ROIs, we were able to determine that the flicker modulations were confined to the ventral surface and M layers of the LGN. In sum, we have developed a temporal frequency paradigm that specifically isolates the M layers of the human LGN.

Disclosures: J.D. Viviano: None. **K.A. Schneider:** None.

Poster

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Topic: D.04. Vision

Support: CAPES

CNPq

FAPERJ

Title: Plasticity inside and outside critical period: Time-course and effects of ligth deprivation on the retinocollicular reorganization induced by monocular enucleation

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Abstract: AIM: Several types of injury are able to induce plasticity of intact circuits in the central nervous system. Monocular enucleation is a widespread model to study injury-induced plasticity that has been used in the past 40 years for the understanding of sensory processing in visual system. However, little is known about the mechanisms involved in plastic reorganization induced by monocular enucleation and the time course of these plastic events inside and outside the critical period. Therefore, we decided to investigate the time course of monocular enucleation-induced plasticity on retinocollicular axons of the remaining eye within and outside the critical period and the effect of light deprivation.
METHODS: Lister Hooded rats were submitted to monocular enucleation on postnatal day ten (PND10), PND21 or PND70 and maintained through different periods after surgery. After eye enucleation at PND21, some animals were kept in dark rearing during 24h or 1 week. Animals received intraocular injections of peroxidase or cholera toxin as anterograde tracers in order to map the topography of ipsilateral axons from intact eye.
RESULTS: During the critical period (PND10), monocular enucleation induced an intense sprouting of intact ipsilateral axons 12 hours after injury. This expansion was maintained up to 1 year after enucleation. At PND21, a phase that marks the end of retinocollicular critical period, monocular enucleation also induced an intense sprouting of intact axons 12 hours after injury. However, this sprouting decreased progressively until 6 months after injury. At PND70 we also detected a robust sprouting 12h after monocular enucleation that was reverted 1 week after. Animals subjected to light deprivation after a monocular enucleation at PND21 continued to exhibit an intense sprouting 24h after injury. However, we observed maintenance of this sprouting one week after enucleation at PND21.
CONCLUSION: Together our results indicate that, contrary to what the literature has been proposing, the plastic capacity of neurons in response to injury is present and strong even after the critical period. It also suggests that the elimination of topographically unrelated fibers appears to depend on visual experience. However, the sprouting induced by enucleation was not dependent on the visual experience.

Disclosures: P. Trindade: None. **A.T. Gomes:** None. **H.R. Mendonça:** None. **A.C. Faria-Melibeu:** None. **P. Campello-Costa:** None. **R. Linden:** None. **C.A. Serfaty:** None.

Poster

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Topic: D.04. Vision

Support: FAPERJ APQ1

CNPq UNIVERSAL

Title: Inhibition of calcineurin and microglial activation alter plasticity of intact retinocollicular pathway following monocular enucleation

Authors: *C. A. SERFATY, L. S. CHAGAS, P. TRINDADE
Neurobio., Federal Fluminense University, Inst. De Biologia, Dept. Neurobiologia, Niteroi, Brazil

Abstract: Lesions to the developing central pathways are able to induce rapid plasticity of intact axonal populations that undergo a rapid compensatory growth during the critical period of development. At latter stages, lesions are able to induce slower and more limited plastic responses. Calcineurin is a serine-threonine phosphatase involved both in synaptic plasticity and microglial-induced inflammatory responses. We used a model of monocular enucleation to study the plastic response of ipsilateral retinocollicular axons from the intact eye in order to address the role of calcineurin and microglial cells in the plasticity that takes place in the collicular visual layers. Therefore we compared the blockade of calcineurin activity and the direct blockade of microglial activation in this form of compensatory plasticity during early postnatal development. Between postnatal days (PND) 7-10 rats were given systemic injections of cyclosporine A (CsA - 10mg/kg), a calcineurin inhibitor or vehicle. At postnatal day 10 (PND10), rat pups were submitted to a monocular enucleation under isoflurane anesthesia. At the same time an intravitreal HRP injection was made in the intact eye to label the ipsilateral axonal population to the denervated superior colliculus. A 24h survival followed both enucleation and axon tracing experiments. Systemic CsA administration inhibited the sprouting of intact ipsilateral axons following a monocular enucleation. CsA also inhibited microglial activation as demonstrated by reduction in immunofluorescence of Iba-1 and IB-4 in the superior colliculus contralateral to eye enucleation. To directly access the role and timing of microglial cells activation in the sprouting reaction, we tested either a microglial inhibitor, minociclin (125 mg/kg, sc) or CsA (50 mg/kg) administered four hours after the enucleation procedure. Both treatments were able to inhibit the sprouting of the ipsilateral pathway after a monocular enucleation. The data suggest that calcineurin-induced microglial activation is required for the lesion-induced plasticity of central visual pathways.

Disclosures: C.A. Serfaty: None. **L.S. Chagas:** None. **P. Trindade:** None.

Poster

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Support: EY016155

EY012716

Title: Ultrastructure of mouse tectogeniculate terminals

Authors: N. ZHOU[1], T. E. KRAHE[2], C. W. D. JURGENS[2], W. GUIDO[2], *M. E. BICKFORD[1]
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Abstract: The mouse dorsal lateral geniculate nucleus (dLGN) is a model system for the study of thalamic circuits. As in other species, the synaptic terminals of the mouse dLGN have been classified into four main categories: large profiles with round vesicles and pale mitochondria (RLP profiles; retinogeniculate terminals), small profiles with round vesicles and dark mitochondria (RSD profiles; corticogeniculate and cholinergic terminals), and GABAergic axon (F1 profiles) and dendritic terminals (F2 profiles). However, whether projections from the superior colliculus (SC) to the dLGN fit one of these categories remains unknown. The ultrastructure of tectogenicualte terminals has been difficult to identify because the injection of traditional tracers into the SC can also label retinogeniculate terminals. To specifically label tectogeniculate terminals, we injected a viral vector into the SC that induced the expression of the red fluorescent protein TdTomato. After a 10 day survival time, TdTomato was robustly expressed in SC neurons as well as their axon terminals in the dLGN, pretectum, lateral posterior nucleus and ventral lateral geniculate nucleus. Within the dLGN, TdTomato-labeled axons were distributed adjacent to the optic tract (lateral shell). This region is the primary recipient zone for a number of identified retinal ganglion cells that respond to the direction of movement. The TdTomato was tagged with a DsRed antibody, horseradish peroxidase, and a diaminobenzidine reaction. The tissue was then processed for electron microscopy. Thus far, we have examined a total of 113 tectogeniculate synapses. The tectogeniculate terminals were of medium size (mean area 0.48 um^2); i.e. larger than RSD profiles (mean 0.22 um^2), but smaller that RLP profiles (mean 1.33 um^2). Similar to retinogeniculate terminals, tectogeniculate terminals contact relatively large caliber dendrites (mean 0.84 um^2), many of which extend small protrusions into the presynaptic terminal. In tissue stained for the type 2 vesicular glutamate transporter (vGLUT2, a marker for retinogeniculate terminals) confocal microscopy indicated that tectogeniculate terminals are closely associated with retinal terminals. This association was confirmed at the ultrastructural level; tectogeniculate terminals were often located adjacent to RLP profiles. Our results indicate that tectogeniculate terminals form a fifth category of dLGN synaptic profiles, and the lateral shell may serve as a point of convergence for the integration of retinal and tectal signals involved in the processing of motion direction.

Disclosures: N. Zhou: None. **T.E. Krahe:** None. **C.W.D. Jurgens:** None. **W. Guido:** None. **M.E. Bickford:** None.

Poster

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Topic: D.04. Vision

Title: Mechanisms governing the response of identified neurons in the superficial layers of the mouse superior colliculus to visual stimuli

Authors: *S. D. GALE, G. J. MURPHY
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Abstract: The diversity and specificity with which individual neurons respond to features of sensory cues has been characterized exhaustively via extracellular recordings of neural activity in vivo. By contrast, the synaptic connectivity and biophysical properties that determine the set of stimuli to which neurons do and do not respond remains largely unclear.
To fill this gap in understanding we have focused on the mechanisms governing signal processing in the superficial layers of the superior colliculus (SC). Neurons here can be grouped into several distinct classes on the basis of (1) morphological features or (2) the specificity with which they respond to the size, speed, and/or direction of visual cues. We used juxtacellular recording and labeling in mice to determine to what degree distinct morphological classes of SC neurons exhibit distinct sensitivities to stimulus characteristics. Complementary whole-cell patch clamp recordings performed in slices, paired with activation of channelrhodopsin-2 (ChR2) expressed in subsets of retinal ganglion cells (RGCs) that project to the SC, enables us to assess the source of synaptic input that specific SC cell types receive and the intrinsic properties that shape responses to RGC input.
Consistent with previous work, we found at least four morphologically distinct classes of superficial-layer SC neurons: wide-field (WF), narrow-field (NF), piriform (P), and horizontal (H) cells. The same classes of neurons can be distinguished unambiguously on the basis of intrinsic biophysical features including input resistance, resting potential, spike shape, and spike rate in response to current injection. Preliminary in vivo results further suggest that WF, NF, P, and H neurons respond preferentially to distinct features of visual stimuli. For example, while both WF and H cells have broad dendritic arbors large spatial receptive fields, WF cells prefer small and moving stimuli whereas H cells respond better to large stimuli. NF and P cells have comparably more restricted dendritic arbors and spatial receptive fields. While most SC cells

were weakly or non-selective for movement direction, the majority of strongly directionally selective (DS) cells were NF cells. We are in the process of recording responses to synaptic input from specific types of DS-RGCs in brain slices in order to determine (1) which SC cells receive DS input and (2) how intrinsic membrane properties and dendritic architecture shape responses to such input.

Disclosures: S.D. Gale: None. **G.J. Murphy:** None.

Poster

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Program#/Poster#:880.17/BB7

Topic: D.04. Vision

Support: Wellcome Trust G083205

MRC Studentship

Title: Three dimensional topography and projection lines within the mouse dorsal lateral geniculate nucleus (dLGN)

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Abstract: The basic organisation of connections within the mammalian visual system is a series of ordered point to point maps that replicates the visual world. However, such topographical representation in central targets is complicated by the fact that the retina samples visual space through multiple retinal ganglion cell (RGC) channels. How does parallel processing integrate into a unified topographic representation? Classically, for the retino-geniculo-cortical pathway, this is defined by the ‘projection line’: the column of geniculate neurons projecting to a given point in primary visual cortex that sub-sample different RGC inputs. These lines have been best defined in primates and carnivores: along a projection line, the location in visual space is constant but the functional inputs vary. The mouse is an increasingly important model organism for understanding the emergence and organisation of retino-geniculo-cortical circuitry but little is known about the topological organisation within the dLGN. Unlike cortex or colliculus, it is not obvious that the dLGN is reducable to a two-dimensional sheet and linear projection paths.
Our classic approach to the ‘projection line’ is to inject retrograde tracers (<10 nl latex microbeads, LumaFluor) into the V1 of anaesthetised mice. Subsequently, we analysed the foci

of labelled cells in serial coronal sections of the dLGN. However, in order to understand the topology of projection paths in mouse dLGN, we have reconstructed the 3D shape of the dLGN by aligning the sections to a MRI template. This was achieved through manual alignment to the template, followed by fast free-form deformation to correct for histological distortions. This has allowed us to interpret the 3D distribution of labelled neurons within the standard dLGN space. For our range of cortical injections, we find that both the number and regional spread of labelled cells within dLGN do not correlate with the injection site volume. Our new 3D analyses allow us to define the co-ordinates of geniculo-cortical projection paths, and determine which parameters change with injection site location. Furthermore, using the same standard dLGN space allows us to examine information regarding the topography of the geniculo-cortical mapping by pooling data across animals. For instance, we can compare virtual pairs of injections to gain insight into the precision of the geniculo-cortical projection, which can then be verified experimentally. These findings not only describe the 3D topology of the retinotopic representation within the mouse dLGN but also have implications for investigating the functional heterogeneity of thalamic inputs into V1.

Disclosures: M.N. Leiwe: None. **A.S. Lowe:** None. **S.J. Eglen:** None. **I.D. Thompson:** None.

Poster

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Title: Brain state and direction-selective cells in the dorsal lateral geniculate nucleus of awake rabbit.

Authors: *X. HEI[1], C. R. STOELZEL[1], J. ZHUANG[1], Y. BERESHPOLOVA[1], J. HUFF[1], J.-M. ALONSO[2,1], H. A. SWADLOW[1,2]
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Abstract: Awake animals often shift between alert and non-alert states and the shift is correlated with profound changes in the behavior of the dorsal lateral geniculate nucleus (LGNd) concentric cells, such that, in the alert state, these cells have higher spontaneous activity, lower burst rates and stronger visual responses (Bereshpolova et al., 2011; Cano et al, 2006). The alert state is defined by the presence of hippocampal theta activity and cortical desynchronization; the non-

alert state is defined by high voltage irregular hippocampal activity and slow wave cortical activity. While the effects of state on concentric neurons have been well studied, direction-selective (DS) neurons make up a small but significant proportion (6.5-13.5%) of LGNd neurons in rabbit (Levick and Oyster, 1969; Swadlow and Weyand, 1985), and the effects of brain state on these cells are not known. Here we examine the receptive field properties of DS cells in rabbit LGNd in alert and non-alert states. We found that: 1) the distribution of preferred direction for ON-OFF DS cells fall into four groups, which is similar to that of ON-OFF retina DS ganglion cells (Oyster and Barlow, 1967); 2) Similarly to what we have shown for concentric cells, the spontaneous firing rate is higher in alert than non-alert states (13.7 spike/s vs. 7.8 spike/s, ~43% of reduction, p<0.01, n=7), while the burst rate is lower in the alert state (0.06 burst/s vs. 1.9 burst/s, p<0.001; n=6); 3) When compared to the non-alert state, the mean response rate in the preferred direction is significantly higher in the alert state (alert: 65.6 spike/s; non-alert: 51.6 spike/s; p<0.05, n=6). 4) In both states, the mean firing rate in the null direction is lower than the spontaneous firing rate (alert: p<0.01; non-alert: p<0.05; n=5). However, the rate reduction in the null direction is greater in alert state (mean rate=1.7 spikes/s) than in non-alert state (mean rate=3.7 spikes/s, p<0.05, n=6), and the directional selectivity index (DSI) is significantly larger in the alert state (0.95 vs. 0.87; p<0.01, n=5; DSI = (Pref - Null) / (Pref + Null); 5) There is no significant difference in directional tuning in these two states (half width at half height, alert: mean=52.5 degree; non-alert: mean=55.9 degree; p=0.51, n=6). These results show that the distribution of preferred direction is faithfully transmitted from retina to LGNd and the state effect on spontaneous firing and bursting is similar in rabbit LGNd DS cells and concentric cells. Although there is no effect of state on the directional tuning of these ON-OFF DS cells, the signal to noise ratio along the preferred-null axis is increased from non-alert to alert state.

Disclosures: X. Hei: None. **C.R. Stoelzel:** None. **J. Zhuang:** None. **Y. Bereshpolova:** None. **J. Huff:** None. **J. Alonso:** None. **H.A. Swadlow:** None.

Poster

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Topic: D.04. Vision

Support: NEI Grant EY018251

Title: Very long lasting changes in the lateral geniculate nucleus after prolonged visual stimulation

Authors: *J. HUFF[1], C. R. STOELZEL[1], Y. BERESHPOLOVA[1], J. ZHUANG[1], X. HEI[1], J.-M. ALONSO[2,1], H. A. SWADLOW[1,2]
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Abstract: The visual system adapts to prolonged intense visual stimulation at multiple levels, and psychophysical experiments, as well as those in retina and other central visual structures have examined such adaptations. Most single neuron studies have revealed adaptation effects that last from tens of milliseconds to a few minutes, while some psychophysical experiments have examined effects lasting tens of minutes. Here, we describe a novel, very long lasting adaption in the dorsal lateral geniculate nucleus (LGNd) of awake rabbits that persists for > one hour. We recorded single unit activity from concentric neurons in LGNd in awake rabbits. After characterizing the receptive field of the neuron with sparse noise stimulation, we recorded the spontaneous firing rates in the presence of a gray monitor screen. Initially, we studied only sustained LGNd cells, with a flickering high-contrast stimulus (1 sec on, 1 sec off) over the receptive field center for 10 - 30 minutes. This generated a sustained, high-frequency response to either the on or off phase of the stimulation. Following this prolonged stimulation regimen, all cells (n = 4) showed a severe reduction in spontaneous activity for as long as we studied these cells (periods of up to 50 minutes after stimulus, where recovery was still < 50%). All measures were taken in the alert state (Stoelzel et al. 2009). We then studied this phenomenon using less intense and less prolonged visual stimulation. One protocol was to use a drifting, high contrast grating for 10 minutes, after which we recorded the activity to the diffusely illuminated screen again. All trials resulted in a very long lasting pronounced reduction in firing rate (mean response 5-10 minutes after stimulation = 29.6% of the baseline activity, $p < 0.005$, n =4; mean response 70-75 minutes after stimulation = 47.6% of the baseline activity, $p < 0.005$, n=4), with one case not reaching 50% recovery in over two hours. A number of mechanisms could result in such a prolonged reduction in spontaneous firing. For example, such intense stimulation could cause a membrane hyperpolarization through various mechanisms. We propose that this is the case, because we measured a pronounced increase in “bursting” of the LGNd neuron throughout the periods of reduced spontaneous firing. These phenomena and their effects on visual responsiveness are currently under investigation.

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Poster

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Topic: D.04. Vision

Support: NSERC Grant

Title: Abnormal visual system development in human albinism: A comparison of the optic chiasm and lateral geniculate nucleus using high resolution MRI

Authors: *L. MCKETTON, K. A. SCHNEIDER
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Abstract: *Introduction*
Albinism has been characterized as a genetic condition of hypopigmentation caused by melanocyte or melanin depletion due to an abnormality in melanin pigment synthesis. The lack of melanin severely impacts the development of the eye and visual system, including a variety of ophthalmic deficits such as reduced visual acuity and an abnormal decussation pattern of the optic chiasm. Our objective was to measure the extent of abnormal chiasmatic crossing in an albino population and examine the structure of the lateral geniculate nucleus (LGN).
Methods
Participants were scanned using a Siemens Trio 3T MRI scanner and 32-channel head coil in York University's Neuroimaging Laboratory. Anatomical regions of interest (ROIs) were created for the LGN by manually tracing 1 h of registered and averaged proton density weighted images and for the optic nerve and chiasm, from registered and averaged T1 weighted images. All masks were registered to DTI space for probabilistic tractography to visualize and quantify tracts coming from each optic nerve to each LGN as well as the inputs to different LGN layers. A whole-brain DTI scan was acquired with 64 diffusion directions, 8:05 minutes in acquisition time, field of view 192 mm, 56 2mm thick slices, TR = 6900 ms, TE = 86 ms and a 128 x 128 matrix. A second diffusion-weighted scan was acquired for diffusion spectrum imaging (DSI), which can resolve crossing fibers. This scan covered the eyes, optic nerves, optic chiasm, optic tracts and the LGN, using 256 diffusion directions, 10.57 minutes in acquisition time, 20 2mm thick slices, TR = 2500 ms, and TE = 86 ms.
Results
Using high-resolution imaging, we were able to reliably identify portions of each LGN innervated by either the left or right optic nerve. The LGN of albinos were significantly smaller than those of controls. We were able to measure the fraction of crossed and uncrossed fibers through the optic chiasm in the two populations using DTI and DSI.
Conclusions
We have demonstrated that it is possible to visualize and quantify differences in the visual system of subjects with albinism compared to controls using high resolution MRI.

Disclosures: L. McKetton: None. **K.A. Schneider:** None.

Poster

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NIH Grant 1R44NS073417

NSF Graduate Research Fellowship

Title: Functional mapping of the magnocellular and parvocellular subdivisions of human LGN

Authors: *R. N. DENISON[1], A. VU[1,3], D. A. FEINBERG[1,3], E. YACOUB[4], M. A. SILVER[1,2]
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Abstract: Parallel processing in the visual system is well established in the lateral geniculate nucleus (LGN) of the thalamus, which has distinct anatomical subdivisions with complementary functional response properties. In particular, the magnocellular (M) subdivision of the LGN is selective for high temporal frequencies and low spatial frequencies, while the parvocellular (P) subdivision is selective for low temporal frequencies, high spatial frequencies, and color. However, the subdivisions of the LGN have rarely been studied in humans. Research has been limited by the difficulty of measuring neural responses from LGN subdivisions due to their small size and their location deep in the brain. We used high-resolution fMRI at field strengths of 3T and 7T, combined with presentation of visual stimuli designed to differentially activate M and P neurons, to functionally localize the M and P subdivisions in humans. At both 3T and 7T, we found a robust spatial gradient of BOLD responses to M-type and P-type stimuli across the LGN consistent with the known anatomical organization of M and P LGN layers. In addition, we found that the functional SNR in the LGN was significantly higher at 7T, thereby allowing for more reliable measurements or, alternatively, higher spatial resolution for equivalent SNR. This demonstrates the advantage of higher field strengths for studying functional properties of subcortical structures. The ability to reliably localize the M and P subdivisions of the LGN in humans will facilitate investigations of the roles of these specialized early visual pathways in human visual perception, attention, and cognition.

Disclosures: R.N. Denison: None. **A. Vu:** Employment; Advanced MRI Technologies. **D.A. Feinberg:** Employment; Advanced MRI Technologies. **E. Yacoub:** None. **M.A. Silver:** None.

Poster

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Topic: D.04. Vision

Support: NIH Grant EY013588

Title: Assessment of binocular interaction in the primate LGN.

Authors: *B. LANKOW, W. M. USREY
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Abstract: A central hypothesis regarding the eye- and stream-specificity of LGN lamination is that it facilitates the targeting of corticofugal fibers that participate in a feedback control circuit involved in the processing of visual information. Given the binocularity of many of the neurons that project from the visual cortex and from the thalamic reticular nucleus to the LGN, it is predicted that interocular modulation of LGN cell responses can be driven through these feedback pathways. This has been a contentious issue; while some studies have failed to find any reliable interocular influences in the LGN, other studies have suggested that it may be possible to modulate LGN responses with dichoptical stimulation paradigms. Several factors may drive these inconsistencies. On the one hand, because the activity of corticogeniculate neurons is strongly influenced by anesthesia and corticogeniculate synapses display rate-dependent facilitation, experiments testing feedback modulation need to be carried out in the alert animal. On the other hand, the small influence expected from non-dominant eye modulation may be difficult to assess reliably with traditional tuning measurements in the alert animal, where data can be noisy due to eye movements and other factors. We feel that this question of feedback and interocular modulation is important enough to warrant careful, focused testing. We are therefore conducting a series of experiments using a sensitive dichoptic reverse-correlation procedure to test interocular modulation of the response properties of LGN neurons in the individual layers of the LGN in the alert macaque monkey. Using reverse-correlation techniques, we have made measurements of luminance, disparity, chromaticity, and perceptual influences of non-dominant eye stimulation on the impulse response functions of individual LGN neurons. Preliminary results from these experiments suggest a negligible influence of binocular modulation in the visual processing of LGN cells in all layers of the LGN. These results indicate that binocular

factors in visual processing are implemented no earlier than layer 4c of the primary visual cortex.
This work supported by NIH grant EY013588.

Disclosures: B. lankow: None. **W.M. Usrey:** None.

Poster

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Topic: D.04. Vision

Support: Hertie foundation ZEN grant to KPH

Title: Retinal KCC2 and NKCC1 effects on direction selective ON and OFF responses in the rat's nucleus of the optic tract

Authors: *C. DISTLER[1], K. SPOIDA[2], A.-K. TRAMPE[2], K.-P. HOFFMANN[3]
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Abstract: In the present study we investigated in vivo the effects of pharmacological manipulation of retinal processing on the response properties of direction selective retinal slip cells in the rat's nucleus of the optic tract and dorsal terminal nucleus (NOT-DTN), the key visuomotor interface in the pathway underlying the optokinetic reflex. Employing a moving visual stimulus consisting of either a large dark or light edge we could differentiate direction selective ON and OFF responses in retinal slip cells.
To disclose the origin of the retinal slip cells' unexpected OFF response we selectively blocked the retinal ON channels and inactivated the visual cortex by cooling. Cortical cooling had no effect on the direction selectivity of the ON or the OFF response in NOT-DTN retinal slip cells. Blockade of the retinal ON channel with APB led to a loss of the ON and, to a lesser degree, of the OFF response and a reduction in direction selectivity. Subsequent blocking of GABA receptors in the retina with picrotoxin unmasked a vigorous albeit direction unselective OFF response in retinal slip cells of the NOT-DTN. Disturbing the retinal chloride homeostasis by intraocular injections of bumetanide or furosemide led to a loss of direction selectivity in both the NOT-DTN's ON and the OFF response due to a reduced response in the neuron's preferred direction under bumetanide as well as under furosemide and a slightly increased response in the null direction under bumetanide.
Our results indicate that the direction specificity of retinal slip cells in the NOT-DTN of the rat strongly depends on intraretinal chloride homeostasis which was shown to be important for retinal direction selectivity (Gavrikov et al., PNAS 100: 16047-16052, 2003;Gavrikov et al.,

PNAS 103: 18793-18798, 2006). On top of the well established input from ON center direction selective ganglion cells we could demonstrate an equally effective input from the retinal OFF system to the NOT-DTN.

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Poster

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Topic: D.04. Vision

Support: BBSRC PhD Studentship BB/D526145/1

Title: The anatomy of the centrifugal visual system and its influence on eye development.

Authors: *C. M. DILLINGHAM, J. A. GUGGENHEIM, J. T. ERICHSEN
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Abstract: Purpose: We investigated the role of the centrifugal visual system (CVS), which projects from the brain to the retina, in emmetropization
Methods: In Experiment 1, electrolytic lesions of the isthmo-optic nucleus/tract (ION/t) or sham-surgeries were performed on 4/5-day-old chicks (n=54; Shaver-Black) to disrupt centrifugal efferents to the retina. Ocular phenotyping (A-scan ultrasonography, keratometry and retinoscopy) was performed 7 and 21 days post-surgery. In Experiment 2, lesions were performed as above. Then, following surgery, either a plus (n=21), a minus (n=20) or a plano lens (n=14) was fitted in front of the treated (contralateral) eye for a period of 7d, followed by a recovery period of 14d. Ocular phenotyping was performed 7d, 14d and 21d post-surgery. At the completion of both experiments, an intra-vitreal injection of WGA was made to the eye contralateral to the lesion, followed 48hr later by perfusion fixation, sectioning, anti-WGA immunohistochemistry and stereological analysis. Counts of remaining (i.e. labeled) ION/t centrifugal neurons were compared to sham controls to determine lesion success. Subjects were split into quartiles based upon lesion success (Q1-Q4) with Q1 representing the lowest and Q4 the highest lesion success.
Results: An overall lesion effect on refractive state asymmetry was found 7 days post-surgery ($F(3,50) = 7.104$, $p<0.001$): Treated eyes of subjects in the highest percentage lesion success quartile (Q4) showed a mean hyperopic shift, relative to control eyes (T-C) at 7d post-surgery of 2.31 D ± 2.46, $p=0.568$). After 21d post-surgery, the initially observed anisometropia in the highest lesion success quartile was no longer apparent (Q4 anisometropia −0.17 D ± 0.48,

p=0.214). In response to myopic and hyperopic defocus, all lesion groups showed comparable refractive and axial compensation (p=0.534 and 0.213, respectively). While all groups showed a similar recovery capability from lens-removal induced defocus, Q4 subjects treated with plus lenses showed a minor but significant overshoot in correction from induced hyperopia relative to both sham operated controls and fellow control eyes (both $p<0.05$). Pathway tracing experiments reveal brainstem interhemispheric connections within the CVS that may play a role in normal eye development.
Conclusions: Unilateral disruption of centrifugal efferents to the retina of the contralateral eye appears to result in a moderate axial hyperopia that is subsequently reversed in a developmentally induced recovery period. However, compensation to lens induced defocus and subsequent recovery are largely unaffected.

Disclosures: C.M. Dillingham: None. **J.A. Guggenheim:** None. **J.T. Erichsen:** None.

Poster

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Support: NHMRC Grant #1027913

Title: Thalamocortical conduction latencies and latencies of visually evoked responses in marmoset lateral geniculate nucleus.

Authors: S. K. CHEONG[1,2], A. N. J. PIETERSEN[1,2], B. DREHER[3], S. G. SOLOMON[3,2], *P. R. MARTIN[1,2]
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Abstract: ***Purpose:*** To study connectivity of the “Blue-ON” koniocellular (K) visual pathway from the lateral geniculate nucleus (LGN) to the primary visual cortex (V1). We also asked whether the conduction latencies of LGN cells significantly contribute to the latencies of visually evoked responses in the parallel visual pathways. ***Methods:*** Single unit, extracellular recordings were made in the LGN of sufentanil-anaesthetized marmosets (*Callithrix jacchus*, n = 4). Electrical current (0-5 mA, 50 μs) was injected into V1 using bipolar electrodes. Antidromic activation of LGN cells was assessed using the collision test and conduction latencies were measured. Visual response latencies were measured in LGN using a 70% Michelson contrast, spatially uniform stimulus filling the receptive field. Receptive fields were characterized using

drifting sinusoidal achromatic and short wavelength sensitive (S)-cone isolating gratings. ***Results:*** 23 LGN cells were activated antidromically from V1: 4 Blue-ON K cells; 1 "non-Blue" ON K cell; 6 parvocellular (P); and 12 magnocellular (M) cells. The P cells had latencies of 1.89 ± 0.16 ms (mean ± SD); M cells 1.17 ± 0.23 ms; and Blue-ON K cells 1.80 ± 0.27 ms. The ON K cell had a latency of 2.39 ms. Thalamocortical latencies were on average 3 ± 1% (mean ± SD) of the visual response latency and were positively correlated with visual response latencies (r^2 = 0.68, p = 0.002). Orthodromic activation was observed in three M cells with latency 2.04 ± 1.53 ms (mean ± SD). ***Conclusion:*** Blue-ON K LGN cells make direct connections to V1, substantiating the hypothesis that the K pathway carries signals serving the blue-yellow axis of color vision. The latency of this connection is similar to that of P cells. The conduction latency of thalamocortical fibers contributes very little to the overall latency of visually-evoked signals arriving at V1.

Disclosures: S.K. Cheong: None. **A.N.J. Pietersen:** None. **B. Dreher:** None. **S.G. Solomon:** None. **P.R. Martin:** None.

Poster

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Title: The distribution of synapsin I & II in the visual thalamus of bush babies

Authors: J. P. PATEL[1], M. KOO[1], K. LI[2], J. A. MAVITY-HUDSON[3], R. T. MARION[4,5], G. PURUSHOTHAMAN[3], D. YAMPOLSKY[3], Y. JIANG[2], *V. A. CASAGRANDE[3,2,6]
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Abstract: As part of larger project designed to understand the organization of primate visual thalamus, we compared the distribution of two proteins, synapsin I and synapsin II to the location of visuotopic maps and known inputs to the pulvinar and lateral geniculate nucleus

(LGN) of the prosimian primate, Otolemur garnettii. Synapsin I and II are proteins that tether a reserve pool of synaptic vesicles and have been linked to synaptic plasticity in the mouse visual system (Kielland et al., 2006). Additionally, these proteins have been shown to identify tecto-pulvinar terminals in tree shrew as well as cortico-thalamic but not retino-fugal pathways in the both tree shrew and rat (Wei et al.,2011). We used standard immunocytochemical methods to identify these proteins which also were compared to the known distributions of other markers of pulvinar subdivisions and LGN layers in bushbaby such as vesicular glutamate transporter 2, VGluT2 and cytochrome oxidase (CO). Synapsin I and II showed an even above background distribution in pulvinar that did not correlate with pulvinar subdivisions defined by visuotopic mapping, tectal connections or other protein markers but could reflect the broad input that all subdivisions of pulvinar receive from cortex. In contrast, in the LGN synapsin I and II were most dense in koniocellular layer 4 (K4) and least dense in the parvocellular (P) layers. It is noteworthy that LGN layer K4 receives a dense input from the superior colliculus, yet the colliculus also projects to the other K LGN layers which were clearly defined by VGluT2 immunostaining but did not stand out as more heavily labeled than the magnocellular (M) LGN layers with either synapsin; VGluT2 densely and precisely outlined all the K LGN layers. In fact, VGluT2which has been shown to exist in retinogeniculate terminals, appeared to be particularly dense in the terminals within all the LGN K layers and was only weakly evident in the terminals within the P LGN layers and moderately dense in the LGN M layers. VGluT2 also was evident in dense terminal patches in lateral and inferior pulvinar which co-localized to some extent with CO patches in these subdivisions of pulvinar. That could indicate the location of collicular inputs to these subdivisions of pulvinar (Baldwin et al.,2011). These results suggest that the synapsin proteins do not localize specifically in zones of primate pulvinar that receive collicular input as might be the case for VGluT2. Instead, the distribution of synapsins appeared to correlate most closely with the distribution of cortical input which innervates all subdivisions of primate pulvinar.

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Poster

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P30EY12576

Title: Molecular specificity of monkey retinal ganglion cells during development

Authors: *K. D. MURRAY[1], D. VAN DER LIST[2], W. MARTIN USREY[3]
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Abstract: Visual information travels from the eye to the dorsal lateral geniculate nucleus (dLGN) of the thalamus via parallel pathways that originate from distinct classes of retinal ganglion cells (RGCs) distinguished by unique receptive-field properties, cytoarchitecture, and axonal termination patterns. Despite the profound influence of these parallel pathways on subsequent visual processing, we know relatively little about the mechanisms that guide the functional maturation of RGC diversity. To address this issue, we investigated the molecular maturation of parasol and midget RGCs in the macaque monkey; these cell types target the magnocellular and parvocellular layers of the dLGN, respectively. Using a discovery-based approach we identified sets of genes unique to parasol and midget RGCs that might underlie their functional maturation. Parasol and midget RGCs were individually isolated from embryonic (GD140), early postnatal (10 week old) and adult monkey retinas by laser-capture microscopy, and total RNA was processed for Affymetrix microarray analysis. On average 28±2% of arrayed probe sets were positively hybridized by monkey RGC RNA. A probe level analysis was performed on robust multichip-averaged data using GeneSpring GX software (v. 12.0) and genes that satisfied a 2-fold expression difference (based on age or cell-type comparisons) and a p value of ≤ 0.05 (2-way ANOVA) were identified. Overall, significantly more genes were downregulated ($\sim$800) than upregulated ($\sim$400) during development. A surprising number of genes had transiently increased expression at 10 weeks of age ($\sim$600) compared to embryonic or adult RGCs. When we compared cell type, a greater number of genes were differentially expressed between parasol and midget RGCs in adult retina ($\sim$150) compared to early postnatal ($\sim$60) and embryonic ($\sim$80) retinas, suggesting a developmental maturation of cell-type specificity. Analysis of the interrelatedness of differentially expressed genes using Ingenuity Pathway Analysis software suggested molecules associated with intercellular signaling and cellular organization and development were among the top differentiators of RGC identity. Subsequent cellular confirmation of differential expression will identify markers useful for delineating the functional maturation of monkey retinal circuitry.

Disclosures: K.D. Murray: None. **D. van der List:** None. **W. Martin Usrey:** None.

Poster

880. Vision: Retinal Circuits and Subcortical Pathways

Location: Hall F-J

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Program#/Poster#:880.28/BB18

Topic: D.04. Vision

Support: RCN Grant 178892/V30 eNeuro (Norway)

NOTUR Computing Resources (Norway)

Helmholtz Alliance on Systems Biology (Germany)

Title: Firing-rate models for the retina-LGN connection derived from experiments

Authors: T. HEIBERG[1], B. KRIENER[1], T. TETZLAFF[2], A. CASTI[3], G. T. EINEVOLL[1], *H. E. PLESSER[1]
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Abstract: Firing-rate models provide a practical tool for studying the signal processing in the early visual system (Dayan & Abbott, 2001). Nordlie et al (2010) have recently shown that rate-based models can capture the response properties of model neurons with strong synapses, as commonly found in retinogeniculate connections. Here we obtain firing-rate models of lateral geniculate nucleus (LGN) relay cells based on a neuron model with conductance-based synapses and after-hyperpolarizing currents fitted to cat relay cells (Casti et al, 2008) and on a spike-response model fitted to macaque relay cells (Carandini et al, 2007).
Our approach is based on Nordlie et al (2010): Input spike trains were modeled as inhomogeneous Poisson point processes with sinusoidally modulated rate. Average rates, modulation amplitudes, and phases of the period-averaged spike responses were measured for each of the data-fitted neurons and a broad range of stimulus parameters. The resulting responses are well described by a linear first-order low-pass filter. Combining this filter with the nonlinear response characteristic for stationary inputs, we constructed a linear-nonlinear firing-rate model, which accurately predicted the population response for a variety of non-sinusoidal test stimuli.
Spike trains from the retina have been shown to be more regular than a Poisson process (Troy & Robson, 1992). Thus, we further investigated the effect of presynaptic spike-train regularity on the models. We found that more regular spike input can lead to rectification of low-rate and linearization of high-rate input. Overall, the rate models perform well for both regular (high-order Gamma) and irregular (Poisson) input.
Our results indicate that firing-rate models with time constants as low as a few milliseconds can be used to model signal processing at the retina-LGN connection.

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Disclosures: T. Heiberg: None. **B. Kriener:** None. **T. Tetzlaff:** None. **A. Casti:** None. **G.T. Einevoll:** None. **H.E. Plesser:** None.

Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

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Topic: D.08. Pain

Support: NINDS grant NS065091 (to CM)

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PUMC&CAMS/IBMS Dean's Fund 2011RC01 (to CM)

fellowship from the Canadian Institutes of Health Research (CIHR) (to LQ)

Title: Functional coupling between neuronal Fc-gamma receptor and the transient receptor potential canonical 3 (TRPC3) channel in IgG immune complex-induced activation of dorsal root ganglion neurons

Authors: L. QU[1], Y. LI[2], X. PAN[2], P. ZHANG[1], R. H. LAMOTTE[1], *C. MA[3]
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Abstract: Pain may accompany antigen-specific immune diseases but the underlying mechanisms remain obscure. One common feature of these diseases is the elevated serum/tissue level of IgG immune complex (IgG-IC). We previously demonstrated that IgG-IC directly excited a subset of dorsal root ganglion (DRG) neurons in rats through neuronal Fc-gamma receptor I (FcγRI) and may induce pain. However, the signaling pathways and transduction

channels activated downstream of IgG-IC are largely unknown. In the rat DRG neurons, recorded with whole-cell patch-clamp electrophysiology, IgG-IC induced a non-selective cation current (I_{IC}) carried by Ca^{2+} and Na^{+}, and regulated/sensitized by intracellular Ca^{2+}. Single-cell RT-PCR revealed that the mRNA of transient receptor potential canonical 3 (TRPC3) was always coexpressed with FcγRI mRNA in the same DRG neuron. Moreover, the I_{IC} was inhibited by ruthenium red (a general TRP channel blocker), BTP2 (a general TRPC channel inhibitor) or pyrazole-3 (a selective TRPC3 blocker). Specific knockdown of TRPC3 using small interfering RNA attenuated IgG-IC-induced Ca^{2+} response and the I_{IC}. Additionally, the I_{IC} was blocked by tyrosine kinase Syk inhibitor OXSI-2, phospholipase C (PLC) inhibitor neomycin, or the IP_3 receptor antagonist 2-aminoethyldiphenylborinate or heparin, but not affected by DAG analog OAG. These results indicated that TRPC3 functionally couples to neuronal FcγRI through the Syk-PLC-IP_3 pathway, and acts as a key downstream transduction channel mediating the depolarizing effects of IgG-IC on DRG neurons. Our findings may provide new therapeutic strategies for the pain related to antigen-specific immune diseases.

Disclosures: L. Qu: None. **Y. Li:** None. **X. Pan:** None. **P. Zhang:** None. **R.H. LaMotte:** None. **C. Ma:** None.

Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

Location: Hall F-J

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Topic: D.08. Pain

Support: CIHR

Louise and Alan Edwards Foundation

AstraZeneca R&D Montreal

Title: Contribution of TRPC channels to calcium regulation in DRG sensory neurons and nociception

Authors: H. ALKHANI[1], A. R. ASE[1], R. GRANT[2], D. O'DONNELL[2], *P. A. SEGUELA[1]
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Abstract: Pain is an unpleasant sensation experienced following injury, inflammation or ischemia. Pathologically abnormal intracellular calcium levels in DRG neurons are linked to

hyperexcitability and chronic pain. Tight regulation of cytosolic calcium levels is key to maintaining calcium homeostasis and SOCE (store-operated calcium entry) has been implicated as a major control mechanism of intracellular calcium levels. The TRPCs (transient receptor potential, canonical) are tetrameric calcium-permeable cation channels with a documented role in store- and receptor-operated responses linked to phospholipase C. TRPCs have been shown to integrate intracellular and extracellular stimuli, including algesic mediators ATP and glutamate, into membrane potential changes and calcium entry, therefore they could play a critical role in peripheral nociception. Our in situ hybridization data on the cellular distribution of the entire 7-member TRPC gene family indicated that TRPC1 and TRPC3 mRNAs are strongly expressed in adult rat DRG sensory neurons. Using calcium imaging, pharmacology as well as expression of STIM1 and TRPC dominant-negative mutant subunits, we gathered evidence for a significant involvement of both TRPC1 and TRPC3 in calcium responses as store- and GqPCR-operated channels in recombinant and native preparations. We are currently investigating the contribution of TRPC1 and TRPC3 function to DRG neuron excitability and calcium-dependent pathways in physiological conditions and chronic pain states.

Disclosures: H. Alkhani: None. **A.R. Ase:** None. **R. Grant:** Employment; AstraZeneca. **D. O'Donnell:** Employment; AstraZeneca. **P.A. Seguela:** None.

Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

Location: Hall F-J

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Program#/Poster#:881.03/CC1

Topic: D.08. Pain

Support: NIH Grant HT32 HD007325

Title: A genetically tractable model of noxious cold detection in *Drosophila* larvae

Authors: *H. N. TURNER, M. J. GALKO
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Abstract: An organism’s comfort and ultimately its survival depends on the ability to detect and avoid noxious thermal stimuli thus preventing tissue damage. In some disease states, patients can experience painful and maladaptive perception of innocuous cold and heat or an inability to perceive noxious cold or heat. Our current understanding of the basic biology of noxious cold perception is gravely minimal. Our goal is to determine the genetic basis for noxious cold perception using the genetically tractable Drosophila model. We have developed a novel “cold probe” that allows focal application of a defined noxious cold stimulus (3-15 ºC), and found that

Drosophila larvae produce a mutually exclusive set of primary reactive behaviors, distinct from the previously described aversive “corkscrew” behavior seen in response to a high temperature probe (1). These behaviors include a tail raise (TR) of approximately 45-90°, a combined head and tail raise (HT) of 45-90°, and a full-body scrunching (SC) behavior, likely to be similar to the “accordion-like” behavior reported previously (2). Below 12º C, the resulting behaviors occur in approximately 60% of larvae, and are consistent and reproducible. We have attained 13 transient receptor potential (TRP) whole animal mutants and are testing these larvae with the cold probe to determine the possible contributions of each of the TRP channels on producing the cold-specific behaviors. To date we have found that two mutants previously reported to affect perception of noxious heat, pyrexia and dTRPA1, both show an increase in HT behavior and a decrease in SC behavior. Meanwhile, brivido mutants recently reported to affect ambient cold preference, have exactly the opposite phenotype- decreasing HT behavior and increasing SC behavior. We will report on the behavioral phenotypes of these and other TRP mutants. Taken together, our unique tool and assay and the other sophisticated genetic approaches available in the fly should allow us to uncover the conserved molecular/genetic basis of noxious cold perception. (1) Tracey WD, Wilson RI, Laurent G, Benzer S. Painless, a Drosophila gene essential for nociception. Cell 2003. 113(2):261-73. (2) Richard Y. Hwang, Lixian Zhong, Yifan Xu, Trevor Johnson, Feng Zhang, Karl Deisseroth, and W. Daniel Tracy. Nocifensive neurons protect Drosophila larvae from parasitoid wasps. Current Biology 2007. 17, 2105-2116.

Disclosures: H.N. Turner: None. **M.J. Galko:** None.

Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:881.04/CC2

Topic: D.08. Pain

Support: NS078530

Title: Translational profiling approach for the molecular characterization of trpm8 sensory neurons

Authors: *D. MCCOY, D. MCKEMY
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Abstract: The sensory nervous system is responsible for the detection of a wide variety of environmental cues. One sensory pathway in which biological players have been identified is that of temperature sensation. A number of Transient Receptor Potential (TRP) ion channels

have been found to play integral roles in the perception of temperature in that they are thermally-gated, leading to nerve firing upon activation. The channel TRPM8 detects both noxious and innocuous cold and is the molecular basis for the cooling associated with menthol. It has been directly implicated in the cold allodynia associated with inflammation, nerve injury, and certain chemotherapeutic drug treatments. TRPM8 knockout studies have shown that the channel is necessary for the development of many of these injury-induced cold hypersensitivities making it likely that cold-sensitive, TRPM8 positive neurons change in response to injury. Recent work has identified candidate molecules involved in the sensitization to cold stimuli. For example, specific potassium channel Kv1 inhibitors have been shown to increase cold sensitive neuron temperature thresholds in vitro. Additionally, recent work in our lab has shown that Phospholipase C (PLC)-mediated depletion of the phospholipid PIP2 results in reduced cold-induced TRPM8 currents. Thus, a gene expression analysis of cold-sensitive, TRPM8-expressing neurons in models of these hypersensitive states would help in the understanding of the molecular basis for thermal hypersensitivity and could reveal new therapeutic targets for these conditions. Typical gene expression profiling approaches use fluorescence-activated cell sorting (FACS) to collect a specific cell subtype to be profiled. However, TRPM8 is expressed in a small subset of neurons and, due to the inefficiencies in attaining ample amounts of mRNA using FACS, an alternative approach is needed. Here we describe the development of a mouse transgenic model which can circumvent these pitfalls using the (TRAP) Translating Ribosome Affinity Purification technique which employs the use of an eGFP labeled ribosomal protein L10a (eGFP-L10a) as a means to pull down cell-specific translating mRNA sequences when genetically targeted to TRPM8-expressing neurons. Additionally, we have further diversified the use of this technique by developing a CRE-dependent construct in which eGFP-L10a and mCherry-L10a can be selectively targeted. This technique allows for transcripts from two different populations to be simultaneously isolated from the same tissue source and will be particularly beneficial in the characterization of cell types in which distinct subpopulations have been identified.

Disclosures: D. McCoy: None. **D. McKemy:** None.

Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

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Support: BFU2008-04425

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BFU2007-61855

Title: Cold receptor activity in the tongue of TRPM8 and TRPA1-deficient mice

Authors: *B. L. DENLINGER, F. VIANA, C. BELMONTE
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Abstract: The tongue is richly innervated by cold-sensitive nerve afferents. The molecular basis of cold transduction in sensory fibers is not fully understood; TRP channels and leak K+ channels are thought to play important roles. We developed an 'in vitro' preparation of the superfused mouse tongue to record impulse activity in single sensory fibers of the lingual nerve in wild-type, TRPM8 (Dhaka et al., 2007), TRPA1 (Kwan et al., 2006) and TRPM8/TRPA1 null mice and analyzed the effect of controlled thermal stimuli on this activity. Sustained nerve impulse discharges were evoked in a fraction of sensory fibers when temperature of the perfusing solution was decreased from a basal temperature of 34-35º down to 10ºC. Based on their thermal threshold, cold-sensitive fibers were subdivided into low (greater-than or equal to 26ºC, LT) and high threshold (< 26ºC, HT) fibers. Deletion of TRPM8 did not completely abrogate the population of cold-sensitive fibers with those still responding to cold exhibiting thresholds in the low and high ranges. The vast majority of cold-sensitive fibers in TRPA1 -/- were LT fibers, supporting the contention that cold transduction in the population of HT fibers depends largely on TRPA1. In TRPM8/TRPA1 -/- mice, cold-sensitive fibers were still found, all being LT fibers. Chloroform (20 mM), an activator of thermosensitive TREK-1 potassium channels, attenuates or eliminates responses of these fibers to cold. Altogether, these results indicate that activation of lingual sensory fibers by cold depends on several transduction mechanisms. TRPM8 appears to be important but not unique for the detection of discrete temperature reductions. Other channels, presumably background potassium channels appear to be also involved, while TRPA1 seems to mediate transduction of more intense cooling.
We also explored the contribution of cold thermoreceptors in the oral mucosa to drinking preference and overall water intake in mice using an experimental cage in which the water temperature of two drinking bottles could be cooled and heated from a control temperature of 22ºC. After water deprivation during 24 hours, drinking time and volume were measured over a 1 hour period. Wild-type mice drastically preferred water at 22ºC over cooler water, while TRPM8 -/- mice had no preference and TRPM8/TRPA1 -/- mice even preferred the cooler water. TRPA1 -/-mice preference closely resembled that of the wild-type mice. All knockouts also spend a longer total time drinking at each temperature than wild-type mice. These data suggest that cold thermoreceptor signaling, in particular mediated by TRPM8 channels is an important cue in thirst and in preabsorptive signaling of satiety.

Disclosures: B.L. Denlinger: None. **F. Viana:** None. **C. Belmonte:** None.

Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

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Topic: D.08. Pain

Support: NIH Grant R01 N542595

Title: The role of TRPM8 and TRPA1 in the cold responses of mice

Authors: *D. S. BRENNER, R. W. GEREAU, IV
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Abstract: Pain is an important clinical and societal issue, costing $99 billion in federal and state medical expenditures in 2008 alone. Despite this expense, recent studies have concluded that pain management must be improved in key patient populations including postoperative patients and cancer patients. Pain can be subdivided into different categories including mechanical, heat, and cold pain, which can have different mechanisms of transduction and transmission. Cold pain is particularly clinically relevant, as broad populations including multiple sclerosis, chemotherapy, and stroke patients all experience types of cold hypersensitivity.
Work over the last decade has suggested that the Transient receptor potential (TRP) channels TRPM8 and TRPA1 may be physiologically sensitive to cold stimuli. Nevertheless, there is still some controversy over the role of TRPA1 in cold pain. This controversy is due, in part, to limitations in the behavioral assays currently available to measure cold responses in vivo. To complement the assays currently in use, we have applied our new assay of cold response threshold, the cold plantar assay, to further define the roles of TRPM8 and TRPA1 in acute, inflammatory, and neuropathic cold responses.

Disclosures: D.S. Brenner: Research Grant; R01 N542595. **R.W. Gereau:** Research Grant; R01 N542595.

Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

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Topic: D.08. Pain

Support: NIH grant DE018661 to J.G.G

Title: Thermal sensitivity of voltage-gated Na^+ channels and A-type K^+ channels confers sensory discrimination between innocuous and noxious cold by primary afferent neurons

Authors: *J. GU[1], I. SARRIA[2], J. LING[2], R. IKEDA[2]
[1]Univ. Cincinnati Col. of Med., Cincinnati, ; [2]Univ. Cincinnati Col. of Med., Cincinnati, OH

Abstract: Both innocuous and noxious cold activate TRPM8, a principal cold transducer, raising a question how the two subtypes of sensory modalities are discriminated by the sensory system. Since TRPM8 is located on both tetrodotoxin-sensitive (TTXs) and tetrodotoxin-resistant (TTXr) Na^+ channel-expressing primary afferent neurons, we hypothesize that the sensory discrimination between innocuous and noxious cold is partially due to thermal sensitivity differences between TTXs channels and TTXr channels. To test this hypothesis, we investigated whether and how action potential firing properties in TTXs and TTXr neurons of rat dorsal root ganglions were differentially modified by cooling temperatures. We found that action potential firing in response to membrane depolarization was suppressed in TTXs neurons but maintained or facilitated in TTXr neurons at noxious cold temperatures. We showed that cooling temperatures strongly inhibited TTXs Na^+ channels and A-type K^+ currents (IA) but had fewer inhibitory effects on TTXr Na^+ channels, and that the thermal sensitivity of these voltage-gated ion channels determined action potential firing properties in both TTXs and TTXr neurons at cooling temperatures. Finally, we found that TRPM8-expressing TTXs neurons and TRPM8-expressing TTXr neurons fire action potentials at innocuous and noxious cold temperatures respectively, demonstrating sensory discrimination between innocuous and noxious cold by the two groups of cold-sensing neurons.

Disclosures: J. Gu: None. **I. Sarria:** None. **J. Ling:** None. **R. Ikeda:** None.

Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

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Suzuken Memorial Foundation

YOKOYAMA Foundation for Clinical Pharmacology

Title: Acute cold hypersensitivity characteristically induced by oxaliplatin is caused by enhanced responsiveness of TRPA1 in mice

Authors: *M. ZHAO, H. SHIRAKAWA, T. NAKAGAWA, S. KANEKO
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Abstract: Oxaliplatin, a platinum-based chemotherapeutic agent, causes peculiar acute peripheral neuropathy. Oxaliplatin-induced acute peripheral neuropathy appears in almost all patients rapidly after infusion and is triggered or exacerbated by cold, but the mechanisms are poorly understood. In this study, we investigated the involvements of thermosensitive transient receptor potential (TRP) channels, TRPA1, TRPM8 and TRPV1 in the oxaliplatin-induced acute hypersensitivity in mice. A single intraperitoneal administration of oxaliplatin (5 mg/kg) induced cold hypersensitivity within 2 hours, while mechanical hypersensitivity was not observed in the early phase. The oxaliplatin-induced acute cold hypersensitivity was inhibited by calcium (0.5 mmol/kg i.v.) or gabapentin (30 mg/kg, i.p.). Similar with oxaliplatin, a single intraperitoneal administration of oxalate (1.7 mg/kg), a metabolite of oxaliplatin, also induced cold hypersensitivity within 2 hours, while neither cisplatin (5 mg/kg) nor paclitaxel (6 mg/kg) induced mechanical or cold hypersensitivity 2 hours after oxaliplatin. These findings suggest that the rapid-onset cold hypersensitivity is representative of the acute peripheral neuropathy characteristic to oxaliplatin in mice. The oxaliplatin-induced acute cold hypersensitivity was abolished by a TRPA1 antagonist, HC-030031 (100 mg/kg) or TRPA1 deficiency. Furthermore, nocifensive behaviors induced by intraplantar injection of allyl-isothiocyanate (AITC; TRPA1 agonist) and menthol (TRPM8/TRPA1 agonist) were significantly enhanced 2 hours after oxaliplatin, while capsaicin (TRPV1 agonist)-induced nocifensive behaviors were not affected. Similarly, oxalate enhanced the AITC-induced nocifensive behaviors, while cisplatin and paclitaxel had no effects. When mouse cultured dorsal root ganglion (DRG) neurons were pretreated with oxaliplatin (100 μM) for 1, 2 or 4 hours, the number of AITC-sensitive neurons was increased, but neither that of menthol- nor capsaicin-sensitive neurons was changed. In HEK293 cells expressing recombinant human TRPA1, high dose oxaliplatin (1 mM) evoked a calcium response. Taken together, these results suggest that short treatment with oxaliplatin characteristically leads to the enhanced responsiveness of TRPA1, but not TRPM8 and TRPV1, on DRG neurons, which may contribute to the oxaliplatin-induced acute peripheral neuropathy.

Disclosures: M. Zhao: None. **H. Shirakawa:** None. **T. nakagawa:** None. **S. Kaneko:** None.

Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

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Program#/Poster#:881.09/CC7

Topic: D.08. Pain

Support: NS 066091

Title: A high density of TRPM8 expressing sensory neurons in specialized structures of the head

Authors: *J. NEWSOM[1], J. L. HOLT[1], J. K. NEUBERT[2], R. CAUDLE[2], A. H. AHN[1]
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Abstract: Transient receptor potential melastatin 8 (TRPM8) receptor is expressed at high levels in the trigeminal ganglion, and is much more abundant there than in the dorsal root ganglia. Among the possible explanations for this are a higher number and density of cold-receptive nociceptors in the head than in other regions of the body. Alternatively, certain structures in the head that serve their own unique functions could differentially express a high density of TRPM8 expressing terminals, which would include the sensation of cold pain, such as in the eye, teeth, face, and oropharynx. In order to better appreciate the functional significance of high levels of TRPM8 in the trigeminal ganglia, we have characterized the expression of TRPM8 receptor terminals in the above terminal fields. Using a mutant mouse in which a GFP reporter has been mutated directly into the TRPM8 locus (Dhaka et al 2008), we determined the expression of GFP-expressing terminals in whole mount sections of the head. By the immunohistochemical detection of GFP, together with functional markers of primary afferent neurons, we have determined the localization and neurochemical identity of TRPM8-GFP expressing neurons in the head and compared them to other non-trigeminal terminal fields. The neurochemical identity of these neurons was determined in relation to the lectin IB4, a marker of non-peptidergic unmyelinated nociceptors, as well as CGRP, a marker of peptidergic nociceptors. We found only sparse innervation of the mucosa of the nasopharynx and turbinates, optic nerve, and the dura-meninges (as was reported previously by retrograde labeling of the dura). However, we found numerous and dense terminal arbors in the epithelium of the midline oropharynx and distal tongue, cornea, and conjunctiva. Accordingly, we found GFP-expressing axons of passage in the corresponding branches of the trigeminal nerve, such as the superior alveolar nerve, a branch of V2 that lies directly above the oropharynx, as well as the mandibular and lingual nerves, originating in the V3 branch of the trigeminal nerve. These findings will be of translational interest in understanding a number of pain behaviors and experiences related to cold pain and reactivity to cold such as dental pain, trigeminal neuralgia, migraine, and possibly the experience of “ice-cream headache.”

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Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

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Support: SAF2010-14990

consolider ingenio 2010

CSD2007-00023

Title: Cold-activated trpm8 channels are activated by the volatile anaesthetic chloroform

Authors: A. PARRA, J. MANENSCHIJN, O. GONZÁLEZ, H. VARA, C. MORENILLA, C. BELMONTE, *F. VIANA
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Abstract: TRPM8 is a member of the transient receptor potential family of non-selective cationic channels. TRPM8 is activated by cold temperature, cooling compounds and voltage. Recent studies have implicated TRPM8 in the pathophysiology of cold nociception and cold allodynia, leading to a strong interest in the search for novel modulators of TRPM8 activity. Here we describe the effects of chloroform (CLF), a volatile aneasthetic.
Using patch-clamp techniques, we studied CLF-induced responses in a HEK293 cell line stably expressing rat TRPM8. CLF activated whole-cell currents with a typical TRPM8 signature (strong outward rectification and near zero mV reversal). Using ratiometric fluorescence calcium imaging, we found that the effect of CLF was concentration-dependent and reversibly blocked by BCTC, a TRPM8 antagonist. CLF activated HEK293-cells transfected with the menthol-insensitive Y745H-TRPM8 mutant normally. We also investigated effects of CLF on cold-sensitive neurons from the dorsal root ganglia (DRG) of adult TRPA1-KO mice, and found that CLF activates these neurons, an effect which is reversibly blocked by BCTC. Similar effects were found in TRPM8-YFP(+) cold-sensitive DRG neurons driven by the TRPM8 promoter. Our results suggest that a low concentration of CLF is able to decrease

the threshold of TRPM8 by cold.
Finally, we recorded spontaneous Nerve
Terminal Impulse (NTI) activity from cold thermoreceptors innervating the mouse cornea *in vitro*. CLF augmented NTI
resting activity and increased mean frequency during sustained cooling pulses. The excitatory effects of CLF were reversibly blocked by BCTC and did not exist in TRPM8 -/- mice.
In brief, we show robust activation of
heterologously and natively expressed TRPM8 channels by CLF. The mechanism of activation is yet unexplained. However, the absence of the menthol binding site did not affect the effect of CLF on TRPM8, suggesting that this site is not critical for its activation by chloroform.

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Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

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Topic: D.08. Pain

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Title: Splice variants of trpm3 and their roles in sensory transduction

Authors: T. KIM[1], J. JUNG[1], D.-J. YANG[1], *Y. JANG[2], H. CHUN[1], H. KIM[1], J. OH[1], U. OH[1]
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Abstract: Transient receptorpotential melastatin 3 (TRPM3), a member of transient receptor potential channel superfamily, is a non-selective cationic channel with various splice variant forms. TRPM3 is now known to play a role in Ca^{2+}-induced insulin release from pancreatic beta cells or contraction of vascular smooth muscle. Recently, TRPM3 is also known to function as a thermo-sensor in sensory neurons. TRPM3 has many splice variants. However, roles of TRPM3 splice variants are largely unknown. Because TRPM3 is highly expressed in dorsal-root ganglion neurons, thus each splice variant may have different functions in sensory transduction. Thus, we sought to identify genes of splice variants of TRPM3 in sensory neurons and determine their

biophysical responses to various stimuli.
Six of TRPM3 splice variants were cloned based on sequence information of NCBI gene bank. All of six splice variants were expressed in mouse dorsal root ganglion neurons. Especially, TRPM3 *variant 2* (*v2*), *v3* and *v6* showed higher expression levels than *v4 and v5*. We then examined whether these splice variants have different responses to heat and pregnenolone sulfate (PS), an endogenous agonist of TRPM3. Although TRPM3 *Variant 1-5* showed similar current amplitudes (0.23±0.05 nA to 50 μM PS). TRPM3 *v6* showed significantly smaller currents to the PS. In contrast, all of TRPM3 splice variants were activated to heat with identical thresholds and current responses. These results show that splice variants of TRPM3 may have different roles as they have different sensitivities to endogenous stimuli.

Disclosures: T. Kim: None. **J. Jung:** None. **D. Yang:** None. **Y. Jang:** None. **H. Chun:** None. **H. Kim:** None. **J. Oh:** None. **U. Oh:** None.

Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

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Johns Hopkins Brain Sciences Institute

Kao Corporation

Title: Keratinocyte stimulation is sufficient to trigger pain in mice

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Abstract: The close proximity of skin keratinocytes to sensory afferents, the expression by keratinocytes of multiple transducers of physical and chemical stimuli, changes in the expression levels of these transducers in pathological states, and the release by keratinocytes of substances capable of activating or sensitizing nociceptors have provided circumstantial evidence that keratinocytes might initiate acute pain responses. Yet, this concept has not been directly tested in vivo. Here, we describe a “chemogenetic” transgenic mouse strategy in which the capsaicin-

gated cation channel, Transient Receptor Potential Vanilloid 1 (TRPV1) is selectively, inducibly, and ectopically expressed in keratinocytes of mice lacking endogenous TRPV1. In vitro, keratinocytes from these mice exhibit robust capsaicin-evoked calcium influx, whereas their sensory neurons are completely insensitive to capsaicin. Topical administration of capsaicin to these mice in vivo triggers spinal cord c-fos induction, acute pain-related behavior and mechanical hyperalgesia. Together, these findings demonstrate the sufficiency of keratinocyte activation for the initiation of pain and introduce a robust system for the dissection of interaction between keratinocytes and sensory afferents.

Disclosures: M. Caterina: Other Research Support; Kao Corporation. Ownership Interest; UCSF. Consultant/Advisory Board; Hydra Biosciences. **T. Sakamoto:** Employment; Kao corporation. **Z. Pang:** None. **A.D. Guler:** None.

Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

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Topic: D.08. Pain

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Kil Chung-Hee Fellowship Fund

Title: 17(R)-resolvin D1 specifically inhibits epidermal TRPV3 ion channel leading to peripheral antinociception

Authors: *S. HWANG[1], S. BANG[1], S. YOO[1], T.-J. YANG[1], H. CHO[2]
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Abstract: Transient receptor potential ion channel vanilloid 3 (TRPV3) is expressed in epidermal keratinocytes and sensory neurons and plays an important role in thermal and chemical nociceptions in the periphery. The presence of TRPV3 inhibitors would improve our understanding of TRPV3 function and help to develop receptor-specific analgesics. However, little is known about physiological substances that specifically inhibit TRPV3 activity. Here, we investigated whether 17(R)-resolvin D1 (17R-RvD1), a naturally occurring pro-resolving lipid specifically affects TRPV3 activity. We examined the effect of 17R-RvD1 on sensory TRP

channels using Ca(2+) imaging and whole cell electrophysiology experiments in a HEK cell heterologous expression system, cultured sensory neurons and keratinocytes. We also examined changes in sensory TRP agonist-specific acute licking/flicking or flinching behaviours and mechanical and thermal pain behaviours using Hargreaves, Randall-Selitto and von Frey assay systems in the absence and presence of inflammation. We showed that 17R-RvD1 specifically suppresses TRPV3-mediated activity at nanomolar and micromolar concentrations. The voltage-dependence of TRPV3 activation by camphor was shifted rightwards by 17R-RvD1, which indicates its inhibitory mechanism is as a result of a shift in voltage-dependence. Consistently, TRPV3-specific acute pain behaviours were attenuated by locally injected 17R-RvD1. Moreover, the administration of 17R-RvD1 significantly reversed the thermal hypersensitivity that occurs during an inflammatory response. Knockdown of epidermal TRPV3 blunted these antinociceptive effects of 17R-RvD1. 17R-RvD1 is a novel natural inhibitory substance specific for TRPV3. The results of our behavioural studies suggest that 17R-RvD1 has acute analgesic potential via TRPV3-specific mechanisms.

Disclosures: S. Hwang: None. **S. Bang:** None. **S. Yoo:** None. **T. Yang:** None. **H. Cho:** None.

Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

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Title: Tonic inhibition of trpv3 by mg^{2+} in mouse epidermal keratinocytes

Authors: J. LUO[1], R. STEWART[1], R. BERDEAUX[1], A. QIAN[1], *H. HU[2]
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Abstract: The transient receptor potential vanilloid 3 channel (TRPV3) is abundantly expressed in epidermal keratinocytes and plays important roles in sensory biology and skin health. Mg^{2+} deficiency causes skin
disorders associated with pruritus under certain pathological conditions such as type 2 diabetes

mellitus. In this study, we investigated the effect of Mg^{2+} on TRPV3 in primary epidermal keratinocytes. Extracellular Mg^{2+} ($[Mg^{2+}]_o$) inhibited TRPV3-mediated membrane current and calcium influx. TRPV3 activation induced a calcium signaling pathway culminating in activation of the cAMP response element binding (CREB). TRPV3 inhibition by $[Mg^{2+}]_o$, the TRPV3 blocker ruthenium red or TRPV3 siRNA suppressed this response. In TRPV3-expressing Chinese hamster ovary (CHO) cells, both extracellular and intracellular Mg^{2+} inhibited TRPV3 single-channel conductance but not open probability. Neutralization of an aspartic acid residue (D641) in the extracellular pore loop or two acidic residues (E679, E682) in the inner pore region significantly
attenuated the inhibitory effect of extracellular or intracellular Mg^{2+}
on TRPV3-mediated signaling, respectively. Our findings suggest that epidermal TRPV3 is tonically inhibited by both extracellular and intracellular Mg^{2+}, which act on both sides of the channel pore loop. Mg^{2+} deficiency may promote the function of TRPV3 and contribute to the pathogenesis of skin diseases.

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Poster

881. Pain Transduction: TRP Channels Other than A1 and V1

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Topic: D.08. Pain

Title: TRPV2 is involved in bone cancer-induced movement-evoked pain but not ongoing pain

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Abstract: Introduction
The treatment of bone cancer pain is challenging because its mechanisms still remain to be elucidated. This study investigated the contribution of TRPV2 activation to the bone cancer-induced pain behaviors using TRPV2 siRNA in a murine model of bone cancer pain.
Methods
The protocol for this study was approved by the Animal Care Committee of our institution. Male C3H/Hej mice were used in the experiment. Sarcoma cells were injected into the left femur medullary for the development of bone cancer pain. Mice received intrathecal (it) TRPV2 siRNA

or vector alone once a day for 3 consecutive days from day 14 after sarcoma injection Bone cancer pain-related behaviors were assessed before and from day 14 to day 21 after sarcoma injection. The quantification of spontaneous flinches was used to measure ongoing pain. Limb use during ambulation and weight bearing were assessed as indications of movement-evoked pain. In some mice, expression of TRPV2 in DRGs was evaluated using Western blot analysis.
Results
Western blot analysis showed that it TRPV2 siRNA significantly reduced TRPV2 expression in lumbar DRGs compared to the vector alone at day 17 after osteosarcoma injection. Mice exhibited bone cancer-related pain behaviors, including spontaneous flinches, impaired limb use during ambulation, and impaired weight bearing during spontaneous standing at day 14 after sarcoma injection. It vector alone did not affect the 3 bone cancer-related pain behaviors. On the other hand, it TRPV2 siRNA significantly improved limb use and weight bearing but not the number of spontaneous flinches.
Summary and conclusion
The results of this study showed that primary afferent-expressing TRPV2 was involved in bone cancer-induced movement-evoked pain but not ongoing pain.

Disclosures: K. Yamamoto: None. **T. Kawamata:** None. **S. Ide:** None. **T. Ishida:** None. **M. Kawamata:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

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Title: Delta-opioid receptors are involved in the central antinociceptive effect of high doses of oxycodone in mice

Authors: P.-P. YANG[1,2], G.-C. YEH[1], P.-Y. LAW[3], H.-H. LOH[3], *P.-L. TAO[4,2]
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Abstract: Oxycodone has been used clinically for over 80 years. While it is known as a μ-opioid receptor agonist, Ross and Smith (1997) reported the antinociceptive effects of oxycodone (171 nmol, i.c.v.) are induced by putative κ-opioid receptors in SD rat. In this study, we have used mu-opioid receptor knock-out (MOR (-/-)) mice to investigate this issue. We found that systemic administration of oxycodone (above 40 mg/kg, s.c.) could induce significant antinociceptive effect in MOR(-/-) mice by the tail flick test. Either kappa-opioid receptor antagonist (nor-BNI, 20 mg/kg, s.c.) or delta-opioid receptor antagonist (naltrindole, 1 mg/kg, s.c.) could partially block this effect. In order to exclude the possible effect of oxycodone metabolites, we have injected oxycodone directly to brain by intracerebroventricular (i.c.v.) injections. We found that oxycodone induced the antinociceptive effect at doses above 50 nmol in MOR (-/-) mice. Delta-opioid receptor antagonists (naltrindole at 10 nmol or ICI 154,129 at 20 micro g) completely blocked the supraspinal antinociceptive effect of oxycodone in MOR (-/-) mice. These data indicated that delta opioid receptors are involved in the central antinociceptive effect of oxycodone at high doses.

Disclosures: P. Yang: None. **G. Yeh:** None. **P. Law:** None. **H. Loh:** None. **P. Tao:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

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Universite de Strasbourg

Title: Differential role of beta-arrestin 1 and 2 on ligand-directed trafficking of the delta opioid receptor

Authors: A. A. PRADHAN[1], M. L. SMITH[1], W. WALWYN[1], A. BANA[1], B. L. KIEFFER[2], *C. J. EVANS[1]
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Abstract: G protein coupled receptors exist in multiple conformations, and agonists can stabilize different active states. These specific ligand-induced receptor conformations result in distinct receptor-effector complexes, which initiate diverse signaling and trafficking events. This concept, referred to as ligand-directed signaling or functional selectivity, has important biological and therapeutic implications. Delta opioid receptors are currently being developed for the treatment of chronic pain and emotional disorders. Convergent evidence from a number of in vitro and in vivo studies has revealed functional selectivity at the delta opioid receptor. Specifically, we compared agonists with similar binding and analgesic properties, but high-(SNC80) or low-(ARM390) internalization potencies. We found that following chronic administration two distinct forms of tolerance were observed depending on the internalization property of the agonist. SNC80 produced a generalized tolerance resulting in receptor downregulation throughout the central and peripheral nervous systems. In contrast, ARM390 produced an analgesic specific tolerance at the level of the dorsal root ganglia. We recently examined the effects of SNC80 and ARM390 in beta-arrestin 1 and beta-arrestin 2 knockout mice. The behavioral effects of SNC80, but not ARM390, were potentiated in beta-arrestin 1 knockout mice, and these mice did not show receptor desensitization to this particular agonist. In contrast, in beta-arrestin 2 knockout mice, the effects of SNC80 were unaffected, whereas the properties of ARM390 were adversely affected. These results suggest that high and low internalizing delta opioid receptor agonists differentially recruit beta-arrestins, resulting in divergent biological effects.

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Poster

882. Opioid Pharmacology and Other Analgesics

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Title: Distinct physiological role of the MOR-1 splice variants in the mouse brain

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Abstract: We recently developed a new mu-opioid receptor agonist amidino-TAPA, which shows extremely potent antinociception by systemic administration. Unlike other mu-opioid receptor agonists, amidino-TAPA does not show rewarding effect at antinociceptive doses. However, in prodynorphin-deficient mice, amidino-TAPA shows potent rewarding effect. The evidence suggests that amidino-TAPA is a potent analgesics lacking psychological dependence liability by releasing the endogenous kappa-opioid peptides. In the present study, antinociceptive mechanism of amidino-TAPA in supraspinal site was described to investigate the underlying mechanism for lack of the psychological dependence liability for amidino-TAPA. Amidino-TAPA showed extremely high affinity and relatively high selectivity for mu-opioid receptors in opioid receptor binding assays. However, the antinociception induced by i.c.v.-administered amidino-TAPA was attenuated by i.c.v. pretreatment with kappa-opioid receptor antagonist in addition with mu-opioid receptor antagonist. Moreover, i.c.v. pretreatment with antisera against endogenous kappa-opioid peptides dynorphin A and dynorphin B attenuated the antinociception induced by amidino-TAPA. The results suggest that amidino-TAPA given supraspinally stimulates novel mu-opioid receptor, which subsequently causes the release of dynorphins to produce antinociception. Although only one gene of mouse mu-opioid receptors (MOR-1) has been identified, more than 30 of splice variants for MOR-1 have been reported. In mice the splice variants containing exon-1 and exon-13 of MOR-1 have been desensitized using the exon-specific antisense oligodeoxynucleotide, the supraspinal antinociception induced by both amidino-TAPA and morphine was attenuated. The antinociception induced by amidino-TAPA, but not morphine, was also attenuated by the desensitization of MOR-1J and MOR-1L, exon-12 and exon-14 containing splice variants, respectively. Moreover, in the condition MOR-1J was desensitized, the inhibiting effect of antiserum against dynorphin A on amidino-TAPA-induced antinociception was disappeared. In contrast, the inhibiting effect of antisera against dynorphin A and dynorphin B was disappeared in the condition MOR-1L was desensitized. The present results suggest that the release of endogenous kappa opioid peptides by amidino-TAPA in supraspinal site is mediated through the activation of MOR-1J or MOR-1L. The supraspinal release of dynorphins by amidino-TAPA via activation of MOR-1J and MOR-1L may be involved in the lack of the psychological dependence liability for amidino-TAPA.

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Poster

882. Opioid Pharmacology and Other Analgesics

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Support: DGE

Communauté Urbaine de Strasbourg

Région Alsace

Médalis

Title: Involvement of RF-amide peptides in the modulation of nociception and analgesic effects of opiates

Authors: *F. SIMONIN[1], J.-P. HUMBERT[1], I. BERTIN[1], B. PETIT-DEMOULIÈRE[2], H. MEZIANE[3], F. BIHEL[4], M. SCHMITT[4], J.-J. BOURGUIGNON[4], K. ELHABAZI[1]
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Abstract: Mammalian RF-amide peptides are encoded by five different genes and act through five different G protein-coupled receptors. Neuropeptides SF and VF (NPSF and NPVF, also called RFRP1 and RFRP3) have been proposed to represent endogenous peptides for NPFF1 receptor, neuropeptides AF and FF (NPAF and NPFF) for NPFF2 receptor, and Prolactin releasing peptide (PrRP), Kisspeptin (or metastin) and 26RFa for GPR10, GPR54 and GPR103, respectively. However, results from several studies suggest that the selectivity of these peptides for their receptors is low and that expression patterns of these receptors and their corresponding ligands only partially overlap. In this study, we took advantage of the cloning of the five human RF-amide receptors to systematically examine their affinity for and their activation by all human RF-amide peptides. Binding experiments performed with membrane from CHO cells expressing these receptors indicated that GPR10, GPR54 and GPR103 display a remarkably good affinity and selectivity for their endogenous ligands. Conversely, we observed a high affinity of NPFF1 and NPFF2 receptors for all RF-amide peptides. Moreover, GTPgammaS and cAMP assays performed with cells expressing these receptors indicated that almost all RF-amide peptides efficiently activate NPFF1 and NPFF2 receptors. As NPFF has been shown previously to induce

hyperalgesia and/or to prevent the analgesic effect of morphine, we further performed a systematic analysis of the hyperalgesic and anti-morphine analgesic effect of mammalian RF-amide peptides in mice. Our results show that all RF-amide peptides induced hyperalgesia and/or prevented morphine analgesia following icv administration. Moreover, these effects were prevented by administration of RF9, a compound that blocks NPFF1 and NPFF2 receptors but not GPR10, 54 and 103. These results point to NPFF receptors as essential targets for the hyperalgesic and/or anti-morphine analgesic effect of exogenously administered RF-amide peptides. Taken together our data suggest that NPFF receptors could be endogenously activated not only by their known endogenous ligands (NPSF, NPVF, NPAF, NPFF) but also by other RF-amide peptides PrRP, Kisspeptin and 26RFa.

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Poster

882. Opioid Pharmacology and Other Analgesics

Location: Hall F-J

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Program#/Poster#:882.05/CC18

Topic: D.08. Pain

Title: Preclinical pharmacology of mu opioids - A comparison of morphine, oxycodone, hydrocodone and fentanyl in rodent pain models.

Authors: *I. Y. CHOI[1], C. ALI[1], J. EVANS[1], K. GOGAS[1], M. A. GUILLON[1], H. GURSAHANI[1], S. D. HARRISON[1], G. C. KIM[1], D. M. MCWEENEY[1], J. W. PFEIFFER[1], P. QUACH[1], J. RIGGS-SAUTHIER[2], P. C. TRINCHERO[1], T. A. RILEY[2]
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Abstract: The pre-clinical pharmacological effects of mu opioid receptor agonists are well described in literature, but the experimental conditions employed often vary between studies. In most cases, pre-clinical pharmacological studies using mu opioids utilize intravenous or subcutaneous dosing routes. In this study, we evaluated the *in vivo* analgesic and side-effect profiles of commonly used mu opioids following oral dosing. In addition, we characterized the *in vitro* binding and function at the mu opioid receptor for each compound.
We examined binding affinities and efficacy of the opioids in membrane preparations or whole cells expressing human opioid receptors. Analgesia was measured in rodent models of acute (mouse acetic acid writhing, rat hot plate), persistent (rat formalin paw), inflammatory (rat

carrageenan-mechanical hyperalgesia, complete Freunds adjuvant-mechanical hyperalgesia) and neuropathic (rat spinal nerve ligation) pain following oral dosing of the selected mu receptor opioids. Mu opioid receptor mediated side effects were evaluated using the mouse gastrointestinal transit model and the rat Rota-rod test.
The *in vitro* rank order binding affinities were morphine (4.6 ±0.2 nM) > fentanyl (9.7 ±0.6 nM) > oxycodone (16 ±0.6 nM) > hydrocodone (113.4 ±18.7 nM). All of the mu opioids were analgesic across the panel of rodent pain models with fentanyl being the most potent agonist across these tests. As expected, in rodent models of gastrointestinal transit and motor coordination, all four agonists produced some level of impairment. The rank order potencies for side-effect profiles however, did not directly correlate to the analgesic potency. For example, the therapeutic indices for fentanyl and morphine with respect to mouse acetic acid writhing and mouse gastrointestinal transit were ~30 and ~3 respectively.
Taken together, these data show that orally administered mu opioid agonists are potent analgesics that produce complete suppression across a panel of rodent pain models, but differ in their therapeutic indices relative to their mu opioid mediated side-effects. These data will be useful in further benchmarking and differentiating the pharmacological activities of novel oral opioid agonists.

Disclosures: **I.Y. Choi:** Employment; Nektar Therapeutics. **C. Ali:** Employment; Nektar Therapeutics. **J. Evans:** Employment; Nektar Therapeutics. **K. Gogas:** Employment; Nektar Therapeutics. **M.A. Guillon:** Employment; Nektar Therapeutics. **H. Gursahani:** Employment; Nektar Therapeutics. **S.D. Harrison:** Employment; Nektar Therapeutics. **G.C. Kim:** Employment; Nektar Therapeutics. **D.M. McWeeney:** Employment; Nektar Therapeutics. **J.W. Pfeiffer:** Employment; Nektar Therapeutics. **P. Quach:** Employment; Nektar Therapeutics. **J. Riggs-Sauthier:** Employment; Nektar Therapeutics. **P.C. Trinchero:** Employment; Nektar Therapeutics. **T.A. Riley:** Employment; Nektar Therapeutics.

Poster

882. Opioid Pharmacology and Other Analgesics

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Topic: D.08. Pain

Support: National Institutes of Health (NINDS-NS-052727)

Title: Assessing the effects of peptide helicity and linker flexibility on opioid receptor binding and antinociceptive efficacy of endorphin-based glycopeptides

Authors: L. ST. LOUIS[1], Y. LI[2], J. BIDLACK[3], R. POLT[2], *E. J. BILSKY[4]
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Abstract: Our group has previously demonstrated that glycosylation of small enkephalin-based peptides (i.e., glycopeptides) can significantly increase their stability, blood-brain barrier transport and systemic antinociceptive potency. Recently we have modified much larger endorphin- and dynorphin-based peptides to explore the delivery of these larger molecular weight compounds into the central nervous system. The current research explores two key aspects of these glycopeptides: (1) the flexibility of the linker segment between the core enkephalin message region and the larger address segment of the molecule, and (2) the effects of varying helicity in the address segment. The helicity was varied at two positions in the address segment to promote or inhibit helix formation (alpha-aminoisobutyric acid, alanine or glycine), while flexibility was altered with other amino acid substitutions in the linker region (L- or D-Proline, L- or D-Alanine, GABA or DAVA). The degree of glycosylation (unglycosylated, monosaccharide or disaccharide) was studied to assess the effects on overall helicity and activity of the compound. Helicity affected the glycopeptides in predictable ways as measured by circular dichroism (CD) in different solvents (H2O, CF3CH2OH and SDS micelles). In general, most of the analogs retained high affinity for mu, delta and kappa opioid receptors, and displayed potent antinociceptive effects when administered intracerebroventricularly (i.c.v.) to mice in the 55°C tail-flick assay. Exceptions included the linker substitution of D-Proline along with other the less flexible linkers. Surprisingly, a number of these glycopetides produced dose-related antinociception when administered intravenously (i.v.). These data indicate that a balance in hydrophilicity/hydrophobicity, helicity and linker flexibility resulted in optimal potency/efficacy following systemic administration. These studies are guiding the development of peptide-based therapeutics to overcome historical obstacles to CNS delivery.

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Poster

882. Opioid Pharmacology and Other Analgesics

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Title: The effect of naltrexone on neuropathic pain in mice locally transfected with the mutant mu-opioid receptor gene in spinal cord

Authors: *J. KAO[1], P.-Y. LAW[2], H. H. LOH[2], P.-L. TAO[3]
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Abstract: The opioid antagonists, such as naloxone and naltrexone, exhibited agonistic properties in the mutated mu-opioid receptor, MORS196ACSTA, in which the conserved Ser196, Thr327, and Cys330 were mutated to Ala, Ala, and Ser, respectively. In our previous study, systemic naloxone (10 mg/kg, s.c.) elicited antinociceptive effect (determined by tail-flick test) without the induction of tolerance, dependence, and rewarding effect in mice 2 weeks after intrathecal administration of dsAAV2-MORS196ACSTA-EGFP. In the present study, we further investigated whether this antinociceptive paradigm could be effective in mice with neuropathic pain. The spinal nerve ligation (SNL) surgery was performed on mice 3 or 4 weeks after intrathecal injection of the lentivirus which carried the gene of MORS196ACSTA-EGFP (called LV-MORS196ACSTA). The von Frey test and tail flick test were used to detect the anti-allodynic and antinociceptive effects of systemic naltrexone before and after gene transfection. After 14 days of naltrexone or morphine treatment (10 mg/kg, s.c., q.d.), the natural withdrawal signs were counted at 22 and 46 hours after the last drug injection. Our results showed that the paw withdrawal pressure and tail flick latency increased after naltrexone (10 mg/kg, s.c.) or morphine (10 mg/kg, s.c.) treatment in mice transfected with LV-MORS196ACSTA in spinal cord. However, chronic treatment of morphine (10 mg/kg, s.c., q.d.) but not naltrexone (10 mg/kg, s.c., q.d.) induced natural withdrawal signs. These data imply that systemic injection of naltrexone may have therapeutic potential for chronic neuropathic pain without the development of dependence after the gene therapy with the expression of MORS196ACSTA in the spinal cord.

Disclosures: J. Kao: None. **P. Law:** None. **H.H. Loh:** None. **P. Tao:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:882.08/DD1

Topic: D.08. Pain

Support: Finska Läkaresällskapet, Finland

Title: Ketamine co-administration increases morphine brain concentrations

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Abstract: Ketamine is widely used in perioperative analgesia with morphine. Here we focus on the effect of ketamine on morphine metabolism and on the effects of ketamine on the development of hyperalgesia in a rat model of postoperative pain (Brennan's model). Morphine is metabolized to morphine-3-glucuronide (M3G) by UDP-glucuronosyl transferase (UGT) 2B1 in rodents. M3G has hyperalgesic effects through mechanisms not related to opioid receptors. Therefore, the metabolism of morphine to M3G is considered harmful. Because ketamine has been shown to inhibit UGT2B1 *in vitro* we studied the effects of ketamine on morphine metabolism and brain concentrations in the rat.
Sprague-Dawley (SD) rats (n = 5) received subcutaneous racemic ketamine (30 mg/kg) with or without 4 mg/kg morphine. Tissue samples were collected at 30 minutes. In the postoperative pain model, SD rats (n = 6) were subjected to a plantar incision in the hindpaw in isoflurane anesthesia. Subanesthetic ketamine (10 mg/kg subcutaneously) was administered at timepoints -20, 0, 60 and 180 minutes relative to the time of the incision. Primary hyperalgesia was measured with a dynamic plantar aesthesiometer after 60 and 180 minutes of the surgery and on postoperative days 1-5.
Ketamine co-administration significantly increased the concentration of morphine in the liver (by 410%) and in the brain (by 22%) but not in the serum (by 10%). The concentration of M3G was significantly decreased in the liver (by 59%) but not in the serum (by 50%) or in the brain (by 44%). No M6G was detected as expected. In the postoperative pain model, the mean force for eliciting the withdrawal response at baseline was 39.6 ± 5.9 g in the saline control group, and after the surgery the threshold force was at its lowest at 180 min (17.6 ± 5.2 g). It returned to the baseline level by postoperative day 4. The administration of ketamine did not significantly alter the development of primary hyperalgesia in this model.
The present results suggest that ketamine increases the brain concentration of morphine and inhibits the formation of M3G, which may contribute to the positive effects of ketamine on morphine analgesia. In the postoperative pain model ketamine did not show antihyperalgesic properties.

Disclosures: T.O. Lilius: None. **V. Jokinen:** None. **P.V. Rauhala:** None. **E.A. Kalso:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

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Program#/Poster#:882.09/DD2

Topic: D.08. Pain

Support: R01DA020469

Title: BU 08073, a kappa, delta, and NOP antagonist with partial agonist activity at mu opioid receptors, can attenuate antinociception induced by kappa and mu opioid agonists

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Abstract: BU 08073 is a buprenorphine analog that binds with high affinity to mu, delta, kappa, and NOP opioid receptors, with Ki values of less than 10 nM at each site. BU 08073 has very low efficacy (less than 10% relative to full agonists) at delta, kappa, and NOP receptors. At mu receptors BU 08073 has moderate efficacy but high potency (EC50 2.9 nM, % stimulation 31.9%), having both higher efficacy and potency than buprenorphine, but having potentially lower brain penetrance compared to buprenorphine. We examined the effects of a single injection of BU 08073 on the tail flick assay, alone or as a pretreatment to morphine or U 50,488 administered 1-h to 10 days post injection from BU 08073. Surprisingly, an acute injection of BU 08073 does not increase tail flick latency in mice at any time point. Despite moderate efficacy at mu receptors, BU 08073 blocks analgesic activity of both morphine (mu-mediated) and U50-488 (kappa-mediated). However, the kinetics of inhibition of opiate-mediated analgesia are unusual. At 1-h post injection BU 08073 is inactive and does not alter mu or kappa-mediated antinociception. However, 6-h following an injection of BU 08073 (3 mg/kg) both morphine and U 50,488-induced analgesia are blocked. BU 08073 still blocks U 50,488 antinociception for 3 days presumably by acting as an antagonist at the kappa receptor. BU 08073 can block morphine-induced antinociception for up to 6 days presumably through its activity at the mu receptor. BU 08073 was also tested for anxiolytic or anxiogenic activity using the elevated zero-maze. BU 08073 alone decreased the time spent in the open quadrant, indicating anxiogenic activity. Surprisingly, a single injection of BU 08073, given as a pretreatment, induced anxiolytic activity for up to 10 days when mice were tested one hour after administration of the anxiogenic compound U 50-488. The reason this compound has no antinociceptive activity, in the tail flick assay, despite greater efficacy at mu receptors than buprenorphine and other mu-opioid partial agonists is unclear. The mechanism by which two anxiogenic compounds can become anxiolytic is also perplexing, however, both of these phenomena probably have to do with the slow onset of action and unusual kinetics of BU 08073.

Disclosures: T.V. Khroyan: None. **L. Toll:** None. **W.E. Polgar:** None. **G. Cami-Kobeci:** None. **S.M. Husbands:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

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Program#/Poster#:882.10/DD3

Topic: D.08. Pain

Support: NIH F30DA03229801

NIH NS027910

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NIH DA027460

Title: Group III mGluR-selective agonist potentiates morphine-induced inhibition of sensitized peripheral nociceptors.

Authors: *A. BAKER, S. CARLTON
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Abstract: Morphine is widely used to treat acute and chronic pain but causes significant deleterious side effects when taken in high doses or for long periods of time. Current studies aim to establish receptor groups and agonists that might potentiate analgesic efficacy of morphine at low doses in order to minimize side effects. One receptor family of interest is the metabotropic glutamate receptors (mGluRs) of which intrathecal and systemic application of Group III agonists have been established to potentiate morphine analgesia. In this study, we aimed to determine if peripheral administration of a Group III agonist potentiates morphine analgesia in acute inflammation. We employed behavior, electrophysiology, and immunohistochemistry techniques to examine the Group III mGluR agonist L-AP-4 plus morphine. In a model of peripheral inflammatory pain (i.e. inflammatory soup (IS) intraplantar), we obtained individual IC50s based on sensitivity to a heat stimulus (Hargreaves plantar test) for morphine (1, 10, 15, 20 mM) and L-AP4 (0.1, 1, 3, 10 µM) after intraplantar injection in the rat hindpaw. Isobolographic analysis of responses to thermal stimuli was conducted to determine the combined IC50 and to reveal if the relationship was synergistic, additive, or antagonistic. Preliminary results indicated that L-AP-4 synergistically potentiated morphine anti-hyperalgesia. Similar experiments were conducted in the *in vitro* skin-nerve preparation to determine if the

combination decreased sensitization at the nociceptor level. Morphine alone, L-AP-4 alone, and the combination were applied to sensitized fibers and responses to thermal and mechanical stimuli were assessed. Preliminary results suggest that the combination reduced mechanical sensitization. We also aimed to examine the anatomical basis underlying the relationship of Group III mGluRs and morphine by double-labeling dorsal root ganglion (DRG) cells for Group III mGluR8 and the mu opioid receptor (μOR). Data indicated that the receptors are co-localized in 28-55% of small and medium DRG cells, which are generally considered to be nociceptors. This will be further confirmed via triple-labeling DRG cells with mGlur8, μOR and either TRPV1 or Isolectin B4. The co-localization evidence hints that the Group III plus morphine combination works through an intracellular mechanism. Overall, preliminary data suggest that peripheral Group III mGluR activation increases morphine-induced inhibition of inflamed nociceptors via modulation of an intracellular pathway.

Disclosures: A. Baker: None. **S. Carlton:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

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Program#/Poster#:882.11/DD4

Topic: D.08. Pain

Support: NIH Grant NS027910

NIH Grant DA027460

Title: Characterization of spontaneous activity in nociceptive fibers from glabrous skin of morphine treated-mice

Authors: *D. HOGAN[1], J. A. MORON CONCEPCION[2], S. M. CARLTON[1]
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Abstract: Repeated use of opioids, either for treatment of chronic pain or as a drug of abuse, induces neuroadaptive changes forming the neurobiological basis for drug dependence and addiction. One consequence of morphine use is increased pain sensitivity (allodynia and/or hyperalgesia), documented in both animal and clinical studies. We analyzed this phenomenon, recording from nociceptors in a skin-nerve preparation. Adult male C57BL6 mice were injected with escalating doses of morphine (5, 8, 10, and 15 mg/kg) or saline every 12h for 48h. Mice were sacrificed ≈16h following the last injection. Recordings were obtained from C and A-delta

fibers innervating the hind paw. Spontaneous activity (SA) was defined as activity greater than 2 standard deviations above background for that fiber type from saline-treated mice. SA was measured during an initial 2 min period and during 5 min periods between heat, cold, and mechanical testing.
Forty fibers (20.4% of total fibers) from 16 of 26 morphine-treated mice showed SA, versus only 2 fibers (1.5%) from 2 of 30 saline-treated mice. In morphine mice, 26.4% of C fibers and 17.8% of A-delta fibers exhibited SA. There were 61 SA events from the 40 morphine fibers. Twelve SA events were characterized by regular firing (average 1.9 Hz.) Seventeen events were characterized by burst-like activity of > 10Hz. The remainder of SA events had slower, irregular firing patterns. Nineteen SA events occurred in the first 2 min recording period before any stimulation. Of the remaining 42, 17 were post-heat, 8 post-cold, and 17 post-mechanical stimulation. Of the post-stimulus events, 17 consisted of continued firing after the end of the stimulus (1 heat, 6 cold, 10 mechanical.) The other 25 post-stimulus SA events occurred an average of 102.7s (range 12-340s) after the end of the stimulus (16 heat, 2 cold, 7 mechanical.) There was no correlation between the type of fiber (heat or cold responsive) and the occurrence of SA events, e.g., heat responsive fibers could show abberent responses after heat, cold or mechanical stimulation. Neither was there any preference for C or A-delta fibers in any category of SA.
In morphine-treated mice, aberrant firing of nociceptors in the absence stimulation could underlie spontaneous pain and contribute to increased pain sensitivity. Sustained firing post-stimulation could underlie hyperalgesia and allodynia. Importantly, this peripheral SA is most likely driving central sensitization which also contributes to hyperalgesic and allodynic states. In human patients, nociceptor activity that is either spontaneous or post-stimulus is likely to be interpreted as pain, and could contribute to opioid-induced hyperalgesia.

Disclosures: D. Hogan: None. **J.A. Moron Concepcion:** None. **S.M. Carlton:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:882.12/DD5

Topic: D.08. Pain

Title: Analgesic efficacy estimates for intrathecally administered opioid agonists in mice

Authors: *S. JASWAL, M. R. GAJJAR, D. M. NAVANI, B. C. YOBURN
Pharm. Sci., Col. of Pharmacy, St. John's Univ., Jamaica, NY

Abstract: The present study used a quantitative model to estimate the analgesic efficacy in mouse spinal cord of four opioid agonists (sufentanil, DAMGO, oxycodone and fentanyl) administered intrathecally (IT). Initially, the time of peak analgesic effect and EC_{50} for each drug were determined using the tailflick assay. Peak effect was 5, 15, 10 and 10 min for sufentanil, DAMGO, oxycodone and fentanyl respectively. The EC_{50}s were: sufentanil 1.8 ng/mouse; DAMGO 0.5 ng/mouse; oxycodone 0.38 µg/mouse; fentanyl 33.0 ng/mouse. ED_{50}s determined using quantal data indicated similar relative potencies. Analgesic efficacy was estimated using the receptor depletion approach with the irreversible µ-opioid receptor antagonist cloccinamox. The operational model of agonism was used to calculate the efficacy parameter (Tau: τ). This approach utilizes graded data (mean latency in sec) and estimation of EC_{50}s. Mice were treated with vehicle (dH_2O containing ≈ 4% DMSO) or cloccinamox (6.4 - 12.8 mg/kg, i.p.), and 24 hrs later IT dose-response studies were conducted for sufentanil, DAMGO, oxycodone and fentanyl. Cloccinamox dose-dependently reduced the potency of all drugs. Cloccinamox (6.4 mg/kg) increased the EC_{50} by 3.9, 4.8, 4.8 and 19-fold for sufentanil, DAMGO, fentanyl and oxycodone respectively. Cloccinamox (12.8 mg/kg) resulted in flat dose-response functions for all drugs except sufentanil, for which the shift in the EC_{50} was 4.7-fold. These shifts in EC_{50}s suggest that sufentanil, fentanyl and DAMGO are higher analgesic efficacy opioids than oxycodone in the mouse spinal cord. When these preliminary data were used to estimate efficacy using the operational model of agonism, the τ values were 62, 54, 47 and 24 for fentanyl, DAMGO, sufentanil and oxycodone; respectively. Studies estimating τ after systemic opioid agonist administration have reported τ values of 58 and 20 for fentanyl and oxycodone respectively, which is in general agreement with the present estimates in spinal cord. The estimation of analgesic efficacy of spinally administered opioids may be useful to characterize the potential utility of opioid agonists used clinically for spinal analgesia.

Disclosures: S. Jaswal: None. **M.R. Gajjar:** None. **D.M. Navani:** None. **B.C. Yoburn:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:882.13/DD6

Topic: D.08. Pain

Title: Analgesic and neuroexcitatory effects of hydrocodone and its metabolites

Authors: *D. M. NAVANI, S. JASWAL, B. C. YOBURN
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Abstract: Hydrocodone (HYC), an opioid agonist for moderate to severe pain, is metabolized to hydromorphone (HYM) and norhydrocodone (NHYC). HYM is believed to be pivotal in HYC induced analgesia, while NHYC is considered to contribute minimally. Although NHYC levels in human plasma and urine following oral HYC may exceed that of HYM, it is not clear if this may impact on analgesic or toxic effects. In this report, we examined analgesia and toxicity produced by NHYC, HYC and HYM in mice. Peak analgesia (tailflick) following s.c. HYC, HYM and NHYC was 15, 45, 45 min respectively and analgesic ED_{50}'s (95%CL) were: HYM: 0.3 mg/kg (0.2-0.4); HYC: 1.4 mg/kg (0.9-2.0); NHYC: 95.8 mg/kg (63.7-146.2). Next, spinal intrathecal (IT) analgesia was determined. The IT ED_{50}'s (95%CL) were: HYM: 7.0 ng/mouse (0.5-100.5); HYC: 1.9 µg/mouse (0.2-48.8). IT NHYC did not produce antinociception at any dose. Naltrexone (1 mg/kg, s.c.) antagonized s.c. analgesia for all three opioids and IT analgesia for HYC and HYM. IT naltrexone (10 µg/mouse) antagonized IT analgesia for HYC and HYM. All compounds produced tonic-clonic seizures at higher IT doses. The ED_{50}'s (95%CL) to induce seizures were: NHYC: 4.3 µg/mouse (2.9-6.8); HYM: 5.2 µg/mouse (3.5-8.3); HYC: 19.4 µg/mouse (12.6-31.6). Naltrexone (1, 10, 30 mg/kg, s.c. or 10 µg/mouse, IT) did not antagonize seizures induced by NHYC (10 µg/mouse, IT). The ratio of IT ED_{50}'s for analgesia and seizures were: HYM: ≈ 740; HYC: ≈ 10; (as noted, NHYC lacked an IT analgesic effect). Displacement binding studies in spinal cord indicated that K_i values (95%CL) for µ (DAMGO) and δ (DPDPE) receptors were, respectively: HYM: 1.3 nM (1.1-1.4), 54.7 nM (27.0-110.9) δ/µ = 42 ; HYC: 76.3 nM (57.4-101.5), 1385 nM (708.5-2706) δ/µ = 18; NHYC: 189.1 nM (127.1-281.4), 4055 nM (2865-5738) δ/µ = 21. Taken together, all compounds produced systemic analgesia, although only IT HYC and HYM produced spinal analgesia. All compounds produced seizures at higher IT doses. Analgesia induced by all compounds was opioid receptor mediated, while seizures induced by IT NHYC were non-opioid mediated. Finally, all three compounds demonstrated µ versus δ-opioid receptor selectivity. These results suggest that NHYC possesses systemic analgesic activity which may contribute to HYC analgesia. However, the finding of spinal neuroexcitatory effects raises the possibility that with repeated administration of HYC, NHYC levels may rise and produce unwanted non-opioid toxicity.

Disclosures: D.M. Navani: None. **S. Jaswal:** None. **B.C. Yoburn:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:882.14/DD7

Topic: D.08. Pain

Support: NIH Grant NS072205

Title: Opioid-induced hyperalgesia is associated with increased MOR-1K gene expression levels

Authors: *F. OLADOSU[1], M. S. CONRAD[3], D. PRUSIK[2], J. GAUTHIER[2], G. SLADE[2], N. U. RASHID[2], L. DIATCHENKO[2], A. G. NACKLEY[2]
[1]Ctr. of Neurosensory Disorders, [2]Neurobio., Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [3]Neurosci., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Opioids commonly used in the treatment of acute and chronic pain have been reported to produce a paradoxical opioid induced hyperalgesia (OIH) in a subset of the population. Recent evidence indicates a potential role for MOR-1K, an alternative splice variant of MOR-1, in OIH. Unlike its parent receptor MOR-1 that mediates opioid analgesia via cellular inhibition, MOR-1K facilitates cellular excitation upon activation. Because of its opposing cellular effects, we hypothesized that MOR-1K contributes to OIH in genetically-susceptible individuals. To test this hypothesis, pain behavioral responses and MOR-1K gene expression were evaluated in three genetically diverse strains of mice (C57BL/6J, CXBK,129SV/EV) receiving subcutaneous injections of vehicle or escalating amounts of morphine sulfate (10, 20, 20, 40 mg/kg) over four days. Responses to mechanical and thermal stimuli were evaluated at baseline, before and following morphine administration on days 1-4, and following morphine cessation on days 5-7. In separate groups of mice, levels of MOR-1K gene expression in spinal cord and brain were measured at baseline and on days 1, 4, and 5. At baseline, C57BL/6J, 129SV/EV, and CXBK mice exhibited different pain sensitivity profiles in response to mechanical and thermal stimuli. Compared to the classic C57BL/6J inbred strain, 129SV/EV mice were the least pain sensitive, exhibiting the highest paw withdrawal threshold and the lowest degree of mechanical and thermal heat hyperalgesia. In contrast, CXBK mice were the most pain sensitive, exhibiting the lowest paw withdrawal threshold and the highest degree of mechanical and thermal heat hyperalgesia. Following morphine administration, C57BL/6J, 129SV/EV, and CXBK mice exhibited different analgesic and hyperalgesic response profiles. C57BL/6J mice displayed analgesia following morphine administration on days 1-4 and hyperalgesia following morphine cessation on days 5-7. 129SV/EV mice displayed analgesia following morphine administration on days 1-4, but failed to develop hyperalgesia at any time point. In contrast, CXBK mice failed to display analgesia, but developed pronounced hyperalgesia as early as day 3 and lasting through day 7. MOR-1K gene expression paralleled pain behavior in the two most extreme strains, such that 129SV/EV mice exhibited decreased expression on days 1 and 4, corresponding to their analgesic behavioral profile, and CXBK mice exhibited increased expression on day 4, corresponding to their hyperalgesic behavioral profile. Together, our findings implicate MOR-1K as a novel druggable target that contributes to the development of OIH.

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Poster

882. Opioid Pharmacology and Other Analgesics

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:882.15/DD8

Topic: D.08. Pain

Support: PSC/CUNY

Title: Spinal and supraspinal mechanisms of morphine hyperalgesia

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Abstract:
Chronic morphine infusion can cause a paradoxical hyperalgesic state in male and female mice that occurs independently of opioid receptor activity (Juni et al. 2006), but sex differences have been elucidated (Juni et al. 2010, Waxman et al. 2010). Whereas both sexes are rendered hyperalgesic by day 4, an acute intracerebroventricular injection of the noncompetitive NMDA receptor antagonist MK-801 reverses hyperalgesia in males after chronic infusion of a high morphine dose (50mg/ml), and both males and females after chronic morphine infusion of a low morphine dose (2mg/ml) (Juni et al. 2010). In contrast, a single intracerebroventricular injection of the selective melanocortin-1 receptor antagonist MSG606 reverses morphine hyperalgesia exclusively in females after chronic morphine infusion of a low morphine dose (Juni et al. 2010). Although we have shown that males and females possess both male- and female-typical hyperalgesic systems, and that subsequent recruitment of these mechanisms is hormonally regulated (Waxman et al. 2010), the precise neural substrates that underlie such processes is still nebulous. Accordingly, in the present study the aforementioned findings were subjected to replication with additional exploration of possible spinal and supraspinal involvement in the regulation of morphine hyperalgesia.

Disclosures: C.A. Arout: None. **M. Caldwell:** None. **G. Rossi:** None. **B. Kest:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

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Title: Acute morphine activates satellite glial cells and up-regulates IL-1beta in dorsal root ganglia via MMP-9 to mask opioid analgesia

Authors: *T. BERTA[1,2], T. LIU[1,2], Y.-C. LIU[3,2], Z.-Z. XU[1,2], R.-R. JI[1,2]
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Abstract: Despite well-known side effects, opioids are still the most used analgesics. It is clinically important to improve the analgesic efficacy of opioids. Activation of spinal cord glial cells such as microglia and astrocytes has been shown to cause chronic opioid-induced anti-nociceptive tolerance and hyperalgesia. However, it is unclear whether acute opioid treatment can activate glial cells in the peripheral nervous system. We examined acute morphine-induced activation of satellite glial cells (SGCs) and up-regulation of IL-1beta in dorsal root ganglia (DRGs), and further investigated the involvement of matrix metalloprotease-9 (MMP-9) in these opioid-induced peripheral changes.
Subcutaneous morphine injection (10 mg/kg) induced robust peripheral glial responses, as evidenced by increased GFAP expression in DRGs but not in spinal cords. Acute morphine treatment also increased MMP-9 expression in DRG neurons, IL-1beta expression in DRG SGCs, and IL-1beta activation in DRGs. Confocal analysis revealed a close proximity of MMP-9 and GFAP immunostaining. Importantly, morphine-induced DRG up-regulation of GFAP expression and IL-1beta activation was abolished after Mmp9 deletion or naloxone pre-treatment. Finally, acute morphine-induced analgesia was enhanced and prolonged after Mmp9 deletion or intrathecal injections of IL-1beta-selective siRNA.
In conclusion, MMP-9 could mask and shorten morphine analgesia via peripheral neuron-glial interactions. Targeting peripheral glial activation might prolong acute opioid analgesia.

Disclosures: T. Berta: None. **T. Liu:** None. **Y. Liu:** None. **Z. Xu:** None. **R. Ji:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

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Program#/Poster#:882.18/DD11

Topic: D.08. Pain

Support: Departmental start up funds

CIHR

Title: The delta-opioid receptor contributes to morphine tolerance in an animal model of chronic inflammatory pain

Authors: *L. HIPOLITO[1], H. BEAUDRY[2], A. GUPTA[3], L. DEVI[3], L. GENDRON[2], J. A. MORON CONCEPCION[1]
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Abstract: Opioid use in chronic inflammatory pain is limited by the development of tolerance and unwelcomed side effects. Although the topic remains controversial, functional and physical interactions between the mu- (MOP) and the delta- (DOP) opioid receptors have been recently reported. Indeed, recent studies suggest that interactions between the MOP and DOP may play an important role in morphine-induced behavioral adaptations. In fact, blockade of the DOP or disruption of MOP/DOP heteromers was shown to enhance morphine-induced analgesia. Here, we investigated the regulation of DOP and the MOP/DOP heteromer during the development of morphine tolerance in a rat model of chronic inflammatory pain. We find an increase in the expression of DOP at the postsynaptic density fraction in the dorsal horn of the spinal cord in morphine tolerant rats and this effect is even more robust in the presence of chronic inflammation. In addition, ELISA studies using a selective MOP/DOP heteromer antibody show an increase in the levels of the MOP/DOP heteromer in the dorsal horn in morphine tolerant rats; interestingly this effect is even more dramatic during chronic inflammatory pain. Thus, our data show that the increase in the levels of postsynaptic DOP correlates with an increase in the DOP/MOP heteromer formation. Furthermore, behavioral analyses show that pretreatment (s.c. or i.t.) with the DOP antagonist, naltrindole, attenuates the development of morphine tolerance. Overall, our data show that the DOP play an important role during morphine tolerance, an effect further amplified in the presence of chronic inflammation. We also show that the increase in DOP levels is associated with an increase in the levels of the MOP-DOP heteromer in the spinal

cord. Altogether, our findings highlight the DOP as a key player in the development of morphine tolerance during chronic inflammation. Further studies are needed to analyze whether the above observed changes in DOP levels and MOP/DOP abundance are also observed in other components of the opioid-sensitive pain-modulating circuit and to define the exact consequences of this phenomenon.
This work was supported by Departmental start up funds to JAMC and by a CIHR grant to LG.

Disclosures: L. Hipolito: None. **H. Beaudry:** None. **A. Gupta:** None. **L. Devi:** None. **L. Gendron:** None. **J.A. Moron Concepcion:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:882.19/DD12

Topic: D.08. Pain

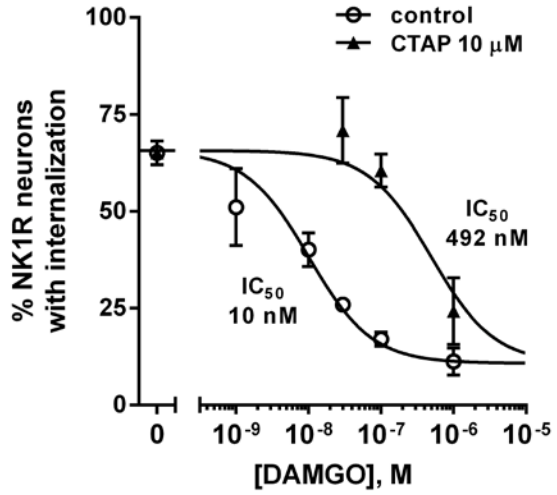
Support: VA grant 1I01-RX000378

Title: Mu-opioid receptors switch NMDA receptors in primary afferents to a non-functional state

Authors: *J. G. MARVIZON[1,2], W. CHEN[2], J. A. MCROBERTS[2]
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Abstract: NMDA receptors present in the central terminals of primary afferents elicit substance P release into the dorsal horn, but this requires phosphorylation of their NR2B subunit by a Src family kinase (SFK) (Chen et al., 2010, Neuroscience 166:924). We recently found that in naïve rats these NMDA receptors are in a dephosphorylated, non-functional state. However, in rats with chronic constriction injury (CCI) of the sciatic nerve (a model of neuropathic pain) these NMDA receptors become functional through a signaling cascade involving microglia activation, BDNF release and SFK phosphorylation. After CCI, these NMDA receptors gradually return to a non-functional state during a period of 3-7 days, suggesting that there is a signal leading to their dephosphorylation. Since mu-opioid receptors (MORs) in primary afferents also activate a SFK, we investigated whether MOR agonists could also turn the NMDA receptors into their functional state. We found the opposite: the MOR agonists DAMGO, morphine and endomorphin-2 abolished NMDA-induced substance P release from rat spinal cord slices (measured as neurokinin 1 receptor internalization). The inhibition by DAMGO was time-dependent, requiring a preincubation of the slices with DAMGO for at least 15 min, whereas DAMGO produced no effect when added together with NMDA. The effect of DAMGO was dose-dependent, with IC50 = 10 nM. The selective MOR antagonist CTAP (10 μM) shifted the dose-response of DAMGO

to the right (IC50 = 492 nM, Fig.), confirming the involvement of MORs. However, a similar inhibition of NMDA-induced substance P release was produced by the delta-opioid receptor agonist DPDPE (1 µM), but not by the GABAB receptor agonist baclofen (10 µM). The inhibition by DAMGO was abolished by the protein tyrosine phosphatase inhibitor BVT 948 (10 µM). This suggests that the MORs present in primary afferent terminals turn these NMDA receptors into a non-functional state by triggering the dephosphorylation of their NR2B subunit by a protein tyrosine phosphatase, reversing the effect of BDNF. This may contribute to the analgesic effect of opioids.



Disclosures: J.G. Marvizon: None. **W. Chen:** None. **J.A. McRoberts:** None.

Poster

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Topic: D.08. Pain

Support: The Nakatomi Fundation

Title: The involvement of RhoA in the up-regulation mechanism of the intestinal P-glycoprotein by repeated orally administered etoposide

Authors: *T. KOBORI[1], S. HARADA[1], K. NAKAMOTO[1], S. KISHIOKA[2], S. TOKUYAMA[1]
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Abstract: *Aims*: Morphine is the most commonly used opioid analgesic for the treatment of pain and is known as a substrate of P-glycoprotein (P-gp), a drug efflux transporter. Recently, in early palliative care, the combined use of opioids and anticancer drugs which are substrate of P-gp for the treatment of cancer patients is increasing. Therefore, drug-drug interaction between opioids and anticancer drugs is known to be watched in the clinical field. We previously reported that repeated administration of oral etoposide (ETP) which is a substrate of P-gp, caused attenuation of the analgesic effect of oral morphine through the up-regulation of intestinal P-gp. However, the detailed up-regulation mechanism of intestinal P-gp by repeated administration of anticancer drugs is still unknown. Recent studies have revealed that RhoA, a small molecular G protein, is involved in the regulation of P-gp expression and activity. The activation of RhoA requires the translocation from cytosol to membrane and its membrane translocation is mediated by posttranslational modification via geranylgeranylation. Moreover, RhoA is known to be involved in various signaling pathways in response to anticancer drugs. Here, we examined the involvement of RhoA in the changes in the intestinal P-gp protein expression and its drug efflux activity induced by repeated administration of oral ETP.
Methods: Intestinall P-gp and RhoA protein expression levels were analyzed using western blot analysis. Rhodamine 123 (Rho123) efflux activity of intestinal P-gp was measured using the *in situ* closed loop method. The analgesic effect of oral morphine was determined with a tail-flick test.
Results: One day after repeated ETP treatment (10 mg/kg, p.o., once a day for 7 days), the analgesic effect of morphine (50 mg/kg, p.o.) was significantly attenuated with decrease in its levels in serum and brain. At the same time, the intestinal P-gp protein expression and Rho123 efflux activity were significantly increased. Moreover, repeated ETP treatment significantly increased RhoA protein expression in the intestinal membrane fraction but not in cytosol. Interestingly, rosuvastatin (5 mg/kg, p.o. once a day for 7 days), an inhibitor of RhoA translocation from cytosol to membrane, prevented these effects. Furthermore, ETP-induced attenuation of the analgesic effect of oral morphine was also significantly suppressed by rosuvastatin.
Conclusion: RhoA activation induced by repeated oral ETP treatment may regulate the changes in the intestinal P-gp protein expression and its drug efflux activity, leading to a decrease in the analgesic effect of oral morphine.

Disclosures: T. Kobori: None. **S. Harada:** None. **K. Nakamoto:** None. **S. Kishioka:** None. **S. Tokuyama:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

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Topic: D.08. Pain

Support: NIH Grant DA014026

NIH Grant DA027811

Title: Bifunctional nociceptin receptor agonists with mu opioid agonist activity show reduced tolerance development to their antinociceptive effects

Authors: *N. T. ZAVERI[1], T. V. KHROYAN[2], W. POLGAR[2], L. TOLL[3], V. B. JOURNIGAN[1], D. YASUDA[1]
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Abstract: The use of mu-opioid agonists such as morphine as analgesics is often hampered by the development of tolerance to its anti-nociceptive effects, particularly during chronic use, necessitating increases in dosage, followed by sequelae of other undesirable side-effects. The nociceptin receptor NOP, the fourth member of the opioid receptor family, and its endogenous peptide ligand nociceptin/orphaninFQ (N/OFQ) have been shown to modulate several opioid-mediated activities, particularly in reward and nociceptive pathways. Intracerebroventricular injection of N/OFQ has been shown to decrease tolerance development to morphine-induced antinociception in rats, suggesting that activation of the NOP receptor system may reduce tolerance development to opioid-mediated antinociception. Further, we have shown that NOP receptor agonists decrease the rewarding effects of morphine in the place-conditioning paradigm. We have hypothesized that 'bifunctional' NOP agonists that also possess mu-opioid agonist activity could have anti-nociceptive activity, and a reduced propensity for opioid-related side-effects (such as reward and tolerance development). We have previously demonstrated that the bifunctional NOP/mu receptor partial agonist SR16435 produces dose-dependent antinociception and exhibits decreased tolerance development to its antinociceptive effects when administered over nine days, compared to morphine. In the present study, we further examined tolerance development to the antinociceptive effects of two other bifunctional NOP/mu agonists, AT-200 and AT-203, using the mouse tail-flick assay. The compounds were administered subcutaneously as daily injections for ten days. The results showed that tolerance developed to morphine antinociception (3 mg/kg) by the fourth day/injection, whereas tolerance did not develop to the antinociception produced by AT-200 or AT-203. When the NOP antagonist SB612111 was given as a pretreatment prior to daily injections of AT-200 and AT-203, tolerance to antinociception developed at a similar rate as that observed with morphine. These latter findings confirm that the inhibition of tolerance development in these bifunctional compounds is mediated by their NOP agonist activity. These results support the therapeutic application of such bifunctional NOP/mu agonists as non tolerance-developing analgesics. This work was supported by grants DA14026 and DA027811.

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Poster

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FRSQ salary award to LSS

CIHR Banting and Best Doctoral Award to AJCD

The Louise and Alan Edwards Foundation's PhD Studentship in Pain Research to AJCD

Title: Lack of opioid-adrenergic spinal analgesic synergy in δ-opioid receptor knock-out mice

Authors: *A.-J. CHABOT-DORE[1,2,3], M. MILLECAMPS[4,2], L. S. STONE[4,2]
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Abstract: Background: When co-administered to the spinal cord, agonists acting at α_2-adrenergic receptors (ARs) and opioid receptors (ORs) interact in a greater-than-additive (i.e. synergistic) manner to produce analgesia. The development of therapeutic approaches that exploit the combination of opioids and α_2-adrenergic agonists is currently limited by the lack of mechanistic knowledge on how these drugs interact at the spinal level. The OR family consists of three subtypes: δ (DOR), μ (MOR) and κ (KOR), all of which can have analgesic actions. It has been demonstrated that both DOR-selective agonists and MOR-selective agonists interact synergistically with α_2AR-selective agonists. However, the relative role of each subtype is not clear as studies have suggested that either DOR or MOR are the primary mediators of synergy with adrenergic agonists. It therefore remains unresolved if opioid-adrenergic synergy requires DOR activation, MOR activation, both or neither.
Objective: Evaluate the role of DOR in spinal opioid-adrenergic synergistic interactions.
Methods: 3-6 month old male WT mice (Charles River, C57Bl6) and DOR-KO mice (The Jackson Laboratory, B6.129S2-*Oprd1*^{tm1Kff}/J) with a deletion in the *Oprd1* gene were used in the substance P (SP) behavioral assay. Drugs were injected intrathecally with substance P (SP) and the inhibition of SP-induced nocifensive behaviors was calculated. Full dose response curves

were generated to evaluate the antinociceptive action of clonidine, morphine, deltorphin II (Delt II) and DAMGO, alone or in combinations. Isobolographic analyses were performed to determine if drug interactions were synergistic, additive or antagonistic.
Results: Clonidine, an α_2AR-selective agonist, interacted synergistically with morphine in WT mice but not in DOR-KO mice. The DOR-selective agonist, Delt II, was not efficacious in DOR-KO mice and did not enhance clonidine efficacy in DOR-KO mice. Clonidine's interaction with DAMGO, a MOR-selective agonist, was additive in both mouse strains.
Conclusion: Our results suggest that opioid-adrenergic synergistic interaction requires activation of DOR since a) synergy is absent when clonidine was combined with either morphine or Delt II in DOR-KO mice and b) the interaction between clonidine and DAMGO is additive in both WT and DOR-KO mice, confirming that MOR activation is not sufficient to produce opioid-adrenergic synergy. The development of combination therapies exploiting this interaction should include activation of DOR in order to take full advantage of the analgesic synergy between opioids and α_2-adrenergic agonists.

Disclosures: A. Chabot-Dore: None. **M. Millecamps:** None. **L.S. Stone:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

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Topic: D.08. Pain

Support: NIH Grant R01 DA015438

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Title: The mechanism underlying spinal analgesic synergy between delta and mu opioid receptors depends on the agonist combination used.

Authors: *D. SCHUSTER[1], M. D. METCALF[2], K. F. KITTO[1], A. C. OVERLAND[4], L. S. STONE[5], R. O. MESSING[6], C. A. FAIRBANKS[3], G. L. WILCOX[1]
[1]Neurosci., [2]Medicinal Chem., [3]Pharmaceutics, Univ. of Minnesota, Twin Cities, MINNEAPOLIS, MN; [4]Pharmacol., UCSD, San Diego, CA; [5]Sch. of Dent., McGill Univ., Montreal, QC, Canada; [6]Ernest Gallo Clin. and Res. Ctr., Univ. of California, San Francisco, San Francisco, CA

Abstract: Spinal analgesic synergy between agonists acting at opioid and adrenergic receptors has been well documented. We have recently shown that the requirement of protein kinase C-

epsilon (PKCε) for analgesic synergy can be used to classify such synergistic interactions as occurring either within a single pre-synaptic terminal, or across multiple neurons. Specifically, we have found that synergistic agonist combinations requiring delta opioid receptors (δORs) and alpha2A adrenergic receptors (α2A-ARs), which co-localize in terminals of peptidergic primary afferent nociceptors (PANs), require PKCε. Additionally, activation of PKCε is not required by combinations requiring receptors not co-localizing in the same neurons, such as those of a delta or mu opioid agonist or α2A-AR agonist paired with a α2C-AR agonist. Here we have used the hot-water tail-flick assay in wild-type mice (PKCε-WT) and mice genetically lacking PKCε (PKCε-KO) to evaluate combinations of agonists acting at δORs and mu opioid receptors (μORs) for the requirement of PKCε for spinal analgesic synergy. Agonists investigated include deltorphin, deltorphin I, deltorphin II, dermorphin, SNC80, DPDPE, leu-enkephalin, beta-endorphin, morphine and endomorphin II. More than a dozen combinations have been tested thus far and all produced analgesic synergy in PKCε-WT mice. Only two combinations, deltorphin I with morphine and deltorphin II with morphine, have shown PKCε dependence. This requirement correlates directly with the amino acid structure of the peptide agonist used. Deltorphin I, deltorphin II, deltorphin and dermorphin are all seven amino acid peptides. The most striking difference among these peptides is the a.a. in the fourth position. In position four, deltorphin I and deltorphin II both contain a negatively charged a.a. (aspartate and glutamate, respectively), whereas dermorphin and deltorphin contain a neutral and a positively charged a.a., respectively (glycine and histidine). This structural difference, along with the behavioral data, indicates that the interaction between a ligand and its receptor may dictate how that receptor interacts with other receptors or signaling molecules. Considering that μORs may be located on PANs where they could co-localize with δORs, or on secondary spinal cord neurons, it is not surprising that synergy between μOR and δOR agonists can occur through multiple mechanisms. In conclusion, these data indicate that analgesic synergy between δORs and μORs can occur through multiple mechanisms, and that specific ligand-receptor interactions may be necessary to facilitate synergy between receptors in the same neuron.

Disclosures: D. Schuster: None. **M.D. Metcalf:** None. **K.F. Kitto:** None. **A.C. Overland:** None. **L.S. Stone:** None. **R.O. Messing:** None. **C.A. Fairbanks:** None. **G.L. Wilcox:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

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Program#/Poster#:882.24/DD17

Topic: D.08. Pain

Support: HL65272

MH45361

Dept Anesthesiology

Title: Leptin replacement in the leptin deficient B6.V-Lep^{ob}/J mouse restores the antinociceptive properties of the adenosine A_1 agonist N^6-p-sulfophenyladenosine

Authors: S. L. WATSON, M. J. FRANK, C. J. WATSON, H. A. BAGHDOYAN, *R. LYDIC
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Abstract: The adenosine A_1 agonist N^6-*p*-sulfophenyladenosine (SPA) and the acetylcholinesterase inhibitor neostigmine bromide cause antinociception when microinjected into the pontine reticular formation (PRF) of C57BL/6J (B6) mouse (J Pain 10:836, 2009). The antinociceptive effects of SPA are concentration dependent and likely receptor mediated (J Pain 13:S45, 2012). These drugs do not have the same effect in congenic leptin-deficient B6.V-Lep^{ob}/J mice. Leptin replacement restores the antinociceptive properties of neostigmine in leptin-deficient mice (J Pain 10:836, 2009). Thus, leptin modulates pain transmission by cholinergic G protein coupled receptors (GPCRs) in the PRF. No previous studies have determined whether pain modulation by leptin is limited to cholinergic GPCRs or can be generalized to adenosine A_1 receptors. This study is testing the hypothesis that replacing leptin in the leptin deficient B6.V-Lep^{ob}/J mouse restores the antinociceptive response to SPA microinjected into the PRF. Adult male mice (n=5) were implanted with guide tubes aimed for the PRF. Antinociception was measured by paw withdrawal latency (PWL) to a thermal stimulus as quantified with an IITC Analgesia Meter. In separate experiments, each mouse received a 50 nL microinjection of saline (control) or SPA (500 pmol). PWL measurements (s) were taken at 10, 20, 30, 60, 90, and 120 min after the microinjection. After the initial set of microinjections, mice were implanted with Alzet osmotic pumps set to deliver 15 μg of leptin per day for 14 days. The microinjection protocol was repeated during weeks 1 and 2 of leptin replacement with each mouse receiving microinjections of saline and SPA in separate experiments. PWL measurements were expressed as percent maximum possible effect (%MPE) to account for individual mouse differences and the thermal stimulus cutoff time of 15 s. After the final experiment, microinjection sites were histologically confirmed to be within the PRF. Two-way ANOVA for repeated measures indicated a significant drug main-effect on %MPE ($F=14.1$; $df=1,16$; $p=0.0199$). Post hoc tests revealed a significant ($p < 0.05$) increase in %MPE caused by SPA in the leptin-replaced mice, but not in the leptin-naïve mice. Therefore, leptin restored the antinociceptive properties of SPA microinjected into the PRF. These data support the conclusions that 1) leptin modulation of pain is not limited to cholinergic GPCRs, and 2) leptin significantly alters SPA modulation of pain via adenosine A_1 GPCRs in the PRF.

Disclosures: S.L. Watson: None. **M.J. Frank:** None. **C.J. Watson:** None. **H.A. Baghdoyan:** None. **R. Lydic:** None.

Poster

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Program#/Poster#:882.25/DD18

Topic: D.08. Pain

Title: Chi shao (Radix Paeoniae Rubra): Anti-inflammatory and analgesic effects of Traditional Chinese Medicine in the rat formalin test

Authors: M. REEDY, *E. P. WIERTELAK
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Abstract: The immense catalog of compounds used in Traditional Chinese Medicine (TCM) offer a compelling source of new treatments for Western medicine. The present studies examined the analgesic and anti-inflammatory properties of chi shao (*Radix Paeoniae Rubra*), a drug widely used in TCM, in the rat formalin test pain assay. All studies were conducted in accordance with protocols approved by the Macalester Institutional Animal Care and Use Committee. In study 1, rats (Sprague-Dawley, Male, +110 days old) received either 20 mg/kg chi shao (P.O., delivered in 10 ml/kg suspension; experimental group) or equivolume vehicle (control group). All subjects received the suspension/vehicle by gavage 30 minutes prior to the onset of testing. Dilute (5%) formalin was then injected subcutaneously into the dorsum of the rats' right hind paws and three subsequent pain-associated behaviors were measured: lifting, shaking, and licking the affected paw. Formalin-induced behaviors were recorded for periods of 1-minute at 5-minute intervals across a 1-hour time course. Behavioral data for each of the three categories collected was collapsed across early/acute phase (first 25 minutes) and later/tonic phase (subsequent 35 minutes) for analysis. Our results indicated no differences between groups in any behaviors during the acute phase, whereas responding in the tonic phase revealed significant differences for licking behavior, as rats administered chi shao exhibited fewer licking behavior. A strong trend was also observed in shaking behavior, where rats administered chi shao exhibited significantly fewer shaking behaviors. Two additional studies to be discussed are currently underway at this time and investigate dose response and time-related characteristics of chi shao. While speculative, chi shao, which is known within TCM practice to be a viable, fast-acting, pain reliever, may offer some guidance for western medicine in the development of potential novel treatments for the control of moderate pain in humans.

Disclosures: M. Reedy: None. **E.P. Wiertelak:** None.

Poster

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Topic: D.08. Pain

Title: Anti-hiperalgesic efect of etanolic extract of Rhodiola rosea in the formalin test in diabetic rats

Authors: *R. M. MONTIEL-RUIZ, M. DÉCIGA-CAMPOS
Escuela Superior De Medicina, IPN, D.F., Mexico

Abstract: Diabetic neuropathy is one of the most common complications in diabetes mellitus and is associated with spontaneous pain, hyperalgesia and allodynia. Currently, there is no available therapies that fully control diabetic neuropathy-induced pain without significant negative side effects. In an attempt to have new therapeutic alternative, the researching medicinal plant extracts has been intensified in the last decade. *Rhodiola rosea* is a plant commonly used in Europe and Asia for its adaptogenic, anti-inflammatory and antioxidant properties. The aim of this study was to evaluate the antihyperalgesic effect of ethanol extract of *Rhodiola rosea* in experimentally diabetic rats. Male Wistar rats were diabetized with streptozotocin (50 mg/kg, i.p.), six weeks later they were pretreated with the extract of *Rhodiola rosea* and hyperalgesia was determined using the formalin test. The administration of 50 μL of formalin (0.5%) in the right hind paw produces a behavior characterized by flinching. Intraperitoneal administration of ethanol extract of *Rhodiola rosea* (1-100 mg/kg) produced an antihyperalgesic dose dependent effect (ED_{50}=46.2 mg/kg) in the formalin test in diabetic rats. The antihyperalgesic effect was also observed following the intraplantar administration of the extract plant concentrations ranging from 100 to 1000 μg/paw. The results suggest that the ethanol extract of *Rhodiola rosea* may be an alternative in the treatment of hyperalgesia associated to the diabetic neuropathy.

Disclosures: R.M. Montiel-Ruiz: None. **M. Déciga-Campos:** None.

Poster

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Program#/Poster#:882.27/DD20

Topic: D.08. Pain

Title: N-acylethanolamine acid amidase (NAAA): A potential novel target for analgesic drugs

Authors: O. SASSO[1], C. MARTUCCI[1], N. REALINI[1], G. MORENO-SANZ[1], R. BERTORELLI[1], A. REGGIANI[1], *D. PIOMELLI[1,2]
[1]Fondazione Inst. Italiano Di Tecnologia, Genova, Italy; [2]Departments of Pharmacol. and Biol. Chem., Univ. of California, Irvine, CA

Abstract: Bioactive N-acylethanolamines, including the endocannabinoid anandamide and anti-inflammatory N-palmytoylethanolamine (PEA), are hydrolyzed to fatty acids and ethanolamine in animal tissues by the catalysis of fatty acid amide hydrolase (FAAH). More recently, N-acylethanolamine-hydrolyzing acid amidase (NAAA), another enzyme catalyzing the same reaction has been cloned. NAAA preferentially hydrolyzes PEA over others fatty acid ethanolamides. Previous studies have shown that PEA exerts profound anti-inflammatory and analgesic effects that are due to a large extent to activation of the nuclear receptor peroxisome proliferator-activated receptor-α (PPARα). We recently developed a selective and potent NAAA inhibitor, ARN077. In the present study we profiled up the in vitro pharmacology of ARN077 and its activity in both inflammatory and neuropathic pain models. Topical administration of ARN077 produced a significant reversal of UVB-induced thermal hyperalgesia as well as carrageenan-induced inflammation and mechanical allodynia after sciatic nerve ligation. When CB1 or CB2 antagonists were injected before ARN077, they did not block the anti-hyperalgesic effect of the NAAA inhibitor, whereas a PPARα antagonist completely reversed ARN077 activity.
Present findings suggest that ARN077 could have therapeutic benefit in inflammatory based cutaneous disease states.

Disclosures: O. Sasso: None. **C. Martucci:** None. **N. Realini:** None. **G. Moreno-Sanz:** None. **R. Bertorelli:** None. **A. Reggiani:** None. **D. Piomelli:** None.

Poster

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Support: NS51667

Craig H. Neilsen Foundation 190848

Title: Evaluation of antinociceptive effect of novel cannabinoid receptor agonists from conus venom in neuropathic pain model.

Authors: *C. PEREZ[1], S. JERGOVA[1], S. GAJAVELLI[1], L. IMPERIAL[2], B. OLIVERA[2], J. SAGEN[1]
[1]Miami Project, Univ. of Miami, Miami, FL; [2]Univ. of Utah, Salt Lake City, UT

Abstract: With the limitations of current long-term pharmacotherapy for chronic pain, the identification of alternative approaches and new therapeutic targets is essential. Cannabinoid (CB) receptor agonists have emerged as potential therapeutic targets, as robust antinociceptive effects have been reported in various pain models. However, most of the available drugs that interact with CB receptors are derived from cannabis and considered clinically unacceptable for long-term therapy due to psychoactive side effects. Therefore specific compounds interacting with CB receptors without aversive side effects are of clinical interest. The venoms of marine snail genus *Conus* are a natural source of various peptides (conopeptides) with potent analgesic effects, some of them already FDA approved. In this study we evaluated the ability of several *Conus* venom extracts to interact with CB_1 receptor.
The venom extracts of six *Conus* species were analyzed in vitro. HEK293 cells expressing CB_1 receptors were treated with venom extracts for 30 minutes and the rate of CB_1 receptor internalization was analyzed by immunofluorescence. Results showed the highest rate of CB_1 internalization in HEK293 cells after treatment with venoms of *C. textile* and *C. miles*. HPLC fractions (seven per species) of these venoms were subsequently analyzed using a similar approach. Based on the analysis, C. *Tex* subfraction with the highest CB_1 agonist activity was evaluated in a formalin test. I.t. injection of the *C.tex* fraction reduced flinching/licking behavior during the second phase of formalin test. The results indicate the presence of CB_1 agonists within the *Conus* venom extract and their potential analgesic effects. To identify the nature of CB_1 agonists, the *C. Tex* fraction was treated with proteolytic enzymes. The effect of treatment on CB_1 internalization was subsequently evaluated. Results showed that proteotolytic treatment attenuated the ability of venom fraction to induce CB_1 receptor internalization. Further evaluation and purification of peptide compounds is under way. The final peptide compounds of screened venoms with CB_1 receptor affinity could subsequently be used as a new analgesic agent in recombinant cell or gene therapy approaches.

Disclosures: C. Perez: None. **S. Jergova:** None. **S. Gajavelli:** None. **L. Imperial:** None. **B. Olivera:** None. **J. Sagen:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:882.29/EE2

Topic: D.08. Pain

Support: NCI CA091007

NIDA DA011471

Title: The cannabinoid receptor agonist WIN 55,212-2 attenuates sensitization of C-fiber nociceptors in a murine model of cancer pain

Authors: *M. L. UHELSKI[1], D. M. CAIN[2], C. HARDING-ROSE[2], D. A. SIMONE[2]
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Abstract:
Evaluating and managing pain related to cancer is a major clinical hurdle. Of the estimated 1.6 million new cancer patients diagnosed each year, between 30 and 60% will develop chronic pain related to tumor growth
and/or treatment. Progress towards understanding the physiological changes associated with pain related to tumor growth is essential for the development of novel therapies. In this study, we utilized a murine model of cancer pain in which osteolytic fibrosarcoma cells are injected directly into and around the calcaneous bone, producing mechanical, thermal, and cold hypersensitivity as the cells proliferate and the tumor enlarges. In addition, electrophysiological studies have identified pathological changes in peripheral nociceptors following tumor development. Behavioral studies in our laboratory demonstrated that local administration of the cannabinoid receptor agonist, WIN 55,212-2, attenuated mechanical hyperalgesia associated with tumor growth through cannabinoid receptors (CB1 and CB2). The purpose of the current study was to examine the impact of WIN 55,212-2 on the sensitization of cutaneous C-fiber nociceptors innervating the plantar surface of the hind paw following tumor cell implantation. Mechanical hyperalgesia of the tumor-bearing paw was confirmed with behavioral assessments prior to electrophysiology. Mice were then deeply anesthetized, the tibial nerve was exposed and action potentials from single afferent fibers were recorded using a teased fiber approach. Well-isolated nociceptors were classified by conduction velocity, and their responses to mechanical and heat stimuli were obtained before and every 15 min for 1 h after drug administration. WIN 55,212-2 (10 µg) or vehicle was injected into the receptive field in a volume of 10 µl. WIN 55,212-2 attenuated tumor-evoked spontaneous ongoing activity and reduced the number of impulses evoked by a suprathreshold von Frey monofilament (0.4 g). Mechanical response threshold was not affected. These results indicate that the attenuation of mechanical hyperalgesia produced by cannabinoids observed in behavioral studies involves a reduction in the sensitization of C-fiber nociceptors. Ongoing studies will determine the contribution of CB1 and CB2 receptors to the effects of WIN 55,212-2 on response properties of C-fiber nociceptors in tumor-bearing mice.

Disclosures: M.L. Uhelski: None. **D.M. Cain:** None. **C. Harding-Rose:** None. **D.A. Simone:** None.

Poster

882. Opioid Pharmacology and Other Analgesics

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:882.30/EE3

Topic: D.08. Pain

Title: Effects of analgesic agents, Neurotropin on neuronal cell

Authors: *R. ISONAKA[1], T. TAKENAMI[2], T. KATAKURA[1], T. KAWAKAMI[1]
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Abstract: Neurotropin® is one of analgesic agents, which is known to treat chronic pain such as lumbago, postherpetic neuralgia and fibromyalgia in the fields of orthopedics, neurology, and anesthesia and it is ordinarily used in Japan. The ingredient of this drug is a non-protein extract from inflamed skin of rabbits inoculated with vaccinia virus. Axonal transport is an important function to supply materials for maintenance of neuronal cells. In this study, we investigated the influence of neurotropin on axonal transport in cultured mouse dorsal root ganglion (DRG) neurons using video-enhanced microscopy. Neurotropin (0.0001-1 Neurotropin Unit (NU/ml)) decreased anterograde and retrograde movement of organelles in a time-and concentration-dependent manner. Neurons treated with neurotropin at any concentration were not morphologically different from those of the control neurons. Axonal transport in sensory neurons is closely related to alleviation of neuropathic pain, therefore these results suggest that the analgesic effect of neurotropin for neuropathic pain is caused by inhibition of axonal transport.

Disclosures: R. Isonaka: None. **T. Takenami:** None. **T. Katakura:** None. **T. Kawakami:** None.

Poster

883. Somatosensory Reorganization and Plasticity

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:883.01/EE4

Topic: D.09. Tactile/Somatosensory

Support: 1R21NS077288-01

Title: *In vivo* imaging of spine dynamics in the barrel cortex during perceptual learning

Authors: *J. I. PARK[1], T. CELIKEL[2], A. W. MCGEE[3]
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Abstract: Understanding how sensory experience modifies neural circuits is central to understanding mechanisms of perceptual learning. Though novel sensory experience and improved motor performance modify functional synaptic connectivity in the cerebral cortex, the specificity and mechanisms of anatomical plasticity that contribute to sensory and motor learning are unclear. We are investigating this question by performing in vivo longitudinal multi-photon imaging of spine dynamics before, during, and after mice are trained on a perceptual learning task.

Our learning task, the gap-crossing task, is a whisker and barrel cortex-dependent object localization paradigm in which a mouse learns to locate a tactile object positioned with a variable distance while perched on an elevated platform. In the absence of visual cues mice locate the target using tactile cues; at distances larger than 5 cm tactile information is exclusively collected by rhythmic palpations of whiskers. However, not all whiskers contribute to tactile exploration on this task. Mice preferentially use C- and to a lesser extent D-row whiskers, while dorsally located A-row whiskers do not contribute to tactile exploration.

We take advantage of this preferential use of whiskers and the topographical nature of their representations in the barrel cortex to image spine dynamics in the apical dendrites of Layer 5 neurons in cortical domains corresponding to whiskers that contribute to gap-crossing performance (e.g. C2 whisker) as well whiskers not involved in the task (e.g. A2 whisker). After cranial window implantation which permits long-term, repetitive imaging, C2 and A2 whisker representations are mapped with intrinsic signal optical imaging. The dendritic spines within the C2 and A2 barrels are then imaged every four days using two-photon laser scanning microscopy. This is done over multiple intervals before beginning the task to determine baseline dynamics, as well as during and after training. This study will reveal anatomical changes associated with perceptual learning while providing within animal controls for the role of sensory experience in shaping anatomical dynamics in the network.

Disclosures: J.I. Park: None. **T. Celikel:** None. **A.W. McGee:** None.

Poster

883. Somatosensory Reorganization and Plasticity

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:883.02/EE5

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NS037070

Title: Functional recovery from infraorbital nerve crush revealed by intracellular recordings from an *in vitro* preparation of neonatal rats

Authors: *F.-S. LO, R. S. ERZURUMLU
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Abstract: Neonatal infraorbital nerve (ION) transection in the rat leads to disappearance of whisker-specific neural patterns along the central trigeminal pathway. Physiological studies show that the complete whisker deafferentation results in conversion of functional synapses into silent synapses and a rapid reactive synaptogenesis in the trigeminal principal nucleus (PrV). Regeneration of the damaged ION fibers is a slow process and never complete. ION crush, on the other hand, leads to a partial blockade and a rapid recovery of ION function. We developed an in vitro trigeminal ganglion preparation in which trigeminal ganglion (TG) cells that innervate the whisker pad are morphologically intact. Intracellular recordings from those TG cells show that there are two classes of cells according to the shape and duration of the action potential (AP): S-type and F-type. S-type cells account for 77% and F-type cells, 23% of the recorded TG cells (n=26). Because TG cells that innervate whisker pad are widespread in the central part of the TG (Klein et al., 1986), we randomly impaled TG cells in the central TG and tested the response to stimulation of ION. Sixty nine percent of recorded TG cells (n=26) revealed an action potential with a fixed latency after ION stimulation indicating that they innervate the whisker pad. Immediately after ION crush, stimulation of ION distal to the crush site induced APs in only 25% recorded cells (n=20) that was significantly lower than the normal side (p&gt0.30). On post-crush day seven, 80% of recorded cells (n=20) responded to the stimulation of the distal ION, that was significantly higher than 3 days after crush (p<0.005), but did not differ from the normal side (p>0.40). These results indicate that function of the ION is partially blocked by crush and recovers in one week. In addition, the ratio of S- and F-type recorded TG cells after ION crush was about the same as normal side (p>0.70), suggesting that ION crush blocks ION fibers non-selectively.

Disclosures: F. Lo: None. **R.S. Erzurumlu:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Program#/Poster#:883.03/EE6

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant 2R01NS048285 (GBS)

NSF GRFP (CAG,DCM)

Title: Adult plasticity in the rodent barrel cortex measured with voltage sensitive dye imaging

Authors: *C. A. GOLLNICK, D. C. MILLARD, A. D. ORTIZ, Q. WANG, R. V. BELLAMKONDA, G. B. STANLEY
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Abstract: Neuroplasticity is the central mechanism the brain uses to recover from disease. Dramatic examples of plastic mechanisms can be observed in the developing brain; however, plastic changes in the adult brain are more subtle, limited and not well understood. In the rodent barrel cortex, the discrete barrel representations in layer 4 are stabilized early in life, while the supragranular layer circuitry remains sensitive to external stimuli. Here we use voltage-sensitive dye imaging to characterize plastic changes in layer 2/3 of the barrel cortex in anesthetized rats (>P60). A DE-paired whisker trimming paradigm is used to drive cortical reorganization. In control animals, the activation of a D-row whisker spreads symmetrically both towards the spared and deprived whisker rows. In contrast, the activation of D-row whisker in a DE-paired animal spreads asymmetrically and is biased towards the spared whisker row. Preliminary results suggest that this asymmetry occurs primarily because of a reduction of spread into deprived columns and not as a result of increased spread into spared columns. Additionally, the direction of the asymmetry can be controlled by flipping the spared and trimmed whisker rows. These results indicate that the cortical response to a whisker deflection remains sensitive to whisker trimming into adulthood, possibly reflecting the potential for whisker loss and regrowth throughout life.

Disclosures: C.A. Gollnick: None. **D.C. Millard:** None. **A.D. Ortiz:** None. **Q. Wang:** None. **R.V. Bellamkonda:** None. **G.B. Stanley:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Topic: D.09. Tactile/Somatosensory

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NIH Grant NS35103

NSF Grant ECCS-0846502

NIH Grant AI47294

Title: Reversible deactivation of motor cortex and posterior parietal cortex in macaques by cooling induces shifts in receptive field size and location in anterior parietal neurons

Authors: *A. B. GOLDRING[1,2], D. F. COOKE[1,2], C. B. WEATHERFORD[2], G. H. RECANZONE[3], S. I. SIMON[4], T. PAN[4], L. KRUBITZER[1,2]
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Abstract: Feed-forward models of somatosensory processing posit that "high-order" somatosensory areas (such as Brodmann's area 2) construct their receptive fields via input from primary areas in anterior parietal cortex (such as 3b and 1), as well as some direct thalamic input. However, reciprocal connections between area 2 and areas within posterior parietal cortex (PPC) suggest that feedback also plays a role in determining the construction of somatosensory receptive fields. For example, focal aspiration lesions of the lateral-most extent of Brodmann's area 5 (area 5L) induce receptive field shifts in area 2 within 60 minutes (Padberg et al., 2010). To study this rapid plasticity more precisely, we used a cooling chip (Cooke et al., 2012) to reversibly deactivate cortex in an anesthetized macaque while mapping the hand and forelimb representations within areas 1 and 2. Cooling cortex to ~20 degrees C disrupts or abolishes local neural activity while neurons in surrounding tissue remain responsive. We cooled restricted portions of the forelimb representation in the lateral portion of the rostral bank of the intraparietal sulcus defined by Seelke et al. (2011) as area 5L. In separate runs, we also cooled portions of areas 7b and M1. Data were collected before cooling, while the cortex was cooled, and after rewarming. We characterized the receptive field properties of neurons in areas 1 or 2 via light cutaneous stimulation as well as hard taps, squeezes, and joint manipulation. Several locations both inside and outside the receptive fields were stimulated via computer-controlled air puffs in order to quantify the responses. Preliminary analysis suggests that deactivation of each region studied produced changes of receptive field boundaries for area 2 neurons. For example, a site within area 2 which was originally responsive to stimulation of proximal portions of dorsal digits 4 and 5 became responsive to stimulation of the entire dorsal surface of digits 3-5 during cooling of area 7b. Cooling of area 5 produced no such shifts within the same site, but cooling of M1 expanded the receptive field to include the entire dorsal surface of the hand. Subsequent

rewarming saw a return of receptive fields to their original extent.
These results imply that a strictly hierarchical model is insufficient to account for the structure of receptive fields within anterior parietal cortex. Additionally, these immediate shifts in receptive field structure provide insight into the immediate recovery period following a stroke or other forms of cortical injury.

Disclosures: A.B. Goldring: None. **D.F. Cooke:** None. **C.B. Weatherford:** None. **G.H. Recanzone:** None. **S.I. Simon:** None. **T. Pan:** None. **L. Krubitzer:** None.

Poster

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Program#/Poster#:883.05/EE8

Topic: D.09. Tactile/Somatosensory

Support: NIH 1R01NS073912

Title: Disinhibition in L2/3 recurrent networks during whisker map plasticity

Authors: *Y. R. SHAO, J. CHUNG, D. E. FELDMAN
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Abstract: Sensory experience drives robust cortical map plasticity, but significant components of its cellular and circuit basis remain unknown. In rat somatosensory cortex (S1), whisker deprivation drives depression of spiking responses in cortical layer (L) 2/3. This plasticity is known to involve a parallel reduction in excitation (E) and inhibition (I) in the feedforward L4-L2/3 projection (House 2011), as well as reduced connection rates between L2/3 pyramidal cell pairs (Cheetham 2007). How sensory deprivation affects overall recurrent network function (including E-I balance) has remained elusive, because of the inability to selectively activate recurrent networks using extracellular stimulation. To overcome this problem, we introduced channelrhodopsin-2 (ChR2) specifically to L2/3 pyramidal cells via in utero electroporation and used light to activate the L2/3 recurrent network in ex vivo across-row S1 slices at P17-24.
To specifically activate the L2/3 recurrent network in a single whisker-related column, we first identified light intensities that drove spiking of ChR2-expressing pyramidal cells only within the stimulated column. We measured recurrent synaptic responses in ChR2-negative pyramidal cells in voltage clamp (31C, with APV and saclofen in bath). A 2 ms light flash evoked recurrent excitation followed by inhibition 2-3 ms later. E-I balance varied with stimulus intensity, with little or no inhibition at minimal activation, preferential recruitment of inhibition as stimulus intensity was increased, and stable E-I ratio dominated by inhibition over a wide range of strong

intensities. We then deprived the D row of whiskers for 5-12 days from P12 to examine the effect of deprivation. Deprived D columns had modestly weaker recurrent excitation, but a major decrease in recurrent inhibition, compared to spared B columns. This resulted in a significant shift in E-I balance toward more excitation. Relative E-I timing was mostly preserved. Deprivation-induced changes were not observed in neighboring spared C and E columns. Thus, the recurrent L2/3 network responds to deprivation with a preferential weakening of inhibition, in contrast to preserved E-I balance in the feedforward L4-L2/3 pathway. Preferential weakening of recurrent inhibition will compensate for decreased sensory drive, suggesting that L2/3 recurrent circuits are a major locus of homeostatic plasticity. In addition, reduced recurrent inhibition may gate or promote subsequent components of sensory map plasticity.

Disclosures: Y.R. Shao: None. **J. Chung:** None. **D.E. Feldman:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: BFU2005-07486

BFU2008-03488

BFU2011-23522

SAF2009-10339 Spanish Grants

Title: Cholinergic-mediated increasing spiking response in barrel cortex layer V pyramidal neurons

Authors: *A. NUNEZ[1], S. DOMINGUEZ[2], W. BUÑO[2], D. FERNANDEZ DE SEVILLA[3]
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Abstract: Neocortical cholinergic activity plays a fundamental role in sensory processing and cognitive functions, but the underlying cellular mechanisms are largely unknown. We analyzed *in vitro* in rat "barrel cortex" $L5_A$ pyramidal neurons the effects of acetylcholine (ACh) on synaptic transmission and cell excitability. ACh through nicotinic and M1 muscarinic receptors enhanced excitatory postsynaptic currents and through nicotinic and M2 muscarinic receptors reduced inhibitory postsynaptic currents. These ACh-induced changes resulted in the generation

of slow all-or-none Ca^{2+} spikes and action potential bursts evoked by electrical stimulation of basal dendrites at $L5_B$. Ca^{2+} spikes were mediated by activation of NMDA receptors and L-type voltage-gated Ca^{2+} channels. Essentially identical effects were observed in the absence of activation of ACh when $GABA_A$–mediated inhibition was blocked by PiTX. Additionally we demonstrate *in vivo* that basal forebrain stimulation induced an atropine-sensitive increase of Layer 5 action potential responses evoked by *vibrissa* deflection; an effect mainly due to the enhancement of a NMDAR component. Therefore, ACh modified the excitatory/inhibitory balance and switched L5 pyramidal neurons to a bursting mode that caused a potent and sustained response enhancement with possible fundamental consequences in the signal processing capacity and plastic properties of the barrel cortex.
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Disclosures: A. Nunez: None. **S. Dominguez:** None. **W. Buño:** None. **D. Fernandez de Sevilla:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: Swiss NSF 31003A_125379

Title: Plasticity-related genes expression is modified by sensory stimulation in the adult mouse barrel cortex.

Authors: *C. SAVARY[1], N. WENGER[1], F. OLIVERI[2], H. FIUMELLI[3], J.-L. MARTIN[3], U. ALBRECHT[4], M. A. RÜEGG[2], E. WELKER[1]
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Abstract: Sensory information helps to shape neuronal circuits during development and adulthood. In the mouse barrel cortex, cells from layer IV are able to adapt their functional state to an increased flow of sensory information from the mystacial whiskers. Previous studies in our group have shown that whisker stimulation induced the formation of inhibitory synapses in the corresponding barrel (Knott et al., Neuron 34: 265-273, 2002) and decreased neuronal responses toward the deflection of the stimulated whisker (Quairiaux et al., J.Neurophysiol. 97: 2130-2147,

2007). Together these observations have turned the barrel cortex into a model to study homeostatic plasticity.
At the cellular level, neuronal activity triggers intracellular signalling cascades leading to a transcriptional response. Experience-dependent gene expression acts then to both modulate gene expression and to directly modify neuronal physiology and morphology. To further characterize the molecular pathways involved in the synaptic changes after whisker stimulation, microarray analysis on laser-dissected barrels in sections through layer IV revealed an upregulation of a series of genes in the stimulated barrels. We then focused on the following plasticity-related genes: the transcription factors, neuronal PAS domain protein 4 (NPAS4) and neuronal activity-regulated pentraxin (NARP); the cytoskeleton protein, cardiac troponin C (TNNC1); and the pituitary adenylate cyclase-activating polypeptide (PACAP).
In this study, adult males from the C57Bl/6J strain had their left C1, C2, C3 whiskers stimulated. For each gene of interest, in situ hybridization and immunohistochemistry revealed different patterns of mRNA and protein expression in the stimulated barrels. NPAS4 and NARP show an increase for both mRNA and protein in the stimulated barrels. The increased level of PACAP immunoreactivity is localized in layers II/III and V of the cerebral cortex, whereas there is no difference of immunostaining inside the stimulated barrels compared to the non-stimulated ones. However, in situ hybridization indicates increased PACAP mRNA expression in the stimulated barrels. Finally, the increase of TNNC1 mRNA and protein is localized in neurons positioned at the interface between layers IV and V.
Altogether, these data confirm the results from the microarray analysis and they suggest a differential role of the tested genes in the structural and physiological reorganization observed after whisker stimulation.

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Poster

883. Somatosensory Reorganization and Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: Royal Society Newton International Fellowship

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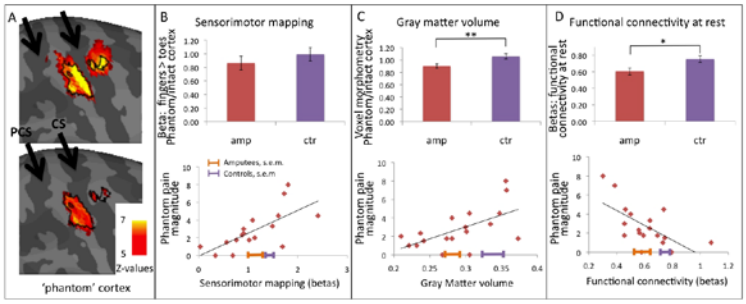
Wellcome Trust WT090955AIA

CIHR MOP-89890

Title: Phantom pain is associated with preserved local activity but disturbed functional connectivity

Authors: *T. R. MAKIN[1], J. SCHOLZ[1,2], N. FILIPPINI[1], D. HENDERSON SLATER[3], I. TRACEY[1], H. JOHANSEN-BERG[1]
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Abstract: Phantom pain has become an influential example of maladaptive cortical plasticity. According to this model, sensory deprivation following limb amputation allows for intra-regional invasion of neighbouring cortical representations into the former hand area of the primary sensorimotor cortex, which gives rise to pain sensations. Over the years, this model was extended to explain other disorders of pain, motor control and tinnitus, and has inspired rehabilitation strategies. Yet, other research, demonstrating that phantom hand representation is maintained in the sensorimotor system, and that phantom pain can be triggered by bottom-up aberrant inputs, may call this model to question. Using fMRI, we identified the cortical area representing the missing hand in a group of 18 arm amputees (Fig. 1A, bottom image and 1B). This allowed us to directly study changes in the "phantom" cortex associated with chronic phantom pain, using functional connectivity (Fig. 1D) and voxel-based morphometry (Fig. 1C). We show that, while loss of sensory input is generally characterized by structural degeneration of the deprived sensorimotor cortex (Fig 1C, top), the experience of persistent pain was associated with preserved intra-regional structure and functional organization (Fig 1B and 1C, bottom panels, respectively). Furthermore, consistent with the dissociative nature of phantom sensations from other sensory experiences, phantom pain is also associated with reduced long-range inter-regional functional connectivity (Fig 1D, bottom panel). We propose that this disrupted inter-regional connectivity may be consequential, rather than causal, of the retained yet isolated local representation of phantom pain. We therefore propose that, contrary to the maladaptive model, cortical plasticity occurs when powerful and long-lasting subjective sensory experience, most likely due to peripheral inputs, is decoupled from the external sensory environment.



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Poster

883. Somatosensory Reorganization and Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: INSERM

CNRS

Ucbl1

McDonnell Foundation

Title: Breaking the boundaries of somatosensory plasticity: Improved touch at the fingers transfers to the lips.

Authors: D.-A. MURET[1], H. R. DINSE[2], C. URQUIZAR[1], O. SILLAN[1], K. REILLY[1], *A. FARNE[1]
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Abstract: It is now well established that cortical plasticity occurs in the adult brain and that plastic phenomena are involved in many brain functions. Understanding the rules governing plasticity and its functional consequences is extremely challenging. A cutaneous coactivation (CA) protocol has been successfully used to study somatosensory plasticity (see Godde et al, 2003). CA relies on the Hebbian postulate and consists of synchronous and passive coactivation of several non-overlapping receptive fields which produces transient somatosensory changes. The aim of this study was to evaluate whether pure somatosensory plasticity, experimentally induced at a specific cutaneous location (i.e., right index fingertip) can cross somatotopically defined boundaries and functionally affect physically distant, but cortically adjacent body-part regions (i.e., the perioral lip region). A two-point discrimination (2PD) task was used to determine the spatial discrimination thresholds at the right and left index finger (RD2 & LD2), and at the right and left sides of the upper lip area. This test was performed during 4 sessions on 2 consecutive days. Between the third and fourth session, a vibrotactile CA was applied for three hours at the RD2 fingertip. Thirty participants were randomly attributed to either of two groups: a test group (n=15, mean age = 20.53 ± 2.26 years), in which subjects received a true CA at the RD2 fingertip; and a control group (n=15, mean age = 23.5 ± 3.25 years), in which the CA was replaced by a sham CA (stimulator fixed at the fingertip but turned off). Results showed that in

the test group three hours of CA led to a significant decrease of the 2PD threshold at RD2 fingertip, from 1.77 ± 0.28 mm to 1.52 ± 0.43 mm (mean decrease of 15.26%), thus replicating previous results. Critically, a significant 2PD threshold decrease was also found at both sides of the upper lip area (from 6.02 ± 0.88 mm to 5.44 ± 1.24 mm, mean decrease of 10.29%). No effects were found at either the LD2 fingertip or at any body-part region of the control group. These results demonstrate that experimentally-induced plasticity following RD2 coactivation improves participants' tactile discrimination performance not only at the coactivated body site, but also at cortically adjacent body sites. Thus, CA-induced somatosensory plasticity appears to cross somatotopically defined boundaries between cortical body-parts representations, spreading its functional consequences to cortically adjacent, but peripherally unstimulated body parts.

Disclosures: D. Muret: None. **H.R. Dinse:** None. **C. Urquizar:** None. **O. Sillan:** None. **K. Reilly:** None. **A. Farne:** None.

Poster

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Program#/Poster#:883.10/EE13

Topic: D.09. Tactile/Somatosensory

Support: NIH 1R01NS072416

Title: Factors influencing the sparse sensory code in L2/3 of mouse somatosensory cortex

Authors: *K. CLANCY, J. ELSTROTT, T. MIYASHITA, D. FELDMAN
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Abstract: Much of rodent neocortex exhibits sparse spiking activity in which only a fraction of neurons are active at one time. In primary sensory cortex, stimulus selectivity is one major cause of sparse firing, but other factors may also contribute, including cell type, brain state and the potential existence of intrinsically high- and low-responsive subcircuits (Wolfe 2010). In primary somatosensory cortex (S1), layer 2/3 (L2/3) has the lowest response probability, and most spiking activity is from a small subpopulation (~ 20%) of more active neurons. Here we examine the factors that determine response probability within the sparsely active L2/3 network.
We performed ensemble 2-photon Ca^{2+} imaging using bulk loaded OGB-1AM in L2/3 of mouse S1 and used the fast-oopsi algorithm (Vogelstein 2010) to detect spikes from ΔF/F traces. Spontaneous and whisker evoked activity were imaged in P23-P45 mice anesthetized with urethane/chlorprothixene. Neurons displayed a range of spontaneous firing rates and response probabilities, however there was a significant correlation ($R^2 = 0.58$) between whisker-evoked

response probability, termed p(r), and spontaneous activity, suggesting that high p(r) neurons may be embedded in a specialized, more active subnetwork. Low p(r) neurons were more narrowly tuned to their preferred whisker. Cells were more likely to respond to a whisker stimulus when the cortex was in a LFP 'down' state. In contrast, spontaneous spikes were more likely to occur when the cortex was in an 'up' state. High p(r) neurons were not exclusively interneurons, as seen in recordings in GAD67-GFP and PV-tdTomato transgenic mice.
The sparse code was plastic in response to whisker deprivation. Animals that underwent 3 days of D-row whisker deprivation beginning at P23-P33 showed a reduced proportion of high p(r) neurons compared to control animals. The spontaneous firing rate and breadth of whisker tuning appeared unaffected. This suggests that the high p(r) neurons are more involved in expression of rapid deprivation-induced plasticity. Together, these results indicate that a population of special, high p(r) neurons exists that are preferentially represented in network activity, and are a locus for rapid experience-dependent plasticity.

Disclosures: K. Clancy: None. **J. Elstrott:** None. **T. Miyashita:** None. **D. Feldman:** None.

Poster

883. Somatosensory Reorganization and Plasticity

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:883.11/EE14

Topic: D.09. Tactile/Somatosensory

Support: NHMRC APP1002190

NHMRC ID601301

Title: Modulation of sensory cortex excitability is dependent on the intensity of peripheral electrical stimulation

Authors: *P. W. HODGES, L. CHIPCHASE, L. SMITH, A. MATUS, S. SCHABRUN
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Abstract: Background: Peripheral electrical stimulation (PES) is commonly applied at sensory intensities in rehabilitation for treatment of pain and at motor intensities to facilitate muscle contraction. These stimuli have opposite effects on excitability of corticomotor pathway: excitability is increased by motor stimulation and reduced by sensory stimulation. It is unknown how the intensity of stimulation affects sensory cortex activity. Methods: Thirteen healthy individuals attended two experimental sessions at least 48 hours apart. Participants received 30 minutes of PES to right abductor pollicis brevis (APB) at: i) sensory intensity (perceptual

threshold), or ii) motor intensity sufficient to induce a muscle contraction. Order of interventions was randomised across participants. Excitability of the corticomotor pathway was measured using transcranial magnetic stimulation (TMS) and activity of the primary sensory cortex using somatosensory evoked potentials (SEPs) before and after each intervention. M-waves were recorded to evaluate changes in the muscle/neuromuscular junction. Results: The amplitude of the N20-P25-N33 complex of SEP, representative of processing in the primary sensory cortex, was increased with stimulation at motor intensity, and decreased with sensory stimulation. Similarly, the amplitude of the motor response evoked by TMS was augmented following motor stimulation and diminished following sensory stimulation. There was no change in the peripheral apparatus with either PES protocol. Conclusion: Altered activity in the primary sensory cortex mirrors that occurring in corticomotor pathways following PES at motor and sensory intensities. This finding suggests co-modulation of changes in primary sensory and motor regions. Transmission of information via cortico-cortical circuits may provide a candidate mechanism for how afferent information in the form of PES alters corticomotor pathway excitability.

Disclosures: P.W. Hodges: None. **L. Chipchase:** None. **L. Smith:** None. **A. Matus:** None. **S. Schabrun:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:883.12/EE15

Topic: D.09. Tactile/Somatosensory

Support: IN 207307-3 DGAPA/UNAM, MEXICO

Title: Tactile stimulation increases the neuronal caudal ambiguous nucleus development of perinatally underfed pups

Authors: D. LOPEZ, C. TORRERO, M. REGALADO, *M. A. SALAS
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Abstract: Undernutrition is a health problem that produces brain morphological alterations and cognitive deficiencies in children of underdeveloped countries. Nutritional rehabilitation could be potentiated by different concurrent therapy techniques. Touch is a modality of sensory system first developed during the fetal and neonatal stages that may be affected by perinatal undernutrition in rats interfering with crying and ultrasound vocalization (USV) emissions. The caudal ambiguous nucleus (AMBc) neurons innervate the pharynx modulating the motor activities underlying USV. The current study analyses the effects of tactile stimulation (TS) on

the AMBc neuronal (Golgi-Cox) development of perinatally underfed rats at 8, 12, and 15 PND. Undernutrition includes the pregnant food intake reduction, followed by the rotation (12h) of a nipple-ligated and a normal dam between litters. The dendritic tree and soma measurements of multipolar neurons in control (C), underfed (U), control stimulated (CS) and underfed stimulated (US) groups were measured. Delicate TS (body massage and passive limb movements) was applied (15 min) daily (days 4-15 of age). In the U subjects multipolar neuronal dendritic scores were reduced ($p<0.05$) in both the number and density at 8 and 15 PND. By contrast TS increases the values of these parameters along the same days of the study ($p<0.05$). Concerning the soma parameters of multipolar neurons, they were reduced in the U subjects, with increments in the perimeter and area of the stimulated groups (CE, US). Additionally, body weight of U animals were consistently reduced; although the stimulated subjects (CE and UE) were increased compared to C and U pups. Data suggest that TS through the increased somatosensory ascending neuronal activity, and the possible release of growth factors, may have an important role to modulate the AMBc neuronal discharges to the pharyngeal motor activity interfering with the USV emissions and the mother-litter interactions at critical stages of life.

Disclosures: D. Lopez: None. **C. Torrero:** None. **M. Regalado:** None. **M.A. Salas:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: Kavli Institute for Brain and Mind Innovative Research Award

Title: Two dimensional changes in the body model for the hand following the brief use of a hand-like tool

Authors: *L. MILLER[1,2], M. R. LONGO[3], A. P. SAYGIN[1,2]
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Abstract: Tool use is a ubiquitous and crucial aspect of human life. It is well known that neural representations of the body in somatosensory and parietal cortices reorganize following long-term changes to the body. We sought to explore effects of brief periods of tool use on dimensional representations of the body called the body model. Recently it has been shown that the use of a long, grabber-like tool leads to a systematic mislocalization of touch along the user's arm (Cardinali et al., 2009, Current Biology), suggesting that the tool became incorporated into

the body model and lead to an elongation of the arm representation in one dimension. Here, we sought to characterize two-dimensional changes to the body model following the use of a custom hand-shaped tool measuring approximately 21 by 45 centimeters. This novel tool was strapped to user's arm, and included five dynamic plastic fingers that moved concurrently with movements of the subject's own fingers. To measure plastic changes to the body model, we used a tactile distance judgment paradigm (de Vignemont, et al. 2005, Current Biology). Wooden points at variable distances (20 mm, 30 mm, 40 mm) were used to touch an experimental site (the dorsum of the hand) and a control site (the forehead). Subjects were then asked to report which touch had the greatest distance between the two points. This procedure was performed before tool use, and repeated after an 8-minute period of tool use (involving grasping, lifting and placing a set of objects into containers). Touches were oriented either horizontally (i.e., *across* the hand or forehead) or vertically (i.e., *along* the hand or forehead). We hypothesized that following tool use, subjects would report that touches to the hand felt larger than to the control site. For horizontal/across touches, the results confirmed our hypothesis, revealing a significant increase consistent with the widening of the hand after tool use. This effect was replicated with the forearm as the control site. Vertical touches however revealed the opposite pattern. Thus we show that the brief use of a hand-like tool can lead to changes in the body model of the hand in two dimensions, and that there may be an interaction between changes in the horizontal and vertical directions. We will use fMRI to determine whether this plasticity is reflected in changes in somatosensory cortex, or in representations of the body in the parietal cortex.

Disclosures: L. Miller: None. **M.R. Longo:** None. **A.P. Saygin:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Program#/Poster#:883.14/EE17

Topic: D.09. Tactile/Somatosensory

Support: CAPES

CNPq

FAPERJ

INNT

Title: Long-distance plasticity. I. Evidence of functional and behavioral correlates of abnormal bundles in callosal dysgenesis.

Authors: *M. C. MONTEIRO[1,2,3], V. LAZAREV[2], R. DEOLIVEIRA-SOUZA[1], L. DEAZEVEDO[2], J. MOLL[1,4], R. LENT[3,4], F. TOVAR-MOLL[1,3,4]
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Abstract: Long-distance plasticity is the anomalous formation or redirection of fiber tracts in the brain, after major disturbances of developmental events. It occurs in callosal dysgenesis (CD), a morphogenetic failure of the corpus callosum (CC), without the split-brain syndrome. Previous studies demonstrated abnormal white matter pathways in CD. To address the functional correlates of some of these bundles, we combined diffusion tensor imaging (DTI) with quantitative electroencephalography, and neuropsychological assessment of interhemispheric information transfer.
Volumetric anatomical (3DT1) and DTI (2,5mm isotropic voxel) images were acquired (3T, Achieva Philips) from 5 patients and 7 healthy volunteers. Neurophysiological assessment was done in these same patients and 15 controls by 16-channel eletroencephalogram recording (Biologic) and analysis of coefficients of coherence (CChr) in 5 frequency bands (Brainsys). Neuropsychological evaluation consisted of tactile recognition and naming tasks and was applied in all acallosals and 12 age-matched normal-IQ volunteers. Groups were compared by Mann-Whitney's (neurophysiology and neuropsychology) and *t test* (neuroimaging).
In patients, structural images showed: partial (n=2) and hypoplasic (n=1) callosal dysgenesis, and agenesis (n=2). DTI and tractography confirmed bilateral Probst bundles (PB) connecting intrahemispheric cortical regions in all patients, and Sigmoid bundles (SB) connecting frontal with contralateral occipitoparietal cortices in partial callosal dysgenesis. The CChr showed increased intrahemispheric and decreased interhemispheric coherence in CD. However, in partial dyscallosals, higher interhemispheric coherence in the frontal area was observed; likewise, higher contralateral coherence between anterior and centroparietooccipital (particularly right-to-left) areas was observed. No impairment was found in tactile object recognition and naming in acallosals. However, they showed a bilateral impairment in transfer of tactile spatial cognition. Changes in electrophysiological connectivity in CD may be the functional counterpart of anomalous bundles resulting from long-distance plasticity, while increased intrahemispheric coherence in CD patients may be attributed to the PB, while anatomical connectivity of SB may underlie the interhemispheric coherence observed between contralateral anterior and posterior cortical areas. Although dyscallosals lack a complete disconnection syndrome, compensatory plasticity seems to have limits, since a behavioral impairment is found, not attributable to cognitive or other cerebral impairment.

Disclosures: M.C. Monteiro: None. **V. Lazarev:** None. **R. deOliveira-Souza:** None. **L. deAzevedo:** None. **J. Moll:** None. **R. Lent:** None. **F. Tovar-Moll:** None.

Poster

883. Somatosensory Reorganization and Plasticity

Location: Hall F-J

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Topic: D.09. Tactile/Somatosensory

Support: NIMH Conte Centre - 1 P50 MH077972

MRC programme grant - G0901299

Title: Normal DISC1 function is required during a neonatal critical period for development of adult plasticity

Authors: *S. GREENHILL, K. FOX
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Abstract: Disrupted in Schizophrenia-1 (DISC1) is the subject of intensive investigation since its dysfunction has been associated with increased likelihood of schizophrenia in humans. Induced malfunction of the DISC1 pathway at P7 produces schizophrenia-like behaviour in adult mice (Li et al, 2007). We have previously demonstrated that the short-term activation of a dominant negative fragment of the DISC1 c-terminal (DISC1-cc) at postnatal day 7 leads to deficits in experience-dependent plasticity in the barrel cortex of adult mice (Juczewski et al, SfN 2010). This would suggest that the activation of DISC1-cc affects neuronal function during a critical period of development. Here, we aimed to further elucidate the period in which juvenile DISC1 disruption leads to deficits in adult experience-dependent plasticity.
DISC1-cc transgenic mouse pups (and wild-type littermates) were injected with tamoxifen (20ug/g body weight in corn oil, subcutaneous) in three groups, each corresponding to a different timepoint of DISC1-cc activation (P7, P11-13 and P28). Tamoxifen injection releases the dominant negative fragment of DISC1 (671-852aa) from a mutant estrogen receptor ligand binding domain (LBD) for approximately 24-48 hours (Li et al, 2007). Pups were allowed to develop until P50 and were then subjected to deprivation of all but the D1 whisker for a period of 18 days, followed by 7-10 days of whisker re-growth. This protocol is normally sufficient to induce strong changes in the receptive field of neurons in barrels surrounding the spared whisker's barrel (Glazewski et al, 1996).
After the deprivation and re-growth period, single unit recordings (n=30 animals) were performed in vivo to measure the receptive fields of neurons in barrels surrounding the D1 barrel. Deprived mutant groups at different timepoints were compared with a one-way ANOVA which found a highly significant effect of injection day ($P<0.0001$, $F=32.1$, $df=2,16$). Post-hoc t-tests revealed that activation of DISC1-cc at P7 completely abolished the response to deprivation, activation at P28 did not inhibit plasticity in adult animals, and activation between

P11 and P13 produced a moderate inhibition of adult somatosensory plasticity. These results suggest a novel critical period of adult plasticity in the barrel cortex, which closes during the second postnatal week.

Disclosures: S. Greenhill: None. **K. Fox:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: NIMH Conte Centre Grant 1P50MH077972

MRC Programme Grant - G0901299

Title: Transient neonatal impairment of DISC1 function delays dendritic and synaptic development of cortical layer III neurons

Authors: *N. R. HARDINGHAM, S. D. GREENHILL, K. FOX
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Abstract: DISC1 has been implicated in schizophrenia and has an important role in brain development. We used a transgenic mouse with a dominant negative fragment of the DISC1 c-terminal (DISC1-cc), activated for 24-48 hours by injection of tamoxifen. Activation of the mutant protein at P7 results in schizophrenic like behaviour (Li *et al*, 2007) and a loss of experience dependent plasticity in layer 2/3 of adult barrel cortex, while activation of DISC1-cc at P28 has no effect on plasticity (Greenhill and Fox, SfN 2012). This suggests that a developmental process taking place between P7 and P28 is affected by the transgene.
To test how structural and synaptic development in the supragranular cortex were affected in the DISC1-cc mutant, we activated DISC1-cc at P7 and made whole cell recordings from layer 3 cells at P8, P14, P21 and P28. Wild type littermates were also injected with tamoxifen for comparison with the mutants. Electrical and synaptic properties of the neurons were recorded at the different ages whilst they were being injected with biocytin and/or Alexa 594 enabling subsequent morphological characterisation of the neurons. We also recorded mEPSPs and tested for LTP in the different experimental groups to measure synaptic efficacy and ability to undergo synaptic plasticity at different developmental stages.
Dendrites were immature at P8 (mean sholl count of 20 ± 2) and in wild types the dendritic growth largely took place between P8 and P14 (P14 mean sholl count of 52 ± 3; P28 mean sholl

count of 58 ± 3). However, in the DISC1-cc mutants, dendritic development was substantially retarded compared to wild types (DISC1-cc: P8 mean sholl count of 20 ± 2 ($p>0.05$ compared to wild types, df=28); P14 mean sholl count of 39 ± 4 ($p<0.05$ compared to wild types, df=31) while a normal developmental stage was reached by P21 (DISC1-cc, P21 mean sholl count of 55 ± 4, P21 mean wild type sholl count of 51 ± 8, $p>0.05$ comparison, df=20). It was found that dendritic growth in horizontal directions, into adjacent barrels was also substantially retarded in the DISC1-cc mutants at P14. Furthermore, the normal development of short term plasticity between P8 and P28 was disrupted in the mutants; in wild types the main reduction in paired-pulse depression took place between P8 and P14; in the mutants this change did not take place until between P14 and P21.
Thus it would seem that normal development of the supragranular barrel cortex is substantially retarded in the DISC1 mouse over the critical period of development and these structural and functional affects have a long-lasting effect on plasticity in the adult brain.

Disclosures: N.R. Hardingham: None. **S.D. Greenhill:** None. **K. Fox:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Program#/Poster#:883.17/EE20

Topic: D.09. Tactile/Somatosensory

Support: CAPES

CNPq

FAPERJ

INNT

Title: Long-distance plasticity. II. Morphological evidence of novel abnormal bundles with possible compensatory functions in human callosal dysgenesis.

Authors: *R. V. BARBOSA[1], T. F. MARINS[1,2], N. D. A. QUEIROZ[1,2], I. BRAMATI[1,2], J. MOLL[2,3], F. TOVAR-MOLL[1,2,3], R. LENT[1,3]
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Abstract: Long-distance plasticity is the anomalous formation or redirection of fiber tracts in the brain, after major disturbances of developmental events. It occurs in callosal dysgenesis (CD), a morphogenetic failure of the corpus callosum (CC) lacking the split-brain syndrome. Two major abnormal tracts in CD subjects have been previously described: the Probst bundle (PB), with a longitudinal course parallel to the midline and the lateral ventricle, and the Sigmoid bundle (SB), an asymmetric tract connecting the frontal lobe to the contralateral occipito-parietal cortex. Since patients with CD do not show the split-brain syndrome, we can expect them to have some kind of inter-hemispheric connection that somehow compensates the connectivity lost by lack of the CC. a hypothesis hardly attributable to these abnormal tracts.
The aim of this study was to look for other white matter changes in patients with CD by using diffusion tensor imaging (DTI) and tractography. Volumetric anatomical (3DT1) and DTI (2.5mm isotropic voxel) images were acquired (3T, Achieva Philips) from 6 patients and 6 healthy volunteers. Regions of interest (ROI) were selected at different parts of cingulum bundle (CB), corticospinal tract and posterior commissure (PC).These bundles were reconstructed by tractography, and values of fractional anisotropy (FA) were extracted. Values of FA of both groups were compared by Mann-Whitney's test.
In patients, structural images showed partial (n=2) and hypoplasic (n=2) callosal dysgenesis, and agenesis (n=2). DTI and tractography confirmed a bilateral PB in all patients, and a SB in dyscallosal but not acallosal subjects. We also found an increase of FA values in the CB of all patients. No difference was found regarding the corticospinal tracts. Surprisingly, tractography of the PC revealed a so far unknown abnormal interhemispheric bundle connecting bilaterally the parietal cortex of 4 patients through this midbrain commissure. This abnormal tract extended from dorsolateral cortical areas along the internal capsule and cerebral peduncle, to cross the midline and ascend with a symmetrical topography towards the homotopic sector of the contralateral cortex.
These results suggest that the early impairment of callosal development in CD subjects causes not only an impact on the morphology of intrahemispheric bundles, but also the formation of abnormal, so far unknown, interhemispheric pathways. It is conceivable that these symmetric connections be associated to the absence of a split-brain syndrome in CD, having a compensatory, rather than pathogenic function in these patients.

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Poster

883. Somatosensory Reorganization and Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NS-055236 to RSW

Title: The cuneate nucleus (CN) may not play a major role in delayed large-scale cortical reorganization in rat forepaw barrel subfield (FBS) cortex

Authors: *R. S. WATERS, C.-X. LI, S. VEMULAPALLI
Dept. of Anat. and Neurobio., Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Introduction: We previously reported that delayed large-scale cortical reorganization in the forepaw barrel subfield (FBS) cortex requires 3 or more weeks before cells in the deafferented cortex begin responding to new input from the shoulder and trunk. At 6 weeks post deafferentation, the majority of sites in the FBS are responsive to the new input. Since new input was shown not to originate from adjacent cortex, we proposed that it must derive from subcortical circuits. In the present study, we examined receptive field input to neurons in the cuneate nucleus (CN) between 1 and 11 weeks following forelimb amputation and report little evidence for CN being a major substrate for large-scale cortical reorganization.
Methodology: Rats (6-8 wks of age) anesthetized with Nembutal received unilateral forelimb amputation. At 1 to 11 wks post-amputation, CN was physiologically mapped using multiunit recording. Rats were anesthetized with Ketamine/Xylazine (100 mg/kg), CN was exposed and an electrode was inserted to a depth of 100 microns, advanced in 50 or 100-micron steps to a depth of 900 microns, and receptive fields were recorded at each step. Lesions were made to mark recording sites, animals were perfused, brains were removed and sectioned, tissue was stained with cytochrome oxidase (CO) and electrode penetrations were reconstructed. Detailed mapping was carried out in CN in the obex region. For analysis, CN was divided into medial and lateral agranular zones, and a central CO-rich cluster zone.
Results: (a) CN, in the vicinity of obex, contains a central barrel region and surrounding agranular regions. (b) The central barrel region is unresponsive to receptive field input during the first week following amputation. (c) At weeks 2 and 3, approximately 22% of the sites respond to new input from trunk and shoulder. (d) After week 3, the central region becomes largely unresponsive again. (e) In contrast, new input is found in the agranular regions as early as post amputation week 2 and continues throughout week 11.
Interpretation: We hypothesized that the CN central barrel-like region would reorganize prior to reorganization in the FBS. The modest reorganization observed during weeks 2 and 3 is unlikely to form the hypothesized substrate for delayed large-scale cortical reorganization.

Disclosures: R.S. Waters: None. **C. Li:** None. **S. Vemulapalli:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: NIH NCRR TL1RR025753

Title: Changes in cortical connectivity underlie altered sensory discrimination after stroke

Authors: *A. L. BORSTAD[1], S. CHOI[2], P. SCHMALBROCK[2], D. NICHOLS-LARSEN[3]
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Abstract: Background: Sensory discrimination is an important yet frequently overlooked aspect of human hand function. Following stroke discrimination impairment is common. Functional imaging studies suggest a bilateral network of brain areas support sensory discrimination. The purpose of this study was to quantify white matter connectivity in the right hand sensory discrimination network in stroke and control participants using diffusion imaging. We hypothesized that impaired interhemisphereic connectivity would correlate with sensory discrimination. Subjects and methods: Sensory function was evaluated, using monofilaments and the Hand Active Sensation Test (HASTe). Thiry-two direction diffusion MRI scans were obtained for 19 individuals with stroke (mean age=62.8 years), (9 right hemisphere) and 16 controls (mean age 59.2 years). Diffusion parameters were used to quantify connectivity of four white matter tracts that support sensory function. Data were analyzed using Oxford Centre for Functional MRI of the Brain Software Library (FSL). Results: Stroke participants had poorer sensory discrimination than controls (p=0.04). Touch perception was not statistically different between groups. Mean diffusivity was statistically higher for the stroke group than control participants for all four tracts measured. Left (L) thalamus to primary sensory (S1) (p<0.001), S1 to S1 interhemisphereic (p=<0.005), L S1 to L middle frontal (p=0.003), L S1 to L S2 (p=0.014). A negative linear relationship was identified between touch perception and thalamocortical connectivity (R= -.37, p=0.03) and between sensory discrimination and S1 interhemisphereic connectivity (R= -.42, p=0.02). In a multivariate regression 28% of sensory discrimination score can be predicted based on connectivity in the networks studied. Conclusions: Right sensory discrimination was poorer in stroke participants and connectivity was diminished in all tracts measured. Interhemisphereic connectivity was most closely correlated to sensory discrimination ability and may reflect the bilateral nature of this task.

Disclosures: A.L. Borstad: None. **S. Choi:** None. **P. Schmalbrock:** None. **D. Nichols-Larsen:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: DARPA Grant N66001-10-C-2008

Title: S1 somatotopy in adult rats and macaques is not pkmζ dependent

Authors: *J. C. IORDANOU, L. VON KRAUS, A. TARIGOPPULA, J. FERBINTEANU, J. T. FRANCIS
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Abstract: Zeta Inhibitory Peptide (ZIP), a pseudosubstrate for protein kinase mζ has been shown to abolish long-term potentiation in vitro and in-vivo and has been shown to decrease performance of learned tasks. ZIP has also been proposed as a possible treatment for focal hand dystonia and chronic pain; however the effects of intracortical ZIP infusions in sensorimotor cortex of normal untrained animals have not been examined. In this study we examined the effect of intracortical ZIP injections in the somatosensory cortex of naive rats (n = 7) and a Macaque. The subjects were implanted with arrays of electrodes in the primary somatosensory cortex, specifically in areas which showed clear response to tactile stimulation of the fingers. Additionally cannulas (1 in the rat, 3 in the macaque) were implanted to allow for direct ZIP injections into the region of cortex being recorded from. Subsequently the rats (Anesthetized) and macaque (Awake) received repeated tactile stimulation on the hand and fingers using a force-sensitive thwacker. Cortical responses to stimulation were recorded before (baseline) and after a 1.0μl infusion of either ZIP (10nmol/ml), scrambled ZIP(10nmol/ml), or vehicle was administered through each cannula. To assess ZIP effects at its maximum (2 hours after infusion) tactile stimulations with recordings resumed in rats at intervals of 20 minutes up to 3 hours post-infusion. In the macaque, one recording session was performed approximately 2 hours post-infusion. The neural recordings were examined for changes in receptive field size and strength, stimulus field size and strength, as well as mutual information between neurons. To assess motor coordination, some of the rats were tested using a rotorod, grid walking test, and paw placement test. No significant differences were found between ZIP and control infusions (scrambled ZIP or vehicle) on either physiological or behavioral measurements. Thus the data suggest that maintenance of the size and strength of the somatotopic representation is not dependent on LTP processes involving PKMzeta.

Disclosures: J.C. Iordanou: None. **L. von Kraus:** None. **A. Tarigoppula:** None. **J. Ferbinteanu:** None. **J.T. Francis:** None.

Poster

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Title: Ipsilateral responses and interhemispheric interactions in area 3b after dorsal column spinal cord injury in New World monkeys

Authors: *J. L. REED, H.-X. QI, J. H. KAAS
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Abstract: Lesions of the spinal cord dorsal columns (DC) produce permanent loss of afferents, depriving cortex of sensory input. We evaluated primary somatosensory cortex (area 3b) in New World owl monkeys and squirrel monkeys 5-9 weeks after incomplete unilateral cervical DC lesions. Over the first month after injury, area 3b reactivates and hand use improves; thus, we expected 3b neurons of deprived monkeys to recover nearly normal response properties. Neurons in the 3b hand representation do not typically respond to ipsilateral stimulation, but simultaneous tactile stimulation of both hands tends to suppress activity in 3b (Tommerdahl et al. 2006; Reed et al. 2011), and loss of input from a digit can reveal interhemispheric interactions (Calford and Tweedale 1990); thus, we investigated how DC lesions affected interhemispheric interactions in area 3b. We implanted a 100-electrode array in the 3b hand representation of normal monkeys and monkeys several weeks after lesions, then delivered tactile stimulation to both hands to examine intra- and inter-hemispheric modulation. We compared responses from 4 normal monkeys and 3 deprived monkeys and tested ipsilateral responses and interhemispheric interactions in 1 of the deprived monkeys, compared with results from such tests in 2 normal monkeys (Reed et al. 2011). We have not encountered responses to ipsilateral hand stimulation in normal 3b neurons; but in a monkey after DC lesion, 10.5% (6/57) of neurons showed significant firing rate increases in response to stimulation of the ipsilateral hand, while 61% (36/59) responded to stimulation of the contralateral hand on digits that mirrored those tested on the ipsilateral hand. Latencies of ipsilateral responses (mean = 29 ms) were similar to latencies of

contralateral responses in monkeys after DC lesions (31 ms; N = 3 monkeys); and ipsilateral response magnitudes (mean = 16 spikes/s) were similar to contralateral response magnitudes in deprived and normal monkeys (17 spikes/s). When both hands were stimulated simultaneously, 63% (44/70) of units showed no significant difference in response magnitude compared to that of the contralateral stimulation alone, 23% (16/70) were suppressed, and 14% (10/70) were facilitated. These findings may indicate that facilitative interactions occur more often in cortex reactivated after DC lesions than in normal cortex as reported in previous studies. Results from neurons responding to ipsilateral hand stimulation after unilateral DC lesions suggest that reorganization affects activity across cortical hemispheres and provide a foundation for investigations needed to examine interhemispheric interactions that occur after injury.

Disclosures: J.L. Reed: None. **H. Qi:** None. **J.H. Kaas:** None.

Poster

883. Somatosensory Reorganization and Plasticity

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:883.22/FF5

Topic: D.09. Tactile/Somatosensory

Support: global Center of Excellence (GCOE) program of the Japanese Ministry of Education, Culture, Sports and Technology

Title: The shorthanded brain; functional connectivity across anterior putamen in top-notch expertise of stenography.

Authors: T. ITO[1], *T. MATSUDA[1], S. SHIMOJO[2]
[1]Brain Sci. Inst., Tamagawa Univ., Tokyo, Japan; [2]Biol./Comp. Neur. Systems, Caltech, Pasadena, CA

Abstract: Stenography, or shorthand, is a very unique set of skills, which necessitates orchestration of various functional modules in the brain, including auditory, linguistic, cognitive, mnemonic and motor, owing to intensive, nearly-life time training. Now that it is quickly replaced with voice recognition technology, we took the last opportunity to scan the top expert's brains in Japanese language, to obtain insights into how the brain can plastically reorganize itself under extreme pressures on accuracy/speed with nearly life-time overtraining. The fMRI data revealed that neural circuits for complex motor control were plastically reorganized especially around the left putamen, and that there was a morphological change. Though the putamen is known as the execution center of the acquired sensory-motor skills in its posterior region, the anterior putamen of stenographers had a high covariation with the cerebellum and the midbrian;

the stenographers had obviously different neural circuits from the well-known automated brain circuits. Indeed, the posterior putamen of the controls exhibited a high covariation with the cerebellum and the midbrain. The data indicate a vigorous plasticity in this area and its connectivity to those other relevant areas. The current study provides a rare dataset indicating such vigorous plastic reorganization of the brain, with massive overtraining but only in the adulthood.

Disclosures: T. Ito: None. **T. Matsuda:** None. **S. Shimojo:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Program#/Poster#:883.23/FF6

Topic: D.09. Tactile/Somatosensory

Support: CIHR

Heart and Stroke Foundation of BC and the Yukon

Title: Selective acute reductions in motor cortex function by targeted ischemia in Channelrhodopsin-2 transgenic mice

Authors: *E. ANENBERG[1], P. ARSTIKAITIS[3], Y. NIITSU[4], J. D. BOYD[2], J. LEDUE[2], T. H. MURPHY[2]
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Abstract: Previously we have evaluated the effects of targeted occlusion of individual pial arterioles on somatosensory cortex function. We now evaluate the effect of targeted ischemia on motor function in Thy-1 line 18 channelrhodopsin-2 transgenic mice. Occlusion of individual arterioles within the motor cortex led to a micro-infarct that was verified using laser speckle contrast imaging. We assessed motor function using light based mapping procedure in channelrhodopsin-2 mice. This method allowed us to acquire a functional representation of forelimb muscles, thus allowing us to detect any ischemia-induced changes in its cortical representation within the first hours after stroke. Our results demonstrate that motor cortex appears to be very acutely sensitive to the effects of ischemia. Contrasting the differences in the immediate post-stroke changes between averaged electroencephalographic (EEG) (18±11% decrease) and electromyographic (EMG) (68 ±7% decrease) responses collected from each stimulation site within 40 minutes of stroke revealed a mismatch indicative of a substantial

amount of viable neurons that were incapable of transmitting to the muscle. Ischemia within a relatively small region of motor cortex severely impaired amplitude of EMG responses from stimulation in the ischemic region to 13 ± 4% of baseline. Notably, in areas that were non-ischemic, and selected for assessment based on the criterion of being the cortical location of greatest baseline motor output, there was a comparable decrease in EMG amplitude to 32.5 ± 5 % of baseline (n=6). Currently we are relating our electrophysiological findings to measures of blood flow. This relatively homogeneous deficit in motor output is present in regions adjacent to the infarct and is likely mediated by deficits in synaptic transmission. These results imply that motor cortex function is dependent a widely distributed synaptic network.

Disclosures: E. Anenberg: None. **P. Arstikaitis:** None. **Y. Niitsu:** None. **J.D. Boyd:** None. **J. LeDue:** None. **T.H. Murphy:** None.

Poster

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Topic: D.09. Tactile/Somatosensory

Support: CIHR Vanier Scholarship

MSFHR Graduate Scholarship

CIHR MOP-12675

Title: Light-based mapping of reorganization after targeted stroke in sensorimotor cortex of Channelrhodopsin-2 mice

Authors: *T. C. HARRISON[1], G. SILASI[2], T. MURPHY[2]
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Abstract: Recovery from stroke is hypothesized to involve the reorganization of surviving cortical areas. For example, the mouse forelimb motor cortex (mFL) often begins responding to sensory stimulation if the neighbouring forelimb sensory area (sfL) is damaged by stroke. When a cortical area is forced to take on a new function after stroke, is it impaired in the performance of its original role? To study the organization of sensorimotor cortex before and after stroke, we developed techniques for chronic light-based motor mapping of Channelrhodopsin-2 (ChR2) transgenic mice. This light-based method allowed us to perform longitudinal studies of motor

cortex that would not be feasible with conventional electrical motor mapping.
In our experiments, brief pulses of light stimulation were targeted to an array of cortical points and the motor activity evoked by stimulation was recorded at each of these points using non-invasive motion sensors. In animals with chronically implanted cranial windows, the brain could be mapped repeatedly for months. Used in combination with intrinsic optical signal imaging of somatosensory cortex, light-based mapping permitted ongoing evaluation of the functional organization of sensorimotor cortex before and after the creation of photothrombotic infarcts targeted to either forelimb motor or sensory areas.
During the pre-stroke baseline period, sFL and mFL were stable (mean weekly drift in motor map center = 0.55 ± 0.37 mm, n = 23 mice). Strokes in sFL, but not mFL or sham strokes, caused the sFL map to shift anteromedially toward mFL (n = 6,7, and 6 respectively, $p<.001$, 2-way ANOVA). The mFL representation was displaced from its baseline position by infarcts targeted to either sFL or mFL, but not sham strokes (n = 7,7, and 8 respectively, $p<0.01$, 2-way ANOVA). Despite the large-scale reorganization observed in sensorimotor cortex, we found that displacement of mFL (defined as displacement of the forelimb motor map from its pre-stroke position) was uncorrelated with displacement of sFL and vice-versa.
The rapid recovery of sensory and motor maps is an encouraging demonstration of the impressive capacity for spontaneous reorganization of cortical circuits. Although the sensory and motor cortices are heavily integrated in rodents, destruction of one of these regions had only limited consequences for neighbouring cortical areas. These results suggest that sensorimotor cortical neurons are adept at vicariously performing the function of stroke-damaged circuits. Future experiments will attempt to identify means of enhancing cortical reorganization after stroke.

Disclosures: T.C. Harrison: None. **G. Silasi:** None. **T. Murphy:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: NIBIB Grant 1T32EB006350-05

Renz Foundation

Title: A sound-to-touch sensory substitution device for the severely hearing impaired

Authors: *S. NOVICH[1,2], D. M. EAGLEMAN[1,2]
[1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Dept. of Electrical & Computer Engin., Rice Univ., Houston, TX

Abstract: There are at least 2 million functionally deaf individuals in the United States alone and an estimated 53 million worldwide. The cochlear implant (CI) is an effective solution for regaining hearing capabilities for certain populations within this group, but not for all. First, CIs are expensive, ranging from $40,000 to $90,000 depending on the age of the recipient. This places CIs out of economic reach for many. Second, CIs require invasive surgery. Third, there is low efficacy of CI implantation in early-onset deaf adults over the age of 12. Given this, there exists an important population of deaf individuals who would benefit from a hearing replacement that has low cost, does not involve an invasive procedure, and may have a higher efficacy for early-deaf adults over the age of 12.
To address these problems, we are developing a low-cost, non-invasive, plasticity-based solution to deliver auditory information to the brain. Specifically, we are developing a "vibratory vest" by which auditory information is captured, digitally processed, and delivered to the skin of the torso using small vibratory motors. We call our device the Vibrotactile Acoustic Coder, or the "VAC." The term for such a technique is sensory substitution, and has previously proven successful in allowing those who are blind to have visual experience through the tongue or skin. Although auditory sensory substitution systems (known as "tactile hearing aids") have been prototyped as early as the 1970s (and proposed as early as the 1920s), a successful project is only now made possible by recent strides in digital signal processing techniques, speech codecs, machine learning, and processor power and cost. Our device leverages all of these advances to develop tactile encoding algorithms that extend beyond the simple filtering and mapping of an incoming audio stream as found in previous tactile aids. Our device will cost an order of magnitude less than a CI, be non-invasive, and –we hypothesize– not require critical period plasticity for use. From a broader perspective, this endeavor will enable us to better understand and model how the brain processes any sort of sensory information, understand the properties and capabilities of the brain's plasticity, and help us develop a generic framework for designing effective sensory-substitution devices. In this poster, we will present the current development status of the VAC and preliminary results of a haptic time perception experiment.

Disclosures: S. Novich: Research Grant; NIBIB. Other Research Support; Renz Foundation. **D.M. Eagleman:** None.

Poster

883. Somatosensory Reorganization and Plasticity

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Topic: D.09. Tactile/Somatosensory

Support: NSERC CGSD

CIHR Operating Grant

Title: *In vivo* activation using channelrhodopsin-2 and voltage sensitive dye imaging in a stroke model reveals altered cortical relationships

Authors: *D. H. LIM, J. LEDUE, M. H. MOHAJERANI, T. H. MURPHY
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Recently, we have developed a method of combining optogenetic cortical point stimulation with voltage sensitive dye (VSD) imaging in order to map functional relationships between cortical areas within the mouse brain (Lim et al., 2012). Here, we extend this method to a stroke model in order to map altered functional relationships after injury. B6.Cg-Tg (Thy1-COP4/EYFP)18Gfng/J transgenic mice, expressing channelrhodopsin-2 (ChR2) in subsets of deep layer pyramidal neurons, were given a targeted photothrombotic stroke to the primary sensory cortex and allowed to recover in their home cages. Eight weeks later, mice were given a large bilateral craniotomy and VSD (RH-1692) was applied to the cortex for imaging. Sensory stimulation of forelimb, hindlimb, whisker, and vision was used to map the primary sensory areas. These areas were then targeted for photostimulation, along with motor and associational areas that cannot be directly stimulated through peripheral activation. We found that the VSD response following sensory stimulation was delayed and prolonged in the injured hemisphere following stroke, as reported previously using VSD imaging in mice with a forelimb-targeted photothrombotic stroke (Brown et al., 2009). While sensory stimulation can be used to investigate connectivity in sensory circuits, it cannot be used to directly investigate associational or motor areas which may also be affected by stroke. Here, because ChR2 stimulation can be targeted to any cortical area in the transgenic mouse, we will evaluate the large-scale dysfunction caused by stroke in regions near and distant from the lesion. We chose twenty cortical sites to investigate using ChR2 point stimulation coupled with large-scale VSD imaging. Changes in intra- and interhemispheric connections between the cortical sites, including homotopic connections, were examined to determine which areas were most affected after stroke. Sham controls showed relatively equal intra- and interhemispheric connection strength in sensory, motor, and associational areas, while stroke animals showed decreased intracortical connection strength in the sensory and motor areas in the injured hemisphere, but increased intercortical connection strength in the motor areas of the injured hemisphere. The evaluation of the large-scale effects of stroke will be important for our understanding of the functional consequences of stroke, and may lead to better treatment strategies.

Disclosures: D.H. Lim: None. **J. LeDue:** None. **M.H. Mohajerani:** None. **T.H. Murphy:** None.

Poster

884. Functional Studies

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Program#/Poster#:884.01/FF10

Topic: D.09. Tactile/Somatosensory

Support: Fulton Undergraduate Research Initiative

Title: Size discrimination in the absence of tactile feedback

Authors: *D. L. FREAR[1,2], C. K. OVERSTREET[2], E. H. MOUGHARBEL[2], S. I. HELMS TILLERY[2]
[1]Phoenix, AZ; [2]Sch. of Biol. and Hlth. Systems Engin., Arizona State Univ., Tempe, AZ

Abstract: Qualities like size, shape, texture, and compliance allow human hands to discriminate between objects in the environment. Tactile, proprioceptive, and visual feedback contribute to these judgments and allow skilled interaction with objects. We designed a series of experiments to identify the contribution of tactile sensation to performance in discrimination of small size differences. Subjects were instructed to grasp a set of seven boxes (ranging in width from 44 to 56 mm) within a virtual reality environment. The positions of their index finger and thumb were tracked by a motion capture system. After the subjects were acclimated to the virtual environment, visual feedback of the locations of the box and their fingers was removed. Instead, binary visual or auditory signals corresponding to the thumb or index finger were presented when the subject's fingers contacted the virtual object. The subject was then asked to identify if the current box was smaller, larger, or the same size as the previous object. Similar size discrimination experiments using physical objects, where tactile information was available, were also performed. Subjects correctly identified the size difference between objects more often during visual feedback than auditory feedback trials; discrimination was most accurate in the physical object condition where tactile feedback was available. Subjects correctly identified large size differences most often. In the visual feedback task, subjects responded correctly in approximately two-thirds of the trials with objects of a size difference -6mm and below and 10mm and above. In the auditory task, subjects correctly discriminated at this level only for the largest size difference (12mm). Although accuracy was low for some experimental conditions, subjects always discriminated at above chance levels. Subjects were more likely to indicate that objects were the same size during experimental conditions that lacked tactile feedback. In

follow-up experiments, we are examining size discrimination performance when artificial tactile feedback cues are presented.

Disclosures: D.L. Frear: None. **C.K. Overstreet:** None. **E.H. Mougharbel:** None. **S.I. Helms Tillery:** None.

Poster

884. Functional Studies

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Topic: D.09. Tactile/Somatosensory

Support: NIH grant 5R01NS072171

Title: Discrepancy between neuronal activity and its associated hemodynamic response

Authors: *N. LI[1], P. VAN ZIJL[1], N. THAKOR[2], G. PELLED[1]
[1]F. M. Kirby Res. Ctr. for Functional Brain Imaging, Radiology, Kennedy Krieger Institute, Johns Hopkins University, Sch. of Med., Baltimore, MD; [2]Biomed. Engin., Johns Hopkins University, Sch. of Med., Baltimore, MD

Abstract: Improvements in MRI hardware are now being translated into detailed investigations of neuronal mechanisms associated with plasticity of cortical networks in human and animal models. Similar to other hemodynamics-based functional imaging techniques, the blood oxygenation level dependent (BOLD) fMRI signal is an indirect measurement of neuronal activity. Determining the degree of plasticity that occurs within and along cortical representations boundaries relies on the assumptions that the hemodynamic responses are co-localized and tightly correlate to neuronal activity. However, the exact relationship between the neuronal responses and hemodynamic responses remain unclear and under debates. The focus of our work was to investigate whether BOLD fMRI signal can be used to deduce about the underlying neuronal activity, and to what degree it co-localizes with neuronal activity. Combining optogenetic tools with electrophysiology, optical imaging of cerebral blood flow (CBF), and high-resolution BOLD fMRI, we sought to determine the correlation between the hemodynamic responses and neuronal activity. By localized excitatory neuronal activation of channelrhodopsin-2 (ChR2) stimulation in the rat primary somatosensory cortex (S1) we induced neuronal responses that were limited to the light stimulation S1 region (<500 μm). The neuronal activities in response to ChR2 stimulation differed in their oscillation frequencies, their cortical laminar profiles and spatial activation patterns across S1 representation compared to conventional forepaw stimulation. However, the cortical laminar profiles and the spatial extents

of the hemodynamic responses measured by CBF and BOLD fMRI induced by ChR2 and forepaw stimulation were indistinguishable. Both stimulations resulted in hemodynamic responses that were greater than 3 mm and only partially co-localized with the electrophysiology measured neuronal responses.
Thus, our results confirm the fundamental discrepancy between neuronal and hemodynamic responses, and suggest that resolving changes in cortical representation boundaries associated with plasticity may benefit from combining hemodynamic based measurements with neuronal stimulation via electrodes and optogenetics.

Disclosures: N. Li: None. **P. van Zijl:** None. **N. Thakor:** None. **G. Pelled:** None.

Poster

884. Functional Studies

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Topic: D.09. Tactile/Somatosensory

Support: BSF grant 2007121

ISF grant 749/10

Title: Sniffing - whisking coordination in freely exploring mice

Authors: E. FONIO, G. GORDON, I. SARAF-SINIK, *E. AHISSAR
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Abstract: Smell and touch convey most of the information that nocturnal rodents collect in their natural environments. Being active senses, a critical factor determining the integration of their sensations relates to the degree of their coordination. While it has been known for nearly fifty years that sniffing and whisking can be coordinated, the dynamics of such coordination and its dependency on behavioral and environmental conditions are not known. Here we used high resolution video recordings and novel image-processing methods, allowing for the first time non-invasive and fully automatic acquisition of two sensing modalities (smell and touch) simultaneously. Alleviating the animals from external constraints enabled us to inspect freely ranging groups of mice in a natural like environment.
We analyzed the use of whisking and sniffing independently, their co-interactions and their relation to head movements in mice during free exploration. Data were recorded during emergence epochs of mice from their home cage to an exposed area. Active sensing parameters of each modality showed significant dependency on head velocity and on the direction of

locomotion (outbound or inbound movements with respect to the home cage). Correlations between sniffing and whisking were lower than those between left and right whiskers ($p < 0.001$), and were significantly larger during outbound exploration compared with inbound ($p < 0.01$). Both bilateral whisking correlations and sniffing – whisking correlations increased with whisking and sniffing power ($r > 0.9$, $p < 0.001$).
Overall, these data indicate that sniffing-whisking coordination is a dynamic process, possibly mediated by sets of coupled oscillators and likely to be controlled by high-level inter-modal motor-sensory loops.

Disclosures: E. Fonio: None. **G. Gordon:** None. **I. Saraf-Sinik:** None. **E. Ahissar:** None.

Poster

884. Functional Studies

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Topic: D.09. Tactile/Somatosensory

Support: EC Marie Curie Fellowship PITN-GA-2010-256301 (TRACKDEV).

Title: Somatotopic and motor mapping of speech articulators in human cortex

Authors: *D. CAREY[1], S. KRISHNAN[2], F. DICK[3], M. I. SERENO[3]
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Abstract: The articulation of speech sounds requires precise control of and coordination between different parts of the upper vocal tract, such as the lips, tongue, soft palate, jaw, and pharynx. Talkers are also exquisitely adaptable to perturbations of the position or movement of the articulators (such as the presence of food or other obstructions), suggesting a high degree of integration across somatosensory and motor representations. Due in part to the difficulty in stimulating inside the mouth, and in isolating movement to discrete articulators, there are few detailed fMRI maps of cortical representations of the vocal apparatus. Moreover, it is unclear how such maps mesh with those of more abstract speech representations such as place of articulation. Here, we used phase-encoded fMRI to map motor and somatosensory representations of the relative position of articulators by having phonetically-trained participants produce trains of individual voiceless stops (/pə/, /tə/, /ʈə/, /cə/, /kə/, /ʔə/) that varied systematically from front to back place of articulation and then sulcus-based spherical surface-

averaging to combine complex-valued mapping data across subjects. Across participants, there was a consistent graded map of place of articulation generally spanning the central sulcus and primary motor and somatosensory areas, and moving from superior to inferior as place of articulation changed from front to back. Production of bilabials preferentially activated somatosensory regions slightly more than halfway along the medial to lateral length of the central sulcus, with activation related to alveolar, palatal, and retroflex stops moving more inferiorly, and tending to occur in curved bands across primary somatosensory areas, primary motor areas, and secondary somatosensory areas. Stops produced at back places of articulation (velar and glottal) activated the inferior aspect of the central sulcus near the subcentral gyrus but less robustly and more variably across participants. Control block design experiments supported the mapping of front to back place of articulation to parallel sequences of medial to lateral positions in S-I and M-I. To our chagrin, there was little evidence for articulation-specific mapping in inferior or middle frontal gyri.

Disclosures: D. Carey: None. **S. Krishnan:** None. **F. Dick:** None. **M.I. Sereno:** None.

Poster

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Topic: D.09. Tactile/Somatosensory

Support: FP7 BIOTACT ICT-215910

FP7 EFAA ICT-270490

Title: Whisker movements may reveal spatial attention: A computational model of active sensing control in the rat

Authors: *T. J. PRESCOTT[1], B. MITCHINSON[2]
[1]Psychology, Univ. Sheffield, Sheffield, United Kingdom; [2]Psychology, Univ. of Sheffield, Sheffield, United Kingdom

Abstract: Much effort continues to be focused on understanding signal processing in the rodent whisker sensory system. In contrast, the control of whisker movement has been the subject of relatively limited investigation. However, an understanding of whisker movement is critical to understanding the sensory signals present in the behaving rat, further, whisker control may also provide a new window into the internal state of the animal.
Several distinct observations of relationships between whisker movement and other variables

have now been reported that indicate the influence of the environment, or the animal's intentions, on whisking behavior. These include (i) that whisker protraction angles are adjusted bilaterally toward the direction of a future head movement (head-turning asymmetry); (ii) that protraction angles are adjusted to be reduced/increased on the side of the animal near to/away from a nearby object (contact-induced asymmetry); (iii) that the angular spread between the whiskers is reduced during surface contacts (spread reduction); (iv) that there is an increase in protraction angle towards the location of an anticipated contact; and (v) that animals engaged in rapid goal-directed locomotion often employ strong protraction. We are motivated to identify a model that can account for all of the above through a single mechanism. One clue, is the observation that unilateral contact often elicits head-turning towards the contact point suggesting that contact triggers attention, which, in turn, typically elicits orienting. We hypothesize, accordingly, that an important hidden variable driving the modulation of whisker movement is the locus of spatial attention--that is, the region of the local environment to which the animal's attention is currently directed, directs both orienting head movements and appropriate regulation of the movement of the vibrissae.

Here we report on a concrete model that confirms this expectation, showing that the model also expresses head-turning asymmetry, contact-induced asymmetry and spread reduction. We further argue that both reaching towards anticipated contact and strong protraction during locomotion, would also be expressed by the model were appropriate inputs available. We conclude that this attentional model can account, in principle, for all major observations to date of the modulation of periodic whisker movement, suggesting that whisker movements could also be used as an experimental tool to reveal the locus of attention of a whiskered animal. The current model is abstract in form, however, we propose that the superior colliculus (SC) is a candidate neural substrate for the implementation of such a mechanism.

Disclosures: T.J. Prescott: None. **B. Mitchinson:** None.

Poster

884. Functional Studies

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Topic: D.09. Tactile/Somatosensory

Support: BIOTACT: R121221111

Title: The relationship between locomotion and whisker touch sensing behaviours in the adult rat is mediated by familiarity with the environment

Authors: *K. P. ARKLEY, R. A. GRANT, B. MITCHINSON, T. J. PRESCOTT
Univ. of Sheffield, Sheffield, United Kingdom

Abstract: As rats are nocturnal, they cannot depend on vision to navigate through dark burrows. Instead, they rely heavily on their sense of touch to explore and interpret their environment, predominantly using their whiskers. Research has implicated that whiskers are used to perform behaviours requiring precise and coordinated movement such as climbing (King et al., J Zool, 285, 172-179, 2001), swimming (Ahl, Vet Res Commun, 10, 245-268, 1986), food acquisition and prey capture (Favaro et al., Neuroscience, 176, 318-327, 2011), but a detailed description of the behaviour (performed by whiskers, head and body) has been ignored. Furthermore, whisking and locomotion behaviour is well correlated over the course of development (Grant et al., Dev Psychobiol, 54, 151-168, 2012), leading us to hypothesise that whisking and locomotion are closely coupled in the adult rat.

We used high-speed videography to record the whisking and locomotion behaviour of food-deprived rats trained to run a circuit for food reward. The experimental arena was manipulated to either disrupt whisking during locomotion (by inclusion of obstacles), or disrupt continual locomotion (by means of a holey floor); a smooth floor with no obstacles was used as a control condition. We filmed the animals moving along the central trackway of our arena using a Photron high-speed camera (500fps) and used a front-silvered mirror to obtain both side-on and overhead views. Beneath the glass floor, illumination from a custom-built light box ensured a clear silhouette of the whiskers in both views, and a second camera provided a good view of foot placements from our pedobarograph. Recording of several seconds duration were made on each training day, automated whisker tracking and Fourier analysis were then used to extract locomotion speed, head lift, and whisking amplitude and set-point. The cross correlation between whisker and front feet positioning was examined, as well as the relationship between locomotion and whisking variables as mediated by familiarity with the environment.

Our findings indicate that increasing familiarity with the environment is correlated with increasing locomotion speed and head lift, and non-exploratory (static) whisker positions that serve as a ‘look-ahead’ object-detector (i.e. lower amplitude, more protracted). However, when rats previously trained in the control environment are introduced to unexpected obstacles, high running speeds are also accompanied with more exploratory whisking (higher amplitude, less protracted). This suggests that locomotion and whisker movements can be controlled independently of one another to produce a more flexible behavioural repertoire.

Disclosures: K.P. Arkley: None. **R.A. Grant:** None. **B. Mitchinson:** None. **T.J. Prescott:** None.

Poster

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Title: Induction of vasodilation predicts increased sensory-evoked firing

Authors: *D. L. VIERLING-CLAASSEN, C. I. MOORE
Neurosci., Brown Univ., Providence, RI

Abstract: We analyzed the impact of induced vasodilation on sensory evoked responses in single units recorded in anesthetized (ketamine/xylazine) mouse barrel cortex. Electrophysiological data were obtained using Neuronexus tetrodes and hemodynamics tracked using Laser Doppler (Perimed) measurements of blood flow. Analyses were conducted on 9 experiments (13 units) in which dilation was successfully induced by Pinacidil application, a pharmacological approach previously shown to selectively target vascular smooth muscle in the neocortex. These data were compared to 7 experiments (18 units) in which identical procedures were followed but drug was not administered.

Following induction of vasodilation and increased blood flow rate, a majority of neurons showed increased sensory evoked firing. This finding generally replicates prior observations from rat barrel cortex obtained under thiopental anesthesia (Cardin et al., 2008 *SfN* Abstracts). This increase in evoked firing showed two apparently distinct epochs, one proximal to the initial dilation event, and one several minutes later. For the initial epoch, the relative amount of increased evoked firing rate was positively correlated with change in blood flow. Firing increases were typically not observed in units in the background firing measured in the inter-stimulus interval between sensory stimuli (5 second ISI). In aggregate, these findings imply increased signal to noise ratio for the relay of sensory information induced by vasodilation and/or blood flow changes.

We also observed a strong correlation between blood flow and the change in mean firing rate in the absence of stimulation 15 minutes post-dilation induction. Analysis of control runs showed no systematic increase over time in sensory evoked firing rate but did show positive correlations between spontaneous changes in blood flow and sensory evoked rate.

Disclosures: D.L. Vierling-Claassen: None. **C.I. Moore:** None.

Poster

884. Functional Studies

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:884.08/FF17

Topic: D.09. Tactile/Somatosensory

Support: Grant from Toyoresin Co.

Title: Gentle cutaneous stimulation inhibits noxious heat-induced autonomic responses in conscious humans independently of perception and texture discrimination

Authors: *H. HOTTA[1], N. WATANABE[1], S. MIYAZAKI[2], Y. MUKAINO[3]
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Abstract: Background and purpose.
Pain can be reduced by touching the skin near the painful site. However, the mechanism has not yet been fully clarified. Recently, we found that gentle mechanical cutaneous stimulation can inhibit the somato-cardiac sympathetic C-reflex elicited by stimulating unmyelinated C afferent fibers of the plantar nerve in anesthetized rats. The effect was dependent on the texture of touching object. Using the two different textures, the present study examined whether touch affects autonomic responses and subjective pain intensity induced by noxious stimulation in humans, independently of perception and texture discrimination.
Methods.
The present study was designed as a cross-over, double-blind, randomized trial using two different types of skin stimulation tools of 11-mm diameter - an elastomer brush (with 417 microcones) and a flat elastomer disc. Twenty healthy adult males (24.4 ± 6.3 years old) participated in the study. The experiment consisted of two 40-minute sessions. While heart rate (HR) and amplitude of digital pulse wave were measured, heat stimulation (46 degree Celsius for 45 sec) was delivered by a Peltier thermode. Subjective pain intensity was measured with a 0-10 visual analogue scale (VAS). A cutaneous stimulation tool was applied, over the right medial malleolus with an adhesive plaster, for a period of 10 minutes. We asked the participants whether they perceived differences in the two stimulation tools, and confirmed that the difference in the texture had not been discriminated.
Results.
Heat stimulation induced pain (4.3 to 9.7 on the 0-10 VAS scale), and changed HR (by 1 to 22 bpm) and pulse wave amplitude (by 0.0023 to 0.33 V). Although VAS score was not significantly affected, magnitudes of autonomic responses were reduced by the touch with the microcone. The heat-induced HR response was significantly inhibited (by 22%) during the touch compared to the pre-touch level. Heat-induced changes in pulse wave amplitude were also significantly inhibited during the touch (by 27%), continuing after the touch was ended (by 28%). On the contrary, touch with the flat disc did not produce constant changes in neither heat-induced autonomic responses nor VAS scores.

Conclusion.
The present study demonstrated that skin touch can inhibit autonomic responses to noxious heat, depending on the texture of the object, but independently of texture discrimination. This result is consistent with our previous study on anesthetized animals. Therefore, we conclude that an inhibition of nociceptive transmission into autonomic responses by gentle mechanical cutaneous stimulation could occur independently of sensation and cognition.

Disclosures: H. Hotta: Research Grant; Toyoresin Co.. **N. Watanabe:** None. **S. Miyazaki:** None. **Y. Mukaino:** None.

Poster

884. Functional Studies

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:884.09/FF18

Topic: D.09. Tactile/Somatosensory

Title: Peripheral ultrasonic neurostimulation combined with fMRI and EEG for the mapping of somatotopic brain circuitry.

Authors: W. LEGON, A. ROWLANDS, A. OPITZ, T. SATO, *W. TYLER
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Abstract: A variety of peripheral neurostimulation procedures are used in the study and clinical evaluation of somatosensory nervous system function. The energy modalities employed by these methods span mechanical, thermal, electromagnetic, and photonic domains. Broadly implemented tools for evoking somatosensations include lasers, contact peltier probes, Von Frey hairs and other mechanical probes, electromagnetic coils, and piezoelectric elements. Less implemented, ultrasound (US) applied to the periphery has been described to be capable of evoking diverse somatosensations. However, these reports have been primarily subjective and have been lacking neurophysiological validation. To substantiate the use of US for somatosensory activation, we recorded evoked-potentials and BOLD response to both right and left US finger stimulation. Results indicate US to be capable of eliciting reliable evoked potentials including a large double-peaked vertex potential (N1/N2) peaking at 110 ms and 160 ms respectively. The same stimulation resulted in BOLD activation in areas involved in somatosensory discrimination including the primary somatosensory cortex (S1), parietal operculum (Op) as well as brain
regions involved in hierarchical somatosensory processing such as supramarginal gyrus (SMg) and areas commonly activated in response to salient and noxious stimulation including anterior

middle cingulate cortex (aMCC) and insula. We also demonstrate the ability to elicit an increase in subjective report of noxious and thermal sensation concomitant with
a change in evoked potentials and BOLD activations as a result of changing ultrasound stimulus parameters. Based upon these observations, we conclude focused pulsed ultrasound can be useful for interrogating and mapping somatosensory brain circuits, which may permit new paths for the study, diagnosis, and treatment of peripheral nerve injury, dysfunction, and disease.

Disclosures: W. Legon: None. **A. Rowlands:** None. **A. Opitz:** None. **T. Sato:** Ownership Interest; Neurotrek. **W. Tyler:** Ownership Interest; Neurotrek. Consultant/Advisory Board; Neurotrek.

Poster

884. Functional Studies

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:884.10/FF19

Topic: D.09. Tactile/Somatosensory

Support: Whitehall Foundation

Sloan Foundation

Title: Statistics of tactile exploration during multimodal sensory navigation

Authors: *T. CELIKEL[1], D. H. HERMAN[2]
[1]USC, LOS ANGELES, CA; [2]USC, Los Angeles, CA

Abstract: Navigation requires sensory information, and most sensory information during navigation arrives across modalities. To address the relative contribution of visual and tactile information in temporal statistics of navigation, we trained adult mice to locate a target platform on the gap-crossing task while illumination of the environment was randomly varied between white-light (i.e. light) and infrared-light (i.e. dark=no visible light) conditions across sessions. Animal mobility on the task and the time animals spent locating the target while positioned at the gap were quantified using motion sensors. The results showed that illumination condition does not contribute to overall likelihood of successful object localization, maximum gap-distance at which mice can locate the target of interest, duration of sensory exploration at the gap or the trial duration which suggest that information that arrive from visual and tactile modalities are highly redundant in determining the location of the target, and likelihood for mice to locate the object. The only independent variable that significantly varied across light and dark conditions was the amount of time that mice spent exploring the gap when they failed to cross onto the target

platform which is probably due to preferential exploration of visual information other than the target platform at the gap. These results argue that tactile modality is the predominant modality mice use to locate objects on the gap-crossing task independent from whether or not there is simultaneous visual information.

Disclosures: T. Celikel: None. **D.H. Herman:** None.

Poster

884. Functional Studies

Location: Hall F-J

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Program#/Poster#:884.11/FF20

Topic: D.09. Tactile/Somatosensory

Support: CIHR

Craig Nielsen foundation

Rick Hansen Institute

Title: Spinal cord and brainstem response to stepwise and block design thermal stimulation paradigms: An fMRI study

Authors: *R. BOSMA[1], C. DOBEK[2], M. BEYNON[2], P. STROMAN[2]
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Abstract: Spinal cord functional magnetic resonance imaging (fMRI) can be used to produce maps of neural function and therefore enables non-invasive visualization of spinal cord responses to peripheral thermal sensory stimuli. Given the limited temporal resolution of this technique, long stimulation paradigms are required to obtain enough data to accurately characterize the spinal cord response. However, repeated stimulation of unmyelinated C-polymodal fibers and thermoreceptors results in response depression, and these receptors quickly acclimate to an adaptation temperature. Therefore, careful selection of the stimulus paradigm is required to maximize the spinal cord response. The purpose of this study is to evaluate the characteristics of the spinal cord and brainstem response to peripheral stimulation in which temperature is raised in a stepwise fashion, in order to enhance receptor responses, compared to the conventional block design.
Functional MRI studies of the spinal cord and brainstem were carried out in healthy volunteers in a 3T Siemens Magnetom Trio. An MR-compatible Peltier thermode (Medoc) was placed on the right hand of participants and provided thermal stimuli during the experiments. Two sensory

stimulation paradigms were employed; a stepwise design and a block design. In the stepwise condition the temperature was raised from 32 ^{0}C (baseline) to 37 ^{0}C, held for 13 seconds and raised to 42 ^{0}C, held for 14 seconds and raised a final time to 45 ^{0}C for 20 seconds. In the block design condition the temperature was raised from baseline to 45 ^{0}C and sustained at that temperature for 47 seconds. The ramp rate was 2 ^{0}C/second for all conditions.
Spinal cord data were analyzed using custom software (written in MatLab). A time series analysis was carried out by means of a general linear model (based on SPM) to identify areas responding to the thermal stimulation. Connectivity analyses were then conducted to identify the sensory network and determine interregional spinal cord and brainstem activity correlations during thermal stimulation.
Robust ipsilateral dorsal horn activity was evident in the stepwise condition, while weaker contralateral dorsal and ventral activity was more prominent in the block design. Stepwise results also revealed consistent activity in regions of the rostral medulla known to be involved in sensory processing and in a region in close proximity to the periaqueductal grey region in the midbrain.
Results from this study lay the foundation for ongoing studies to assess the changes that occur at each of these relay points as a result of trauma and may elucidate neurological changes which affect functional outcomes.

Disclosures: R. Bosma: None. **C. Dobek:** None. **M. Beynon:** None. **P. Stroman:** None.

Poster

884. Functional Studies

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Program#/Poster#:884.12/GG1

Topic: D.09. Tactile/Somatosensory

Support: BSF grant 2007121

ISF grant 749/10

Title: Motor-Sensory strategies of object localization in rats

Authors: *E. ASSA, I. SARAF - SINIK, E. AHISSAR
Weizmann Inst., Rehovot, Israel

Abstract: Perception is the process of gaining information about the outside world. The dynamical nature of perception dictates a mode of operation in which motion of the sensory organ and sensation are mutually dependent. Motor-sensory interactions should probably be

optimized to the perceived features. A motor-sensory strategy is defined here as the motor plan selected by the animal during a specific perceptual task.
Here, we seek to identify the motor-sensory strategy underlying object detection and localization in rats in terms of “invariant” and “modulated” parameters. Both types are subjected to brain control, which maintains invariant parameters constant between task repetitions and under different environmental conditions by changing modulated variables. Next, we demonstrate how motor-sensory strategies exhibited by freely behaving rats facilitate acquisition of information during perception.
Rats were trained to report the presence (‘detection’) and locations (‘localization’) of two vertical poles, bilaterally positioned in their path. The report was based on a three-alternatives-forced-choice scheme, in which the location of the rewarding drinking tube (‘sipper’) was associated with the presence and relative location of the two poles. The task was repeated with and without wind perturbation. Head and whiskers were tracked with high-speed video and analyzed off-line.
Motion parameters of the head and whiskers were significantly modulated along a trial, in a stereotypic manner, such that task progress could be divided into five sequential behavioral phases: (*i*) detection (*ii*) early and (*iii*) late localization (*iv*) sipper approach and (*v*) sipper localization. Analysis of the localization phases (ii and iii) indicate that relative object location is first encoded by bilateral temporal difference of contacts and later by whisker angular difference. The motor-sensory strategy displayed by the rats supported this coding scheme by keeping whisking velocity and head angle relatively invariant during localization also under wind perturbations while modulating whisker set-points and radial distance of whisker-object contact. These observations emphasize the active nature of perception and suggest that motor-sensory strategies are inherent components of perception.

Disclosures: E. Assa: None. **I. Saraf - Sinik:** None. **E. Ahissar:** None.

Poster

884. Functional Studies

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:884.13/GG2

Topic: D.09. Tactile/Somatosensory

Support: NWO 453-10-003

Title: Ownership and disownership of hands in a patient recovering from somatoparaphrenia

Authors: *C. DIJKERMAN[1,2], H. E. VAN STRALEN[1,2], L. J. KAPPELLE[2], M. J. E. VAN ZANDVOORT[1,2]
[1]Helmholtz Inst., Utrecht Univ., Utrecht, Netherlands; [2]Dept. of Neurol. and Neurosurg., UMC Utrecht, Utrecht, Netherlands

Abstract: It may appear self-evident that we have ownership over our own body. Nevertheless, ownership of (part of) our body can be disrupted after cerebral damage, as for example is the case in somatoparaphrenia. In this disorder, patients attribute ownership of their contralesional limb to another person. So far, most studies of patients have been descriptive, depending on a verbal report of their experience. In the current study we aimed to experimentally manipulate body ownership in a patient recovering from somatoparaphrenia using the rubber hand illusion (RHI). In this illusion synchronous visuo-tactile stimulation results in the subjective experience of a rubber hand being part of the body, and reduced ownership over their own hand. This can be measured through questions about the disowned hand, a proprioceptive displacement of the perceived position of the hand towards the rubber hand or the feeling of a lower temperature in the disowned hand.
We assessed a 78-year-old female, who suffered a right-hemisphere stroke about two weeks prior to the testing. Shortly after the stroke, she did complain that she experienced her left hand no longer belonging to her body, but felt as if her arm belonged to her father in law. This feeling was subsided at the time of testing. Clinical assessment showed limited contralesional sensorimotor impairments and no cognitive deficits.
The RHI was induced for her left disowned hand as well as for her unimpaired right hand through synchronous stroking of her own hand and a rubber hand. Asynchronous stroking was used as control condition. All measures of the RHI, questions, proprioceptive displacement as well as skin temperature, indicated that she experienced an enhanced RHI with her left compared to her right hand. Moreover, there was no difference in the strength of the RHI between the synchronous and asynchronous stroking for the left hand. This suggests that ownership over the left hand was more easily disrupted than over the normal right hand and that visual presentation of a left rubber hand was sufficient to induce this disruption.

Disclosures: C. Dijkerman: None. **H.E. van Stralen:** None. **L.J. Kappelle:** None. **M.J.E. van Zandvoort:** None.

Poster

884. Functional Studies

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Program#/Poster#:884.14/GG3

Topic: D.09. Tactile/Somatosensory

Support: BSF grant 2007121

ISF grant 749/10

Title: Emergent hierarchical control via novelty management of multiple motor-sensory loops

Authors: *G. GORDON[1], E. FONIO[2], E. AHISSAR[2]
[2]Neurobio., [1]Weizmann Inst. of Sci., Rehovot, Israel

Abstract: When encountering novel environments animals perform complex yet structured exploratory behaviors composed of elementary and repeatable motor primitives. We present a hierarchical model that manifests a delicate balance between learned novelty-seeking and novelty-avoidance primitives.
Exploration motor primitives are learned via hierarchical curiosity loops, in which each loop is a manifestation of intrinsic-reward reinforcement learning. Each loop converges to a behavior that optimizes the learning process of a specific feature of the environment. The transition between primitives is governed by novelty management principles, which dictate that novelty should be pursued; yet when too much novelty is accumulated, the agent retreats to a ``base state".
We implement the model in two scenarios: the rodent whisking system and mice exploration of novel arenas. In the former, we model two loops, the first learning whisker self-motion and the second seeks to localize objects in the environment. We show that periodic whisking and object palpation emerge as the converged behaviors of these loops.
The structured increase in extent and complexity of mice exploration of novel arenas is also captured by the same model. The hierarchical curiosity loops account for the increased dimensionality of behavior, whereas the novelty management principle accounts for the gradual increase in scope and the recurrent retreats to the home base. Here we model locomotion behavior by three loops, which converge to circling in corners, wall attraction and open-space exploration, respectively.
This work shows that the same set of novelty management principles can explain the exploratory behavior in two different modalities: whisking and locomotion. We propose that these principles may be common to exploratory behavior in general.

Disclosures: G. Gordon: None. **E. Fonio:** None. **E. Ahissar:** None.

Poster

884. Functional Studies

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Program#/Poster#:884.15/GG4

Topic: D.09. Tactile/Somatosensory

Support: Whitehall Foundation

Sloan Foundation

Title: Sensorimotor computation for adaptive whisking during multimodal sensory navigation

Authors: *D. H. HERMAN[1], K. WALLIN[2], T. CELIKEL[2]
[1]Neurosci, USC, LOS ANGELES, CA; [2]Neurosci., USC, los angeles, CA

Abstract: Previously we have shown that mice perform close-loop sensorimotor computation to locate objects of interests in their immediate environment. When animals were allowed to explore their environment using their whiskers in darkness, whisking amplitude was influenced by the recent history of tactile information collected during navigation. In natural environments however, most sensory information arrives across multiple sensory modalities, driving animal to integrate multimodal sensory information. In this study, we built on our previous observations on the rules of sensorimotor computation during adaptive whisking and quantify the role of visual information on tactile exploration during object localization while mice collect visuotactile information about their environment by randomly varying the illumination between (white) light and dark across sessions on the gap-crossing task. Our results show that, independent of the illumination, mice continue the adaptive whisking strategy where the protraction setpoint is predictive of the relative distance to the target. In contrast, in the dark, the whisking protraction and retraction setpoints are positioned rostrally as compared to whisking in the light, and that the magnitude of this difference is monotonically related to both the nose distance to the target and the current gap-distance. Although there were no differences in whisking frequency, bilateral whisking symmetry, and head-whisker motion across the illumination conditions, there was significantly greater variability in whisking amplitude in dark which decreased as the animal approached to the target. These results argue that visual information during multimodal visuotactile exploration contributes to development of search strategies (i.e. where to whisk) although motor control of whisker position relies on the tactile information.

Disclosures: D.H. Herman: None. **K. Wallin:** None. **T. Celikel:** None.

Poster

884. Functional Studies

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Topic: D.09. Tactile/Somatosensory

Support: Prodex

European Space Agency

Interuniversity Attraction Poles (Belgium)

FNRS (BELGIUM)

FRSM (BELGIUM)

ARC (BELGIUM)

NanoBioTouch (Commission of the European Union)

Title: Fingertip deformation during the onset of a tangential sliding movement.

Authors: *B. DELHAYE[1,2], P. LEFÈVRE[1,2], J.-L. THONNARD[1]
[1]Inst. of Neurosci. (IoNS), Univ. catholique de Louvain, Brussels, Belgium; [2]Inst. of Information and Communication Technologies, Electronics and Applied Mathematics(ICTEAM), Univ. catholique de Louvain, Louvain-la-Neuve, Belgium

Abstract: In daily life, we manipulate objects that have different physical properties (mass, surface, shape). Most of the time, we succeed in this task and prevent the objects dropping from the hand. This is explained by the fine coordination between the load force (LF), the tangential force due to the object weight and inertia, and the grip force (GF), the normal force applied to the objects to counteract the LF. This fine coordination is possible with the help of the cutaneous feedback from the fingertips mechanoreceptors. These respond to the stresses and strains created by the deformation of the skin during object grasping and manipulation. Measuring the fingertip skin deformation before and during slipping may thus contribute to better understand how we can prevent the objects from slipping during their manipulation.
In this study, we used an imaging system to record the fingertip deformation during the onset of tangential sliding movements of the fingertips on a glass surface. The constraints were applied passively to the fingertip of subjects in different directions: front-rear translations, left-right translations and clockwise-counterclockwise rotations. A robotic system controlled the normal force applied to the fingertip and the tangential speed. The contact forces and torques were recorded. Using computer vision techniques, the contact area was extracted from the images and the relative velocity field between the glass and the fingertip contact area was computed during the whole sliding phase. An ellipse was fitted to the contact area during the whole slip in order to measure the displacement (for translations) and the tilt angle (for rotations) of the contact area. Results showed that the skin deforms in a complex way from a full stuck state to a full slipping contact. During this transition, while the tangential force was monotonically increasing, the skin progressively slipped on the plate, beginning from the border of the contact area and ending at the central zone, and forming a velocity gradient from the border to the center. This behavior was observed for both translational and rotational movements. This transition from a full stuck state

to a full slip was accompanied by a reduction of the contact area, which then stayed constant during full slip. The skin displacement, due to the elasticity of the finger skin, was measured. This displacement was significantly larger in lateral movements in comparison with front-rear movements. The direction of rotation did not affect the absolute value of the tilt angle significantly. The form of the decreasing stuck region was also critically dependent on the loading direction and the moisture of the fingertip.

Disclosures: B. Delhaye: None. **P. Lefèvre:** None. **J. Thonnard:** None.

Poster

884. Functional Studies

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Topic: D.09. Tactile/Somatosensory

Support: NIH RO1 DC003311

NIH P30 HD02528

The Sutherland Foundation

Title: Short-term adaptation in the human somatosensory cortical network due to repeated stimulation of the hand and face

Authors: *L. VENKATESAN[1], M. POPESCU[4], E. POPESCU[4], S. M. BARLOW[1,2,3]
[1]Neurosci., [2]Speech-Language-Hearing, [3]Human Biol., Univ. of Kansas, Lawrence, KS;
[4]Hoglund Brain Imaging Ctr., Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: OBJECTIVE: To use magnetoencephalography (MEG) to study the short-term adaptation response of the human somatosensory cortical network due to repeated stimulation of the hand and face using a custom-built pneumotactile stimulator called the TAC-CELL. METHODS: The TAC-CELL is a custom molded (O.D. = 15mm, I.D. = 6mm, H = 6mm) pneumatic actuator with a rise time (10-90%) of 8.5 ms. A custom non-commutated servo motor operating under position feedback and computer control was used to pneumatically charge the TAC-CELL. A whole-head 151-channel MEG scanner was used to record the cortical response to a pneumatic tactile stimulus that consisted of a repeating 6-pulse train (50-ms pulse width, intertrain interval = 5 s, 125 reps/train rate, train rates [2, & 4 Hz]). Nine healthy females (Mean age = 24.2 years [SD = 3.2]) with no history of neurological disorders participated in this study. A double-adhesive tape collar secured the TAC-CELL at two locations, including the 1) glabrous

surface of the right index finger, and 2) right non-glabrous surface of oral angle. Source reconstruction, dipole localization, estimation of dipole strength peak amplitudes, and latencies were performed using CURRY software.
RESULTS: Peak dipoles were localized to the left primary somatosensory cortex (SI) and posterior parietal cortex (PPC) for hand stimulation, and to the contralateral left SI for face stimulation. Dipole locations for both hand and face stimulation were consistent with the somatotopic organization in SI. The PPC component for hand stimulation was more medial (mean $\Delta x = 3$ mm, SD = 5 mm), more posterior (mean $\Delta y = -4$ mm, SD = 10 mm), and more superior (mean $\Delta z = 2$ mm, SD = 3 mm) when compared to SI. Peak dipole strength was significantly dependent on stimulation frequency ($p=.026$), cortical response component ($p<.0001$), and pulse-index number ($p<.0001$). Peak SI and PPC latency for hand stimulation was observed at 66± 6 and 96± 6 ms respectively. Peak SI latency for face stimulation was detected at 52 ± 6 ms.
CONCLUSIONS: TAC-CELL stimulation of either the face or hand with patterned pneumatic stimulus was highly effective in inducing short-term adaptation of somatosensory cortical network. The significant difference between the latencies of peak dipole strengths of hand and lip SI, and hand PPC is attributable to the difference in axon length and distance from the mechanosensory nerve terminals in the face and hand to their central targets in SI. A short delay in the PPC response reveals the hierarchy in the serial-processing mechanism that exists in the somatosensory cortical network. The lack of PPC response during face stimulation may be due to the absence of face representation in the PPC.

Disclosures: L. Venkatesan: None. **M. Popescu:** None. **E. Popescu:** None. **S.M. Barlow:** None.

Poster

884. Functional Studies

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Program#/Poster#:884.18/GG7

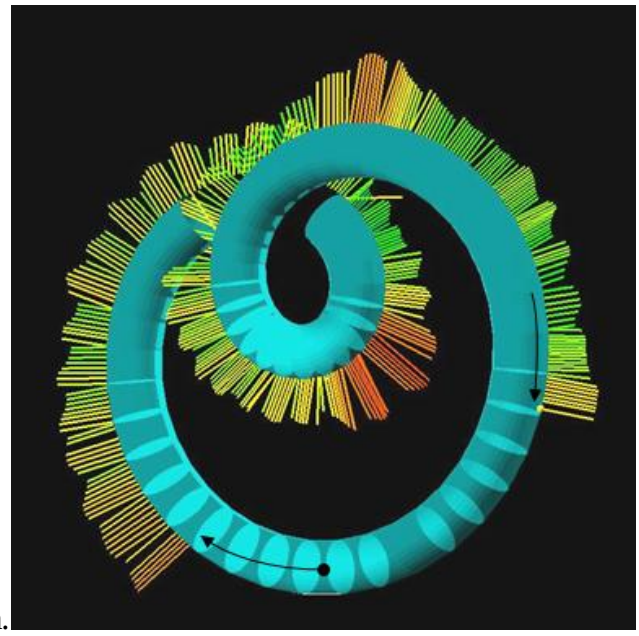
Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NS027484

Title: Somatosensory prediction in haptic tracing

Authors: *M. FLANDERS[1], J. TRAMPER[2]
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Abstract: When you run your index finger along an unfamiliar curved surface, with your eyes closed, haptic guidance mechanisms come into play. Tactile and propriocepive inputs from the hand and arm are used on-line to continuously control the movement, feedback corrections being combined with feedforward predictions. This study sought to determine what sort of feedforward predictions the somatosensory system is capable of. Human subjects interacted with a large-workspace 3D Phantom force-feedback robot. The distal phalanx of the right index finger was strapped into a small, light-weight sled and virtual surfaces were programmed at the fingertip. Tubular shapes were created in vertical planes: various elliptical hoops (40 cm long axis) with abruptly flattened regions, or tilted Limaçon shapes of a similar size, where the surface curvature varied continuously according to cosine functions (see Figure). At the beginning of each trial, the fingertip popped into the lumen of the tube (4 cm diameter) and then moved clockwise along the interior surface pressing outward (contact forces are color-coded for intensity in the Figure). In the ellipse experiment, subjects anticipated abruptly flattened regions very poorly, even after repeated laps around a given hoop, each of which was in the same frontal plane. In contrast, despite the more complex and 3D nature of the Limaçon experiment (with titled shapes in rotated vertical planes), finger speed smoothly varied in phase with the 2/3 power law prediction, and voluntary modulation of contact force also indicated prediction of upcoming curvature increases and decreases. The results suggest that, without vision, it is difficult to anticipate the spatial location of an abrupt somatosensory event (a corner), but in contrast, smooth changes in curvature can be anticipated using a feedforward predictive mechanism.



Disclosures: M. Flanders: None. **J. Tramper:** None.

Poster

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BK21 Project

KI Brand project 2012

Kolon Industries (the next generation haptic user interface project)

Title: A novel stimulation method based on a neuromorphic mechanoreceptor model for haptic illusion

Authors: K. GWAK, J.-C. PARK, *D.-S. KIM
Electrical Engin., KAIST, Daejeon, Korea, Republic of

Abstract: Tactile stimulation has gained increasing importance in several areas including human-computer interface to provide higher intuitiveness and enriched sensory stimulation for users. The principle aim of this study is to provide an illusory tactile sensation such as the shape or the texture of any objects to human. Haptic illusion generation system employing a developed vibrotactile stimulator, 6 by 4 pin-array type with 3mm nominal pitch, and a computational model of human distal finger pad is implemented to improve the veracity of tactile stimulation. The stimulator consists of 24 tiny ultrasonic linear motors operating periodically in the range of 0 to 100Hz within 200μm amplitude. Each motor is calibrated and computationally modeled using laser displacement sensor. Previously reported RA mechanoreceptor models are applied to simulate the human distal pad. The model employs integrate-and-fire neuron model with a specified refractory and hyperexcitability period. For a specified target haptic illusion such as an inclined bar, optimization algorithm with subgradient method derives the corresponding spatio-temporal stimulation patterns for 24 motors based on the stimulator and the distal pad model. Bar orientation discrimination experiments are conducted for human subjects using the developed illusion generation system. The results show that the stimulator can reliably present illusory bar with specified orientations. This preliminary result implicates the important possibility to generate haptic illusion. Not by scanning all possible combinations of stimulus through human

experiments, one can considerably shrink the range of combinations before the human experiments. Furthermore, hints for complicated haptic illusions, which are not able to be just anticipated by researcher, can be derived. Once various illusions are reliably generated, the method will be applied to numerous fields from haptic UI to prosthetic skin.

Disclosures: K. Gwak: None. **J. Park:** None. **D. Kim:** None.

Poster

884. Functional Studies

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:884.20/GG9

Topic: D.09. Tactile/Somatosensory

Support: BrainGain, SmartMix, Ministery of Economic affairs

Title: A methodology for recording and online analysis of electrocorticographical data from freely behaving rats under spatiotemporally controlled tactile stimulation.

Authors: *A. FRANSEN, E. MARIS, G. DIMITRIADIS
Dep. Bio- en NeuroPsychologie, Radboud Univ., Nijmegen, Netherlands

Abstract: We have developed a methodology for the recording of electrocorticographical (ECoG) data from freely behaving rats while controllably stimulating the corresponding somatosensory regions on the rats' snouts. These recordings cover a large part of the rat's brain and involve areas that, up to today, have been accessible only through wire recording experiments with limited precision. This setup allows for both online and offline analysis. Our methodology comprises of a surgery process, an experimental setup with live stimulation and online experiment control, and data analyses that make use of an in-house developed 3D rat brain model.
Our surgery protocol allows us to chronically implant a 32 channel flexible polyimide-based ECoG grid onto the lateral side of the rat's cortex, while leaving the jaw muscles intact. The grid covers motor, somatosensory and auditory regions. This setup provides high quality, stable recordings for more than 5 months.
The experimental setup comprises two parts. One is the use of a head-mounted tactile stimulator on the rat's upper lip. This stimulator is easy to manufacture (utilizing 3D printing and limited post processing) and gives controlled stimulation of the upper lip in a freely behaving rat. The second part of the setup is a software package, which expands on software for human Brain Computer Interface (BCI) experiments (i.c., BCI2000). This makes possible BCI experiments in small animals that involve online feedback about the animal's brain state.

Finally, our grid recordings need to be accurately co-registered with the brain surface over a large cortical area. For this purpose, we constructed a 3D model of the rat's brain using the LONI rat atlas with co-registration of functional areas taken from the Paxinos atlas.

Validation

To show that our data collection and head-mounted stimulator systems operate in unison, we have recorded data both from anesthetized and awake rats. To establish the validity of our method, we first conducted receptive field mapping experiments under anesthesia. These experiments convincingly show that event-related potentials (ERPs) discriminate between the different stimulation areas on the rat's upper lip.

Moreover, we have also conducted stimulation experiments in freely moving rats. Importantly, with identical tactile stimulation, there are important differences between the ERP maps obtained under anesthesia and during wakefulness. With respect to behavior, the rats can be trained to perform simple detection and identification tasks whilst carrying the stimulator. The system therefore allows for a full spectrum of behavioral experiments with controlled sensory stimulation.

Disclosures: A. Fransen: None. **E. Maris:** None. **G. Dimitriadis:** None.

Poster

884. Functional Studies

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:884.21/GG10

Topic: D.09. Tactile/Somatosensory

Support: NSERC

Title: Zinc transporter-3 knockout mice display decreased acuity in a novel whisker-dependent texture-discrimination task

Authors: *H.-P. WU[1,2], R. DYCK[2]
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Abstract: Zinc is intimately involved in the regulation of synaptic function in the mammalian brain. A subset of telencephalic neurons sequesters zinc in their synaptic vesicles where it is co-released with glutamate. At the synapse, zinc can interact with a variety of receptors and modulate neurotransmission. One prominent example is the modulation of NMDA receptor activity by zinc, which implicates zinc in the regulation of synaptic plasticity. We have demonstrated, in previous studies, robust experience-dependent alterations in the levels of

synaptic zinc within the somatosensory and visual cortices of mice. We established that sensory deprivation significantly increased the level of vesicular zinc in the respective sensory cortices, while stimulation decreased levels. In the present study, we investigated the role of zinc in somatosensation. Specifically, we measured texture discrimination by zinc transporter-3 knockout (ZnT3-ko) mice using their mystacial whiskers. ZnT3-ko mice do not have detectable levels of vesicular zinc within the brain. Vesicular zinc is found in high concentrations within limbic structures and is involved in learning and memory processes such that ZnT3-ko mice display age-dependent deficits in the Morris water maze as well as deficits in fear conditioning. Therefore, behavioural tasks that test the somatosensory ability of ZnT3-ko mice may be confounded by processes such as learning and memory. In order to minimize involvement of the hippocampal and amygdaloid systems in our present study, we developed a novel whisker-mediated texture discrimination task to test the sensitivity of the whisker-sensory system. We found that ZnT3-ko mice were significantly impaired in the texture discrimination task compared to wild-type controls. Wild-type mice were able to discriminate between rough and smooth textures separated by 25 μm in mean grit size while ZnT3-ko mice were not able to make the discrimination with a difference of less than 300 μm. We tested mice that were both 3 months of age and greater than 6 months of age, and found that age did not affect the level of impairment observed in ZnT3-ko mice. The results from our study suggest that vesicular zinc may be important in regulating the sensitivity with which the animal processes sensory information.

Disclosures: H. Wu: None. **R. Dyck:** None.

Poster

884. Functional Studies

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:884.22/GG11

Topic: D.09. Tactile/Somatosensory

Support: Whitehall foundation

Sloan foundation

Title: Circuit requirements for spatial touch localization

Authors: *C. HUANG, A. RESNIK, T. CELIKEL
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Abstract: Anatomical extraction of cortical columns (Hutson & Masterton, 1986) or targeted down-regulation of the electrical activity in the supragranular layers of individual cortical

columns in the primary somatosensory cortex (Laudenklos et al, 2005) impair haptic object localization on the gap-crossing task. However, the neural computation that is responsible for localization of touch is not known.
To address the minimal circuit requirements for successful spatial localization we intracellularly recorded sub- and suprathreshold activity from excitatory neurons in the supragranular layers (L2/3) of the barrel cortex, and show that individual L2/3 neurons' subthreshold responses contain as much information about stimulus location (i.e. which whisker is being deflected) as the stimulus entropy. When the action potential (AP) is generated there was a significant information loss (1.86±0.17 vs. 0.36±0.14 bit, $p<0.001$) which occured across all membrane states. To determine whether local computation in L2/3 can rescue the lost information, we developed a 2-layer network model where distribution and identity of neurons were determined after reconstructing the canonical cortical column in the somatosensory cortex in soma resolution (see Ye, Huang, Celikel poster). Simulations on this computational column showed that although APs from single L2/3 neurons are not information rich (0.28±0.15 bit), similar to observations in the biological column above, all the information about the stimulus can be successfully recovered by integrating responses from a pool of 8-20 local L2/3 neurons. Contribution of each neuron to the information recovery depends on the slope of their excitatory postsynaptic potential (EPSP) and their AP threshold, the membrane potential at which they generated an AP. Neurons whose AP thresholds are hyperpolarized carry significantly more information about the stimulus (0.33±0.16 vs 0.21±0.11 bit; -56 to -54 vs -54 to -52 mV respectively). Accordingly faster rise in EPSP slope is positively correlated with the information content of the neuron. These results suggest that subthreshold responses of single L2/3 neurons in the barrel cortex have complete information about the spatial localization of touch however, at the network level, a small but finite number of L2/3 neurons (<20) are required for computing object location. Interestingly contribution of individual neurons' to information recovery at the network level is not stochastic but depends on the rate with which they integrate the incoming synaptic information.

Disclosures: C. Huang: None. **A. Resnik:** None. **T. Celikel:** None.

Poster

885. Reflexes and Reflex Modulation I

Location: Hall F-J

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Program#/Poster#:885.01/GG12

Topic: D.12. Kinematics and EMG

Support: Det obelske Familiefond of Denmark

European Research Council (ERC) via the ERC Advanced Grant DEMOVE (No. 267888)

Title: Short latency contralateral inhibition identified in single motor units in human

Authors: *U. YAVUZ[1], F. NEGRO[1], S. S. GEERTSEN[2], J. B. NIELSEN[2], D. FARINA[1], N. MRACHACZ-KERSTING[3]
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Abstract: In static and dynamic tasks a short latency (37-41 ms) inhibition of the contralateral Soleus (cSOL) muscle occurs following ipsilateral tibial nerve (iTN) stimulation [3, 4]. To gain further insights into the pathways that induce this response we have recently investigated the convergence with the disynaptic inhibitory pathway evoked by common peroneal stimulation on the contralateral side (cCPN) [1]. For such studies it is necessary to determine the exact timing of the responses in relation to the stimuli. Surface electromyography (sEMG) has limitations in this regard as it relies upon the occurrence of action potentials at a particular time in relation to the stimulus and a strong response can synchronize their timing thus distorting their onset [2]. The aim of the current study was to use the peristimulus frequencygram (PSF), a technique which illustrates the time course of the net input current to a motor neuron more accurately compared with sEMG, to study the time-course of the postsynaptic potential in cSOL motor neurons induced by the two stimuli (iTN and cCPN stimulation). Three subjects (age: 26-40 years) were seated in a chair with their cSOL contracted to 5-10% maximum. Bipolar Teflon® insulated silver wires (75 µm in core diameter) were inserted into the cSOL muscle. sEMG was recorded with bipolar electrodes from the same muscle. Three conditions were investigated and the stimuli were applied randomly: 1) iTN at 2×motor threshold, 2) cCPN at motor threshold, and 3) iTN+cCPN stimulation. Signals were recorded at 10 kHz for offline PSF and trigger averaged sEMG analysing. The onset of inhibition measured from trigger averaged sEMG was 44 ± 1.7 ms in the iTN stimulation, 48 ± 2.0 ms for cCPN stimulation, and 41 ± 1.7 ms for the iTN+cCPN condition. Six motor units were decomposed from the intramuscular EMG signals. The PSF showed that the iTN stimulation induced short latency contralateral inhibition on all cSOL motor units with an average latency of 42 ± 7 ms. The amount of inhibition (27.7 ± 10.5%) in the iTN+cCPN condition was above that found for the summation of the inhibition response in iTN (12.9 ± 7.6%) and cCPN (13.8± 2.8%) conditions for all motor units. This is the first study to show a significant change in the firing rate of single motor units of the cSOL following iTN stimulation. It further supports our previous finding that it is at least in part mediated via Ia inhibitory interneurons in the disynaptic reciprocal inhibitory pathway.
1)Geertsen et al. 8th FENS forum, 2012
2)Miles T. S., Türker K. S., Exp Brain Res, 65: 331-6, 1987
3)Stubbs W. S., Mrachacz-Kersting N., J Neurophysiol, 102(6): 3596-605, 2009
4)Stubbs W. S. et al., J Neurophysiol, 105(2): 503-11, 2011

Disclosures: U. Yavuz: None. **F. Negro:** None. **S.S. Geertsen:** None. **J.B. Nielsen:** None. **D. Farina:** None. **N. Mrachacz-Kersting:** None.

Poster

885. Reflexes and Reflex Modulation I

Location: Hall F-J

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Program#/Poster#:885.02/GG13

Topic: D.12. Kinematics and EMG

Support: a Grant-in-Aid for Scientific Research (no. 24390431 to M. Inoue) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan

Title: Effect of chemosensory inputs into the pharynx on voluntary swallows in humans

Authors: *M. INOUE[1], Y. NAKAMURA[1], A. HATAKEYAMA[1], Y. KITADA[2]
[1]Div. of Dysphagia Rehabil., Niigata Univ., Niigata, Japan; [2]Morioka taste and swallowing research institute, Morioka, Japan

Abstract: Introduction: Ability to initiate repetitive voluntary swallowing varies even among healthy humans. In the previous study, we recorded voluntary swallows and showed that the number of swallows was increased with appropriate chemosensory inputs, e.g., activation of water receptors in the pharynx and larynx, which may compensate for difficulty in initiation of swallowing. The aim of this study was to investigate if the pattern of swallow-related electromyographic (EMG) burst in the suprahyoid muscles was also affected by the peripheral conditions. We hypothesized that the rising time (RT) of EMG burst was decreased with increasing the number of swallows in each subject.
Methods: Voluntary swallowing was recorded during infusion of fluid (distilled water [DW] or 0.3 M NaCl solution) into the pharynx at a very low rate in 18 healthy volunteers. DW and NaCl were used to activate and inhibit the water receptors. Swallowing intervals (SIs) were measured between the fourth and ninth swallows, In addition, using rectified suprahyoid EMG burst, the rising time (RT) defined as the time interval between the onset and peak and falling time (FT) defined as the time interval between the peak and offset were measured. Facilitatory effect (FE) for SI was calculated by subtraction of the SI of DW from that of NaCl. Similarly, FE for RT was also obtained. Means values of these variables were compared between DW and NaCl.
Results: SIs were significantly shorter during the infusion of DW than that of NaCl. RT was significantly shorter during the infusion of DW than that of NaCl solution while there was no significant difference in the FT between the conditions. There was a linear positive correlation between the conditions; the longer either the SI or RT with infusion of 0.3 M NaCl solution was,

the stronger the facilitation of swallowing by chemosensory
inputs was. While the percentage of facilitation of both the SI and RT exhibited great variation among the subjects, there was a linear correlation between them.
Conclusion: The present results suggest that not only SI but also RT represent individual potential to perform swallow.

Disclosures: M. Inoue: None. **Y. Nakamura:** None. **A. Hatakeyama:** None. **Y. Kitada:** None.

Poster

885. Reflexes and Reflex Modulation I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:885.03/GG14

Topic: D.12. Kinematics and EMG

Support: NIH Grant HD32571

Title: A quadrupedal postural controller with physiological sensor delays

Authors: *N. E. BUNDERSON[1], T. R. NICHOLS[2]
[2]Sch. of Applied Physiol., [1]Georgia Tech., Atlanta, GA

Abstract: Animals have a multi-layered control system which includes intrinsic viscoelasticity of muscle, and short and long latency neural feedback mechanisms. To what extent each of these contributes to postural stability is a matter of intense debate. We have developed a postural controller which takes into account actual biological complexities such as physiological time delays and redundant joints and actuators. By including the complexities we demonstrate a role for the multi-layered control observed in animals which is not evident in simplified models. We propose that the postural task can be divided into local (joint and limb) and global tasks and that control of these tasks is divided among biological layers of control.
The musculoskeletal model is comprised of a 6 degree of freedom trunk segment and four limbs. Each limb has 6 kinematic degrees of freedom and 31 muscles. The total force in each muscle is the sum of undelayed intrinsic forces, reflexive (20ms delay) forces, and control (60ms delay) forces. The intrinsic forces are modeled with a parallel spring and damper acting on the instantaneous change in muscle length and velocity. Reflexive forces are modeled with a similar parallel spring and damper acting on the delayed length and velocity values.
The control forces are the sum of constant background forces and delayed control forces. The delayed muscle control forces are calculated to minimize ground reaction force magnitudes and generalized coordinate accelerations of an internal model. The internal model is constructed from

delayed state information. The delayed muscle control forces must also result in a net force and torque acting on the internal model center of mass which is equivalent to that which would be produced by a virtual parallel spring damper attached to the internal model center of mass. The controller robustly stabilizes a model of a quadruped against postural perturbations. The model cannot stabilize against perturbations when any of the layers are removed and the mode of failure is different depending on which layer is removed suggesting different roles for each



layer.

Disclosures: **N.E. Bunderson:** Ownership Interest; Biomechanico. **T.R. Nichols:** None.

Poster

885. Reflexes and Reflex Modulation I

Location: Hall F-J

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Topic: D.12. Kinematics and EMG

Support: NIH NICHD-R01

Graig H. Neilsen Foundation

Title: Neuromuscular recovery patterns following robotic gait training in spinal cord injury

Authors: *M. M. MIRBAGHERI[1,2], X. NIU[1,2], D. VAROQUI[1,2], M. KINDIG[2]
[1]Physcal Med. & Rehabil., Northwestern Univ., CHICAGO, IL; [2]Sensory Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL

Abstract: Spasticity is a common consequence of spinal cord injury (SCI), affecting mobility and causing gait impairments. The therapeutic effects of robotic-assisted locomotor (LOKOMAT) training on the neuromuscular abnormality associated with spasticity were characterized in SCI subjects with incomplete motor function loss. LOKOMAT training was provided to subjects 1-hour per session, three days per week for four weeks, with up to 45 minutes of training per session. The neuromuscular properties of the ankle were then assessed using a joint perturbation device prior to and after 1, 2, and 4 weeks of training. A system identification technique was used to quantify the joint stiffness and separate it into intrinsic (muscular) and reflexive components. The modulation of these neuromuscular properties with joint angle was characterized over training time. Reflex stiffness increased slightly from full- to mid-plantarflexion and then increased sharply and reached a maximum between neutral position and mid-dorsiflexion, and then decreased thereafter. This modulation was quantified by a linear regression fit to the sharply increasing portion of the curve. Recovery patterns for the slope and intercept of this regression were classified using “growth mixture” modeling. The ability of baseline measures of walking capacity (gait speed, endurance, and mobility) to predict the slope and intercept recovery patterns was evaluated using logistic regression analysis.
Two distinct classes were observed. Class 1 subjects had initially higher reflex stiffness parameters (i.e., intercept and slope vs. ankle position) and reduced significantly over the training period. Class 2 subjects initially had lower reflex stiffness parameters and experienced minor reductions. Similar results were observed for the intrinsic stiffness intercept; however, intrinsic slope showed no significant improvement over training for either class. The results reveal that subjects with higher reflex intercept were correlated with lower walking capacity. These findings demonstrate that LOKOMAT training is effective in reducing reflex and intrinsic stiffness (which abnormally increase in SCI) and improving the abnormal modulation of reflexes over the ankle range-of-motion. Further, reflex abnormality contributes to walking impairment. Thus, LOKOMOAT training can be considered as effective interventions for improving neuromuscular function in SCI.

Disclosures: M.M. Mirbagheri: None. **X. Niu:** None. **D. Varoqui:** None. **M. Kindig:** None.

Poster

885. Reflexes and Reflex Modulation I

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Program#/Poster#:885.05/GG16

Topic: D.12. Kinematics and EMG

Support: NIH R01 NS069214-02

Title: Persistent inward currents and their role in spasticity

Authors: *S. NANDYALA, C. M. NIU, T. D. SANGER
USC, Los Angeles, CA

Abstract: Spasticity is a condition in which a person suffers from an abnormal, velocity-dependent tightness or stiffness in response to a passive stretch. While a clinical identification of the features of spasticity can be performed, the relationship between spasticity and the pathophysiological phenomena observed in the presence of spasticity remains unclear. One of these phenomena is the development of persistent inward currents which activate the spinal motoneurons to varying degrees. Due to the complexity involved in isolating the phenomena in vivo, the extent to which each of these these phenomena contributes to the production of spastic symptoms remains unknown. However, with advances in computing hardware and technologies, it is now possible to construct a biorealistic system which mimics the actual spinal motor loop using computational models of the required parts. Therefore, we set out to build a hardware system where we can systematically add in each of the phenomena found in the presence of spasticity and quantifiably determine the extent of their involvement. Our system is constructed using models of muscle fibers, muscle spindles, synapses, and spiking neurons. The baseline version of the system uses a regular spiking neuron model to mimic healthy motoneurons. The experimental version of the system uses a spiking neuron model modified to mimic the persistent inward currents (PICs) found in spastic motoneurons. Using the model, we simulated stretch reflexes at a single joint. The results show short-latency stretch reflexes that are abnormally enhanced in the presence of PICs. Analysis of the EMG onset as a function of speed of stretch shows good correspondence to known spasticity patterns as reported in (Levin et. al. 2000). Analysis of the reflex torque in response to a stretch also shows good correspondence to known spasticity patterns as reported in (Rymer et.al 1999). From this model, we conclude that PICs are sufficient to produce the EMG response to stretch as seen in spasticity. While this does not prove that PICs are responsible in vivo, it does suggest that PICs by themselves are sufficient to explain spasticity, and thus if found in patients with spasticity no other explanation would be required.

Disclosures: S. Nandyala: None. **C.M. Niu:** None. **T.D. Sanger:** None.

Poster

885. Reflexes and Reflex Modulation I

Location: Hall F-J

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Program#/Poster#:885.06/GG17

Topic: D.12. Kinematics and EMG

Support: NSERC

Title: Stretch reflex responses in the ankle plantarflexor muscles associated with locomotor adaptations to resisted walking

Authors: C. CHEUNG[1,3], *A. DOMINGO[2,3], L. BOUYER[4,5], T. LAM[1,3]
[1]Kinesiology, [2]Human Kinetics, Univ. British Columbia, Vancouver, BC, Canada; [3]Intl. Collaboration on Repair Discoveries (ICORD), Vancouver, BC, Canada; [4]Dept. de Réadaptation, Univ. Laval, Quebec City, QC, Canada; [5]Ctr. for interdisciplinary research in rehabilitation and social integration (CIRRIS), Quebec City, QC, Canada

Abstract: In order to respond appropriately to changes in the environment, the nervous system has to rapidly adapt its motor output. During walking, sensory feedback pathways play an important role in mediating such adaptations. We have previously shown that a Lokomat-applied velocity-dependent resistance against hip and knee flexion during walking results in an adaptive increase in hip and knee flexor muscle activity during swing. With sustained exposure to the resistance, after-effects in the form of high stepping can be observed. We have also observed increased plantarflexor activity at the end of stance with Lokomat-applied resistance. Such adaptive changes in muscle activity provide a means for understanding how changes in reflex gain may contribute to locomotor adaptations. Thus the purpose of this study was to determine if the there are changes in the strength of stretch reflex pathways from the ankle plantarflexors with the locomotor adaptations seen in resisted walking. Subjects performed multiple walking trials with and without resistance. A robotic gait device (Lokomat) was used to apply velocity dependent resistance against hip and knee motion. Plantarflexor stretch reflexes in the right ankle were elicited with a motorized ankle-foot orthosis (Noel et al., 2009) that applied 4-6 deg dorsiflexor stretches at 150-200 deg/s. Perturbations were timed to coincide with early and late stance (15 ± 5% and 52 ± 4% of the step cycle, respectively). Surface EMG was recorded from the right soleus, medial and lateral gastrocnemius, and tibialis anterior. For each subject, short-latency (35.5 ± 3.4 ms) reflex responses (mean amplitude) were identified by plotting the means of the perturbed and non-perturbed cycles, and analyzed by manually placing cursors. Lokomat-applied resistance against hip and knee movements during walking was associated with an increase in EMG activity in the ankle plantarflexors during late-stance but not during early stance. Rapid dorsiflexion stretch perturbations resulted in a clear stretch reflex response in plantarflexor muscles. During steps against resistance, however, the normalized amplitude of the stretch reflex response was suppressed compared to non-resisted steps. This was observed in the reflex ratios (resisted/baseline) for short latency reflex response elicited in the soleus during late stance (0.67 ± 0.21) as well as during early stance (0.72 ± 0.34). The suppression of stretch reflex responses during late stance was present despite an increase in the background EMG.

These results suggest that changes in the gain of peripheral feedback pathways may not contribute to the production of locomotor adaptations to resistance.

Disclosures: C. Cheung: None. **A. Domingo:** None. **L. Bouyer:** None. **T. Lam:** None.

Poster

885. Reflexes and Reflex Modulation I

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Topic: D.12. Kinematics and EMG

Support: Falk Medical Research Trust

CTSA Grant #UL1RR031973

Title: Increased hip to ankle contribution during a challenging standing tracking task in people with multiple sclerosis

Authors: *M. C. CHUA[1], A. S. HYNGSTROM[2], A. V. NG[3], B. D. SCHMIT[1]
[1]Biomed. Engin., [2]Physical Therapy, [3]Exercise Sci., Marquette Univ., Milwaukee, WI

Abstract: This study demonstrated that people with MS increase hip contributions, and reduce ankle contributions to balance control during a standing arm tracking task, compared to healthy subjects. Although it is well-known that MS affects mobility by disrupting balance and gait, adaptations to control strategies for functional movements are not well described. A better understanding of changes in movement control in MS is important for designing therapies to counteract the effects of MS on movement.
Participants in this study included 10 individuals with MS and 10 neurologically intact subjects. All subjects were able to walk 6-10 meters comfortably at a self-selected pace and were 30-60 years old. Subjects tracked a moving target with their finger along a figure-eight trajectory that was projected on a large touchscreen. The movement of the target either increased linearly (ramp tracking) in frequency from 0.2 - 1.5 Hz over a period of 67 seconds, or maintained a steady speed (steady speed tracking) at seven different frequencies between 0.2 and 1.5 Hz. Motion capture cameras and two force plates were used to capture kinematic and kinetic data.
The MS subjects showed increased ratios of hip to ankle peak-to-peak (PP) moment, as well as hip to ankle PP angular range of motion during both ramp and steady speed tracking, compared to the control subjects. The maximum hip and ankle moments and angles also showed a negative correlation to increasing level of impairment, measured with the Berg Balance Score and 25 feet walk test.

This study demonstrates that people with MS use increased hip and reduced ankle control to maintain balance during a challenging standing tracking task. This altered hip-ankle strategy for maintaining balance in a challenging situation, which might be related to differences in impairment between the hip and ankle.

Disclosures: M.C. Chua: None. **A.S. Hyngstrom:** None. **A.V. Ng:** None. **B.D. Schmit:** None.

Poster

885. Reflexes and Reflex Modulation I

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Four Years Fellowship from the University of British Columbia to YX

Title: Motor output is relatively resistant to global ischemia and recovers faster following reperfusion than sensory processing in Channelrhodopsin-2 transgenic mice.

Authors: *Y. XIE, S. CHEN, R. LIU, E. ANENBERG, T. MURPHY
Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Previously, we have developed an approach to directly probe neuronal excitability during the period beginning with induction of global ischemia and extending after reperfusion using transgenic mice expressing Channelrhodopsin-2 (ChR2). Within these ChR2 mice, we activate deep layer cortical neurons independent of synaptic or sensory stimulation. In the present study, we employed a motor mapping technique using ChR2 transgenic mice combined with electromyographic (EMG) recording to assess dynamic changes in motor output during 5 min of global ischemia and following reperfusion. For comparison, we simultaneously assessed sensory processing by electrically stimulating the forepaw and recording the electroencephalographic (EEG) signal in the somatosensory area and the ChR2 stimulation-evoked EEG response in the motor cortex representation. Consistent with our previously

reported findings using urethane anesthesia, in ketamine/xylazine anesthetized mice, neuronal excitability assessed by the ChR2 stimulation-evoked EEG responses was intact during the period of strong spontaneous EEG suppression, was depressed during ischemic depolarization and mostly recovered (96.3 ± 18.8% of pre-ischemia value, n=5) 10 min after reperfusion. In contrast, the decrease of the somatosensory-evoked potential coincided with the EEG suppression and the recovery was incomplete and delayed after reperfusion (27.8 ± 9.5% of pre-ischemic value at 90 min, n=5). Surprisingly, EMG responses evoked by the ChR2 stimulation in motor area showed significant recovery (51.0 ± 28.1% of pre-ischemic value, n=5) 15 min after reperfusion and further completely recovered to 97.8± 30.5% of pre-ischemic value 55 min after reperfusion. The present results confirmed our previous findings which defined a period where cortical neurons are excitable but synaptically silent after global ischemia using a different anesthesia (ketamine/xylazine) and temperature regulation (brain surface temperature was clamped to 36.5°C). Novel findings from our results demonstrate relatively reduced vulnerability of motor output compared to sensory processing following global ischemia and reperfusion. Differential sensitivity of sensory and motor processing to the effects of stroke may have implications for particular forms of rehabilitation that target these systems.

Disclosures: Y. Xie: None. **S. Chen:** None. **R. Liu:** None. **E. Anenberg:** None. **T. Murphy:** None.

Poster

885. Reflexes and Reflex Modulation I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:885.09/GG20

Topic: D.12. Kinematics and EMG

Support: STW, NeuroSIPE programme. Project 10732: QDISC

Title: Identifying neuromuscular control during low back stabilization: Simultaneously quantifying intrinsic properties and proprioceptive feedback

Authors: *P. DRUNEN[1], E. MAASWINKEL[2], F. C. T. VAN DER HELM[1], J. H. VAN DIEËN[2], R. HAPPEE[1]
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Abstract: Motor control deficits have been suggested as potential cause and/or effect of a-specific low-back pain and its recurrent behaviour. Therefore, the goal of this study was to identify motor control in low back stabilization by quantifying the intrinsic properties and

proprioceptive reflexes simultaneously. Most recent studies in low-back stabilization have focused either on intrinsic properties or on reflexes, by experimentally excluding one component or have lumped both components. However, changes in co-contraction could result in changes in proprioceptive reflexes and vice versa, making combined identification essential.
In fifteen healthy subjects, upper body sway was evoked using continuous anterior force disturbances (0.2-15 Hz, 35 N amplitude, 60 N preload) applied dorsally at T10 level, while the subjects performed a resist or relax task. Torso kinematics and reflexive muscle activation (sEMG) were evaluated using closed loop identification. Intrinsic and reflexive contributions to low-back stabilization were identified using a physiologically-based, neuromuscular model including intrinsic stiffness, intrinsic damping and inertia combined with sensory feedback from muscle spindles (position and velocity) and Golgi tendon organs (force).
Task dependency was found at low frequencies for both kinematics and reflexive back muscle activity (the resist task had reduced displacement and stronger reflex gains). The neuromuscular model was able to predict on average 84% of the kinematics and 36% of the sEMG measurements, while resulting parameter values were consistent over subjects. The methodology presented allows identification of low back motor control by quantifying the intrinsic properties and proprioceptive reflexes simultaneously. Therefore, this methodology has the potential to investigate motor control deficits in LBP patients in detail.
This research is supported by the Dutch Technology Foundation STW, which is part of the Netherlands Organisation for Scientific Research (NWO) and partly funded by the Ministry of Economic Affairs, Agriculture and Innovation. See www.neurosipe.nl - Project 10732: QDISC

Disclosures: P. Drunen: None. **E. Maaswinkel:** None. **F.C.T. van der Helm:** None. **J.H. van Dieën:** None. **R. Happee:** None.

Poster

885. Reflexes and Reflex Modulation I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:885.10/HH1

Topic: D.12. Kinematics and EMG

Title: Fatiguing to exhaustion affects spinal inhibitory mechanisms

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Abstract: Exercised-induced fatigue causes a wide variety of central and peripheral changes in the neuromuscular system. Many neural mechanisms have been implicated during a fatiguing

task. These adaptations are thought occur as an adjustment to the failure of the muscular contraction ability. It is shown, for example that the H-reflex amplitude decreases during sustained submaximal contraction or at post-exercise the Hmax/Mmax ratio decreases. However, changes in the inhibitory circuits of the spinal cord after a prolonged bout of exercise have not been extensively examined. Here, we investigated two important inhibitory mechanisms in spinal circuits (post-activation depression (PAD) and recurrent inhibition-RI), after a heavy exercise to exhaustion (3 hour running) in 6 elite and well-trained runners. Using the method described by Pierrot-Deseilligny (1975) we measured the changes in the activity of the Renshaw cells. We also measured PRD throughout the soleus H-reflex recruitment curve, before and immediately after the termination of running. Subjects sat on a chair and placed both feet on a wedge and exerted an isometric pushing force equal to 10% of their maximum voluntary contraction, while monitoring their force output. The analysis showed that after the fatiguing exercise, the soleus recruitment curve was depressed and shifted to the right with the maximum H-reflex changing from 4.26 mV to 3.75mV (p=.028). In the PAD measurement, fatigue resulted in a decrease in the amount of depression observed. The PAD ratio (conditioned H to unconditioned H ration) was 78.1% which showed a statistically significant depression in H-Prime, while this value was 88.03% after fatigue which suggested no significant difference between the amplitude of the H reflex and the H' value. No significant change in recurrent inhibition was observed after fatigue (recurrent H being 30.42% pre-exercise vs. 31.08% post-exercise, p=0.31). Our results suggest that after a prolonged fatiguing exercise, the feedback loop of the alpha motoneurons do not change (which is an indication of postsynaptic events) while there is a decrease in post-activation depression. This release of inhibition might be a compensatory mechanism in response to the general depression of the H-reflex throughout the recruitment curve. It is also suggested that any change in reflex inhibition to motoneurons after fatigue is most probably due to presynaptic mechanisms and not to an autogenic (negative) feedback regulation.

Disclosures: B. Tahayori: None. **C. Acikada:** None. **R.F. Chapman:** None. **D. Koceja:** None.

Poster

885. Reflexes and Reflex Modulation I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:885.11/HH2

Topic: D.12. Kinematics and EMG

Title: Capacity of young, middle-aged, and old subjects to improve steadiness during a cocontraction task by modulating Ia presynaptic inhibition

Authors: *J. GOULD, M. R. HOLMES, I. PEÑA-GONZÁLEZ, R. M. ENOKA
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Abstract: Old adults tend to perform motor tasks with a greater amount of antagonist muscle activation than young adults, but the reasons underlying this increased coactivation have yet to be identified (Hortobágyi and DeVita, 2006). When performing steady submaximal contractions, old adults accommodate changes in task demands by increasing the coactivation of agonist and antagonist muscles, whereas young adults rely on a strategy of modulating afferent feedback (Baudry et al., 2010). Old adults may prefer the feedforward coactivation strategy due to a decline in the function of the pathways that can provide feedback adjustments. The current study compared the changes in steadiness and the modulation of presynaptic inhibition of soleus Ia afferents in 11 young (24.5 ± 3 yrs, 6 women), 9 middle-aged (52.3 ± 8 yrs, 3 women), and 9 old adults (78.2 ± 4 yrs, 5 women) before and after a single session of practicing a cocontraction task. The task required subjects to sustain a target EMG amplitude for the plantarflexors while concurrently contracting (cocontracting) the dorsiflexors to reduce the net muscle torque about the ankle to zero. There was no difference in the maximal voluntary contraction (MVC) force for the plantarflexor muscles between young and middle-aged subjects, but MVC force was significantly less for old subjects ($P<0.05$). Younger subjects had a greater Hmax/Mmax ratio than middle-aged ($P<0.05$) and old subjects ($P<0.001$), whereas there was no significant difference between middle-aged and old adults. There were no differences in steadiness between groups during an initial cocontraction trial ($P=0.713$). The main finding of the study was that young adults were able to improve steadiness by ~19% ($P<0.001$) during the 50-min practice session, whereas there was no change for the middle-aged adults, and old adults became less steady by ~15% ($P<0.05$). Furthermore, the improvement in steadiness by young adults was accompanied by a significant reduction in the amount of Ia presynaptic inhibition, as indexed by D1 inhibition ($P<0.01$). Conversely, the other two groups exhibited no change in Ia presynaptic inhibition after practicing the cocontraction task. Rather, MVC force declined significantly for middle-aged (17%) and old (20%) groups following the cocontraction practice, whereas there was no significant difference in MVC force for the young adults. The findings indicated that young adults were able to improve steadiness with practice of a cocontraction task by modulating afferent feedback, whereas middle-aged and old adults were not able to improve steadiness despite no significant strength differences between young and middle-aged adults.

Disclosures: J. Gould: None. **M.R. Holmes:** None. **I. Peña-González:** None. **R.M. Enoka:** None.

Poster

885. Reflexes and Reflex Modulation I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:885.12/HH3

Topic: D.12. Kinematics and EMG

Title: Crossed-spinal modulation of the soleus H-reflex: The effects of age and physical activity.

Authors: *R. A. RYDER, K. KITANO, D. M. KOCEJA
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Abstract: A growing body of literature has associated participation in physical activity with higher levels of both physical and mental health during the aging process. One understudied mechanism in neural aging research is the role of physical activity in maintaining or restoring spinal cord reflex function, in particular the impact of physical activity on crossed-spinal reflexes and bilateral lower limb coordination during aging. Therefore, the purpose of this study was to examine the time-course modulation of the soleus H-reflex following a conditioning stimulation from the contralateral common peroneal nerve in both sedentary and highly physically active young and elderly subjects. Data was collected on 20 subjects who were divided into one of four groups: 1) physically active young subjects ages 20-35 (n=5), 2) sedentary young subjects ages 20-35 (n=5), 3) physically active elderly subjects aged ≥65 (n=5), and 4) sedentary elderly subjects aged ≥ 65(n=5). Each subject's physical activity level was determined by their responses on the International Physical Activity Questionnaire. To assess crossed-spinal reflex modulation, conditioning stimuli were delivered to the common peroneal nerve at the fibular head of the left leg (200 Hz train, 20ms duration at 1.2x motor threshold). This was followed by test stimulation to the posterior tibal nerve at the popliteal fossa in the right leg to evoke a soleus H-reflex (the control amplitude of H-reflex was adjusted at 50% of the maximal H response). The following inter-stimulus intervals between the conditioning and the test stimuli were examined: control (H-reflex only), 25ms, 50ms, 75ms, 150ms, and 300ms. All testing was performed with the subjects in the prone position. A significant interaction was found between inter-stimulus interval and physical activity ($F= 3.34$, $p=.027$). Sedentary subjects, regardless of age, demonstrated a significant facilitation in the reflex at 25ms, 50ms, 75ms, and 150ms (by 9%, 32%, 31%, and 22% respectively) whereas physically active subjects show an inhibition at 25ms (9%) and maintain stable reflex amplitudes across all other tested inter-stimulus intervals. It is concluded that participation in physical activity appears to contribute to greater crossed-spinal reflex stability in both young and old subjects.

Disclosures: R.A. Ryder: None. **K. Kitano:** None. **D.M. Koceja:** None.

Poster

885. Reflexes and Reflex Modulation I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:885.13/HH4

Topic: D.12. Kinematics and EMG

Support: NIH-R01

Craig H Neilsen

Title: Effect of an anti-spastic medication (Tizanidine) on velocity-dependent component of the ankle stiffness in spinal cord injury population

Authors: *D. VAROQUI[1,2], M. W. KINDIG[1], X. NIU[1,2], M. M. MIRBAGHERI[1,2]
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Abstract: Two-thirds of spinal cord injury (SCI) patients present spasticity at one year after injury. A major health problem for SCI patients, spasticity limits mobility, affects ambulation and reduces independence in daily activities. Tizanidine is an alpha(2)-adrenoceptor agonist widely used to manage spasticity resulting from SCI. Clinical studies show that tizanidine is an effective anti-spasticity agent, but the mechanism by which the spasticity is reduced remains unclear. Typically, the benefits of tizanidine on spasticity are assessed by clinical assessments of muscle tone, e.g., Ashworth scale, which cannot quantify the effects of this medication on the ankle stiffness. The aim of this study was to quantify the effects of tizanidine on passive and velocity- mediated components of the stiffness at the ankle joint. Twenty-five incomplete chronic SCI participants with long-term spasticity were recruited for this study. Patients were instructed to take tizanidine for four weeks. Patients were assessed four times during the month: at the baseline (pre-medication), and 1-, 2- and 3- weeks after the first administration of medication. A joint stretching position-controlled device applied a series of triangular dorsi-flexion perturbations with a 40° amplitude, while subjects were instructed to maintain no tonic contraction (relaxed condition). Ramps were applied at low velocity (5°/s) and high velocity (100°/s). The resistance torque and the plantar-flexor and dorsi-flexor EMG activity were recorded during all stretches. The resistance torque measured in the 5°/s condition was assumed to measure the passive stiffness, as no reflex activity was evoked in any of the participants at this velocity, as verified by EMG. Velocity-dependent stiffness was computed by subtracting the passive torque from the torque recorded at the 100°/s velocity. From the torque-angle curves, the resistance torque at controlled angular positions, quasi-static stiffness, and the energy loss were calculated. Results showed that the tizanidine did not affect the passive stiffness. However, we observed a reduction of the velocity-dependent stiffness during the high-velocity stretches. This decrease is particularly observed in the dorsi-flexion direction, where the ankle plantar-flexor muscles were stretched. This velocity mediated stiffness reduction appears as early as one week after the first dose of medication. Reduction in velocity-dependent component, which is most if not all reflexive response, demonstrates therapeutic effects of tizanidine on reflex behavior.

Disclosures: D. Varoqui: None. **M.W. Kindig:** None. **X. Niu:** None. **M.M. Mirbagheri:** None.

Poster

885. Reflexes and Reflex Modulation I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:885.14/HH5

Topic: D.12. Kinematics and EMG

Title: The effect of six weeks endurance training on postural control during full body perturbations

Authors: *H. HASSANLOUEI[1], D. FALLA[2,3], L. ARENDT-NIELSEN[4], U. KERSTING[4]
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Abstract: Muscle fatigue is an exercise-related decrease in the maximal voluntary force and is known to adversely affect postural control. This study examines whether six weeks of endurance training can minimize the effects of fatigue on postural control during dynamic postural perturbations. Twenty healthy volunteers participated in the study and were randomly assigned to either a 6-week endurance training program or a control group (no training). For the training group, exercise was performed on a bicycle ergometer 3 times per week. The load intensity ranged between 50 to 70% of the heart rate reserve and the duration of the sessions were between 20 and 45 min. Maximum voluntary contraction (MVC) of the knee extensors was assessed at baseline and on week 7. Furthermore, at baseline and on week 7, dynamic exercise was performed on a SRM bicycle which consisted of light cycling followed by a ramping protocol starting at 100 W with the workload increased by 15 W. min $^{-1}$ until exhaustion. Simultaneous measures of anterior-posterior (*Fy*) and medial-lateral (*Fx*) ground reaction forces were analyzed during full body perturbations performed before and after the high intensity exercise protocol. Subjects stood on a moveable platform which produced an 8 cm forward slide over 0.5 ms at random time points so that the subject could not anticipate the perturbation. Changes in the center of pressure were assessed from the onset of platform movement until 300 ms after completion of platform movement. Preliminary results indicate a significant increase in knee extension MVC post training for the training group (pre: 541.5 ± 123.8 N.m, post: 712.1 ± 119.6; $P<0.05$) but not for the control group (pre: 585.65 ± 161.2 N.m, post: 468.01 ± 45.82;

P>0.05). At baseline, fatigue induced an increase in the anterior-posterior (*Fx*) ground reaction forces during the perturbations in both groups (pre fatigue: 106.92 ± 43.66 mm; post fatigue: 127.20 ±59.19 mm; P<0.05). However for the training group the ground reaction forces were not altered by the fatiguing protocol when assessed at week 7 (pre-fatigue: 96.44 ± 37.82 mm; post fatigue: 100.46 mm; P>0.05). In conclusion, six weeks of endurance training improved the ability to control balance along the anterior-posterior axis in response to rapid full body postural perturbations in the presence of muscle fatigue.

Disclosures: H. Hassanlouei: None. **D. Falla:** None. **L. Arendt-Nielsen:** None. **U. Kersting:** None.

Poster

885. Reflexes and Reflex Modulation I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:885.15/HH6

Topic: D.12. Kinematics and EMG

Title: Long-latency reflexes of the upper limb are insensitive to skin stretch

Authors: *I. KURTZER
Neurosci. and Histology, New York Col. of Osteo. Med., Old Westbury, NY

Abstract: A growing body of evidence indicates that long-latency reflexes - the muscular responses occurring ≈50-100 ms after a limb displacement - depend on multiple neural pathways. SAII cutaneous afferents may provide a sensory source for long-latency reflexes (LLRs) since these receptors are excited by the change in skin strain which naturally accompanies joint displacement. Accordingly, LLRs would be augmented by skin stretch that is congruent with the direction of joint displacement and attenuated by incongruent skin stretch. In this preliminary study we tested whether LLRs of the upper limb utilize this relevance source of information. Three healthy subjects participated (all male, 26-30 yro). Surface muscle activity was obtained from major muscles spanning their elbow joint (brachioradialis & triceps lateral), shoulder joint (pectoralis major& posterior deltoid) and both joints (biceps brachii & triceps longus). A programmable robot (KINARM, BKiN Technolgies) allowed free movement of the arm in the horizontal plane and applied brief torque pulses to displace the elbow joint. Elbow flexion evoked LLRs from the arm's extensor muscles whereas elbow extension evoked LLRs from its flexor muscles. Another stimulus was a brief skin stretch via a light-weight solenoid. We attached the device in two different arm positions to create a skin stretch congruent with elbow extension or elbow flexion. Elbow displacement, skin stretch, and paired stimuli occurred in

random sequence. Efficacy of these stimuli was assayed by the grand mean of each subject's t-value and general trends were assayed by linear regression.
Similar to our earlier studies, elbow displacement evoked robust LLRs in all of the arm muscles (grand mean t = 7.7). We did not find that skin stretch congruent with elbow extension or flexion evoked a LLR in any arm muscle (grand mean t = −0.6). Moreover, the LLR during elbow displacement did not differ from the LLR during elbow displacement paired with congruent skin stretch (grand mean t = −0.2) or incongruent skin stretch (grand mean t = −0.2). A similar pattern emerged in the patterns of linear regression. LLRs during elbow displacement accurately predicted LLRs during elbow displacement paired with skin stretch ($r = 0.95$, $P < 0.01$). The prediction was not improved by including activity from the skin stretch condition ($r = 0.93$, $P < 0.01$) nor did skin stretch predict the residual activity after removing elbow displacement as a factor (partial $r = -0.1$, $P > .2$),
Despite the relevance of skin stretch to signal limb displacement and its utilization in the conscious appreciation of limb position, we found no evidence that skin stretch contributes to the arm's LLRs.

Disclosures: I. Kurtzer: None.

Poster

885. Reflexes and Reflex Modulation I

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Topic: D.12. Kinematics and EMG

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Dept of VA RR&D grant 1 I01 RX000243- 01A1

Center Grant, State of Florida BSCIRTF

Title: Reorganization of heterogenic inhibitory force feedback between hindlimb extensors after both acute and chronic time points post spinal cord injury

Authors: *I. F. NIAZI[1], T. NICHOLS[1], A. RISING[2,3], L. LITTLE[3], S. MONDELLO[3], D. HOWLAND[2,3]
[1]Applied Physiol., Georgia Inst. of Technol., Atlanta, GA; [2]Brain Rehabil. and Res. Ctr., VA Med. Ctr., Gainesville, FL; [3]Dept. of Neurosci. and McKnight Brain Inst., Univ. of Florida, Gainesville, FL

Abstract: One of the major clinical issues associated with spinal cord injury (SCI) in mammals is the lack of animal's ability to maintain balance and weight support. We are interested to know how do the neural networks in the lumbar spinal cord of mammals and their interactions with muscles changes after SCI that may interfere with balance control. We hypothesize that following SCI there is reorganization of the postural limb reflexes driven by load receptors. Heterogenic force feedback provides cross-joint and axis coupling (Nichols 1994). Also, as proposed earlier (Biewener and Daley 2007) the distal joints provide the main mechanical interface with the ground, while the proximal joints serve mainly to drive the movement. In control decerebrate animals there is a proximal to distal gradient in the strength of inhibitory force feedback among the limb muscles. In decerebrate animals with acute to chronic (2-20 weeks) lateralized T10 spinal cord injuries, this gradient is reversed with strong inhibition onto the gastrocnemius muscles. This reversal was seen in cats regardless of whether the cat required assistance with weight support and balance or could walk independently across a runway. In contrast, cats that received dorsal hemisections did not show this reversal. Thus, we propose that ventral/ventrolateral horn pathways in the spinal cord are important for regulating the distribution of intermuscular force feedback.

Disclosures: I.F. Niazi: None. **T. Nichols:** None. **A. Rising:** None. **L. Little:** None. **S. Mondello:** None. **D. Howland:** None.

Poster

886. Reflexes and Reflex Modulation II

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Program#/Poster#:886.01/HH8

Topic: D.12. Kinematics and EMG

Support: University of Victoria

Candian Foundation for Innovation

BCKFD

Title: The effect of age on responses to TIbial Nerve STIMULATION during walking

Authors: *S. R. HUNDZA, R. BRODIE, A. GAUR, D. COMMANDEUR, T. GIBNEY, M. D. KLIMSTRA
Univ. Victoria, Victoria, BC, Canada

Abstract: While walking can become limited or impaired with age, the specific changes in neural control across the adult lifespan that are associated with this behavioural change are not well understood. In young healthy subjects, studies have used muscle and cutaneous reflexes to probe the neural control of rhythmic locomotor tasks revealing characteristic reflex modulation patterns both within and between limbs. These stereotypical reflex responses are specifically sculpted to serve important regulatory functions during human locomotion such as assisting with obstacle avoidance during swing phase. In this way, cutaneous reflexes can be used as an indicator of neurological function during locomotion as well as to characterize deficits in neural control. To date, age-related differences in electrically-evoked cutaneous reflex responses during walking have not been investigated. Understanding age-related differences in cutaneous reflex control is vital given their putative functional relevance during walking for obstacle and trip avoidance. We have compared cutaneous reflex responses during locomotion in between young (18-30 yrs) and healthy older adults (>70 yrs). EMG recordings were made from limb and trunk muscles using a tethered surface EMG system. Reflexes were evoked with electrical stimulation (5 x 1 ms pulse @ 300Hz) to the distal tibial nerve at the ankle during walking on a treadmill. EMG during stimulation and control conditions (without stimulation) were averaged separately and subtracted for each phase of the walking cycle to yield a cutaneous reflex response. Additionally 3D motion analysis was performed to assess any kinematic changes associated with the tibial nerve stimulation. Preliminary analyses showed age-related differences in responses. This understanding may be useful to establish mechanisms of age-related neural changes and present potential markers for deterioration in functional ability related to fall risk.

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Poster

886. Reflexes and Reflex Modulation II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:886.02/HH9

Topic: D.12. Kinematics and EMG

Support: NSERC

CIHR

CFI

Title: Technical considerations for gait analysis using wearable inertial measurement units.

Authors: *M. D. KLIMSTRA[1], R. CATER[2], S. MONSEES[3], K. GALLAGHER[3], N. LIVINGSTON[4], S. MACDONALD[5], G. MULLIGAN[3], D. COMMANDEUR[3], S. HUNDZA[3]
[2]Biol., [3]Exercise Science, Physical and Hlth. Educ., [4]CanAssist, [5]Psychology, [1]Univ. of Victoria, Victoria, BC, Canada

Abstract: When performing gait analysis clinicians and researchers must carefully consider their measurement tool, variable of interest as well as analysis technique. Each of these factors can alter the ability to accurately detect differences between gait conditions as well as clinical populations. Therefore the major aim of this study was to evaluate each of these factors across different experiments in multiple clinical populations. There are currently a number of instruments that can be used to measure gait events such as complex motion analysis systems, pressure sensing mats, pressure sensing insoles and wearable inertial measurement units (IMU). Each device has different capabilities with respect to ease of use, portability and set of measureable gait parameters. Additionally, it is very important to consider the reliability of a measurement device in collecting specific gait variables proven valuable at detecting gait deficiencies. As a prime example measures of gait variability have been recently used as a marker for gait performance, future mobility status, cognitive status, and falls. Therefore this suggests that a tool which is able to reliably detect subtle changes in gait variability would be clinical useful. As part I of this study we evaluated the use of an IMU, with a novel gyroscopic measurement algorithm, for measuring gait variability compared to a pressure sensing mat and video analysis. During this experiment we evaluated changes in stride time variability induced by wearing shoes with reduced stability (i.e. high heels) during walking. Also we determined changes in stride time variability for those with Parkinson's diseases as a result of engaging in a targeted exercise program. In Part II of this study we sought to further compare the IMU and pressure sensing walkway for measures of gait variability in an aging population during gait under varying cognitive demands. This data was analyzed for differences between groups when comparing the raw data as well and a novel "data residualization" technique. This analysis technique has potential to allow us to predict inter-individual differences in gait deficiencies beyond gross correlates such as age, gender and disease.

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Poster

886. Reflexes and Reflex Modulation II

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Program#/Poster#:886.03/HH10

Topic: D.12. Kinematics and EMG

Support: WVU Startup Fund

Title: Adaptation to asymmetric locomotor task

Authors: *S. YAKOVENKO, V. GRITSENKO
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Abstract: The neural motor pathways evolved a combination of hierarchical and pluralistic control mechanisms to generate movements of increasing dexterity and complexity. Any movement including “simple” stereotypical locomotion is a product of interactions between descending and recursive feedback pathways that span several levels of the hierarchy. These interactions ensure integration of multiple objectives that ultimately result in the emergent sequence of muscle activations. While most studies focus on the specific pathways of the control hierarchy using predominantly a reductionist approach, the mechanisms of interactions between multiple pathways that require holistic and interdisciplinary methodology are not common. The main objective of this study is to describe the emergent properties of locomotor control and their task-dependent plasticity. We studied the interlimb interactions of spinal pathways that contribute to the control of human gait during adaptation to an asymmetric locomotion task. Subjects walked along curved paths that modified the speed of stepping of each leg. During this task, activity of 16 muscles was recorded using surface electromyography, and whole body 3D kinematics was recorded using an active-marker motion capture system. Additionally, soleus H-reflexes were evoked unilaterally multiple times during each step cycle. H-reflex modulation during the asymmetric locomotion task was compared with predictions of a biomechanical model of neural hierarchy. Specifically, we estimated optimal bilateral scaling of spinal contribution to the regulation of locomotor phases using the model and then tested these predictions experimentally during human gait by comparing evoked responses to transcranial magnetic stimulation and H-reflexes. The findings support the hypothesis that contralateral interactions modulate optimally sensory feedback gains, to drive the speed of stepping of each limb in an asymmetric locomotion task.

Disclosures: S. Yakovenko: None. **V. Gritsenko:** None.

Poster

886. Reflexes and Reflex Modulation II

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Topic: D.12. Kinematics and EMG

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Title: Crossed-spinal influence on the human soleus H-reflex and efficiency of postural recovery from perturbation in young and elderly subjects.

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Abstract: The purpose of the study was to investigate a possible connection between crossed-spinal mechanisms in spinal reflexes and bilateral coordination in the lower limbs. Participants were recruited from two age groups; elderly (n=29, age: >65 yr) and young (n = 14, age: 21-30 yr). The H-reflex was evoked in the right soleus muscle and data were collected while subjects were in the prone position. The soleus H-reflex was conditioned through the common peroneal nerve (CPN) in the contralateral side. A train of 4 consecutive pulses was used for conditioning (1ms duration with 6ms interval for 20ms). Inter-stimulus intervals between conditioning and test stimulation were set at 25ms, 50ms, 75ms, 150ms, and 300ms. Intensities were adjusted to evoke 50% of Hmax for test stimulation and 1.2x motor threshold for conditioning stimulation. Efficiency of postural recovery was assessed by principle component analysis (PCA) of the center of pressure (COP). Subjects were perturbed during quiet standing by electrical stimulations applied to the tibial nerve either bilaterally or unilaterally. COP trajectories were analyzed for 1 sec from the timing of stimulation. For H-reflex modulation, a significant interaction was found in age group by latency ($F = 3.49$, $p < .05$). Soleus H-reflex amplitudes were significantly facilitated at 50ms and 75ms intervals in both age groups (by 15% and 17% in the elderly group and by 19% and 15% in the young group, respectively) while elderly subjects showed extended facilitation to 150ms (by 31%). For the efficiency of postural recovery, the first components from bilateral, right, and left perturbation were significantly lower in the elderly compared with the young group (.951 vs .987, .905 vs .961, and .876 vs .966 respectively). It is suggested that the facilitatory effect in H-reflex by the conditioning stimulation applied in the contralateral CPN may be associated with the crossed-spinal connection in the spinal cord which may be related to the bilateral coordination in motor behavior. Moreover, attenuated efficiency of the postural recovery measured by PCA in the elderly may be associated with extended facilitatory effects in the soleus H-reflex. Taken together, the present study demonstrated a possible relationship between efficiency in bilateral coordination and mechanism in crossed-spinal neural network.

Disclosures: K. Kitano: None. **R.A. Ryder:** None. **B. Tahayori:** None. **D.M. Koceja:** None.

Poster

886. Reflexes and Reflex Modulation II

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Program#/Poster#:886.05/HH12

Topic: D.12. Kinematics and EMG

Support: NIH Grant HD32571

Title: Stiffness of the Forelimb of the Decerebrate Cat

Authors: *R. S. MARTIN, T. BURKHOLDER, T. NICHOLS
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Abstract: The cat forelimb differs from the hindlimb both in anatomy and neural wiring. These differences manifest themselves in the diverse ways each is used during locomotion and in different postures. Studies of limb stiffness were performed in order to (i) evaluate the mechanical properties, (ii) validate the performance of computational models, and (iii) infer the unique reflex organization brought about by these dissimilarities in the limbs. Measurements of endpoint forces of both forelimbs were obtained in a pre-collicular decerebrate preparation using 2 robotic actuators mounted with force sensors. Each scapula was immobilized with a clamp and, while in isoflurane-anaesthetized (passive) and unanesthetized (active) states, recordings were taken, in a natural standing position and a crouched position (5 cm above natural standing), of the natural spontaneous activity of the limb. Each perturbation of the supporting surface was a 1 cm displacement in 16 directions in the sagittal plane and 2 in the medio-lateral plane. The results indicated that the greater stiffness component was within 10° of the limb axis in all positions and states. The limb demonstrated more stiffness in compression and more compliance in extension in the natural standing position, while exhibiting the opposite behavior in the crouched position. Further analysis revealed that the mechanical properties of the forelimb during postural maintenance cannot be described as spring-like. These results suggest a substantial asymmetry in inter-muscular reflex organization.

Disclosures: R.S. Martin: None. **T. Burkholder:** None. **T. Nichols:** None.

Poster

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Topic: D.12. Kinematics and EMG

Support: STW10736 http://www.neurosipe.nl/project.php?id=21

Title: Human neck reflex adaptation towards the frequency content of anterior-posterior torso perturbations

Authors: *R. HAPPEE[1], E. DE BRUIJN[1], A. SCHOUTEN[1,2], F. VAN DER HELM[1,2], P. FORBES[1]
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Abstract: Reflex modulation has been extensively reported during posture maintenance in response to task instructions, and to perturbation type, bandwidth and amplitude. For the head-neck system the modulation of the vestibulocollic (VCR) and cervicocollic (CCR) reflexes is essential to maintain upright head posture during unexpected disturbances. Previous studies have estimated that VCR and CCR contribute equally during perturbations in the sagittal plane; however, their modulation with respect to the properties of the disturbance remains unclear. This study seeks to establish how neck reflexes are modulated during perturbations with varying properties and how each reflex contributes to stabilization behavior. We hypothesized that VCR and CCR (a) modulate according to the perturbation bandwidth, (b) are unaffected by the perturbation amplitude and (c) increase when performing a visual acuity task.
Methods: Twelve subjects were perturbed via the torso while restrained in a seated position on a motion platform. The anterior-posterior perturbations varied in bandwidth from 0.3 Hz to a maximum frequency of 1.2, 2.0, 4.0 and 8.0 Hz, at three different amplitudes, and with eyes open and closed.
Results: Head kinematics and neck muscle EMG demonstrated significant ($P < 0.05$) changes due to bandwidth, which through modeling and closed loop identification were attributed to modulation of VCR and CCR gains. VCR and CCR demonstrated dominant contributions to stabilization during high (8.0 Hz) and low bandwidth (1.2 and 2.0 Hz) perturbations respectively, and equivalent contributions during mid bandwidth perturbations (4.0 Hz). However both were attenuated when perturbations exceeded the systems natural frequency (~2-3 Hz). Amplitude had an effect only for the lowest amplitude relative to other conditions attributed to thresholding properties of the semicircular canals. With eyes closed reflex gains decreased, attributed to the reduced ability to discriminate self-motion without vision.
Conclusions: To maintain head-upright posture adaptations of neck reflexes are observed to occur due to perturbation frequency and visual task conditions but not amplitude. Estimation of reflex contributions demonstrates that previous literature has underestimated the contribution of CCR, particularly during low frequency perturbations.

Disclosures: R. Happee: None. **E. de Bruijn:** None. **A. Schouten:** None. **F. van der Helm:** None. **P. Forbes:** None.

Poster

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Support: FAPESP (proc n. 2010/15522-4) to RAM

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FAPESP (proc n. 2009/15802-0) to LAE

Title: Responsiveness of H- and T-reflexes of soleus muscle to presynaptic inhibition induced by a low frequency train of stimuli

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Abstract: The differences between the responsiveness of mechanically (T) and electrically (H) evoked reflexes of the soleus (SO) muscle to different conditioning were addressed. Previous studies have shown that the T-reflex is less sensitive to presynaptic inhibition (PSI), induced by conditioning stimulation to the common peroneal nerve (CPN), as compared to H-reflex. Recently, it was reported that increased frequency of the conditioning stimulus (up to 1Hz) to the CPN reinforces the effect of PSI onto SO H-reflex. Here, the following hypotheses will be tested: 1) the delivery of a train of conditioning stimuli at low frequency will enhance PSI of the T-reflex to a similar extent as observed for the H-reflex; 2) these effects will be the same for both conditioning to test intervals: 15 ms (to induce D1 inhibition) and 100 ms (D2 inhibition). Nine subjects, aged 31.5 ± 6.5 y (mean ± STD), were seated in an armchair with the legs immobilized. The mechanical stimulus was a sine wave with 10 ms duration applied to the Achilles tendon through a vibratory device. The electrical stimulus was a rectangular pulse (1 ms duration) applied to the posterior tibial nerve (PTN). T- and H-reflexes were conditioned by either a "single" (1 ms duration) or a "train" of 7-10 conditioning stimuli applied at 1Hz to the neck of the fibula 15 or 100 ms before PTN stimulation. A 3-way ANOVA was used to detect differences and interactions among conditions. H-reflex was more responsive to a single conditioning stimulus than T-reflex ($P<0.05$). The train of stimuli induced a significant reduction in amplitude for both reflexes ($P<0.05$) compared to the "single" condition. There was no significant interaction between the factors "reflex" and "type of conditioning stimulus" ($P>0.05$), which indicates that the conditioning train reduced T and H-reflexes to a similar extent. Given the lower sensitiveness of T-reflex to "single" conditioning, these results suggest that the effectiveness of the train in reinforcing PSI was proportionally higher for the T-reflex, as

compared to H-reflex. No interaction between either “reflex” or “type of stimulus” and the factor “type of inhibition” (D1 or D2) was detected (P>0.05), suggesting that D1 and D2 are part of the same inhibitory pathway. One explanation for the increased PSI level in response to the train of stimuli is that the post activation depression in inhibitory interneurons interposed in the oligosynaptic pathway could, as a final result, lead to a facilitation of PSI in both reflexes. The proportional higher effect for T-reflex might be explained by a possible less saturated PSI effect due to interaction between afferent volleys (bursts of firing) and mechanisms of PSI.

Disclosures: R.A. Mezzarane: None. **F.H. Magalhães:** None. **V.M. Chaud:** None. **L.A. Elias:** None. **A.F. Kohn:** None.

Poster

886. Reflexes and Reflex Modulation II

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Title: The effect of cycling speed on control of knee extensor and flexor muscles in bicycling.

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Abstract: Introduction: Investigating electrical muscle activities (EMG) of healthy persons’ during cycling is used to build functional electrical stimulation (FES) patterns for spinal cord injured (SCI) people. If the velocity of FES cycling is planned to be increased than how the stimulation of knee muscles should be changed? If muscle activities of healthy individuals are known than similar activity patterns should be planned for people with paralyzed limbs. As cycling velocity increases, the electrical activities of quadriceps and hamstring muscles increase, but it is a question how the control of flexor-extensor muscle pairs is changing and whether the rate of change of activities differs for the 2 muscle groups?
Methods: Surface EMGs of quadriceps and hamstrings muscle groups were measured for 41 healthy subjects’ (16-33 years aged) with the ZEBRIS movement analyzing system during cycling on a recumbent stationary bike equipped to apply 8 pedal resistance. Cycling was

executed with 2 velocities (about 45 and 60 rpm) and against 3 resistance (gear 1, 5, 8). The sampling frequency of EMG was 1000 Hz. Filtered (Butterworth) and smoothed (RMS) data were averaged across time. Muscle activities in the 2 speed conditions were given by the mean EMG amplitudes for each muscle for each resistance condition. A ratio was computed (mean EMG in fast cycling divided by mean EMG in slow cycling) to show change in EMGs as a result of speed change. The difference in ratios obtained for the 2 muscles was tested with Student's T-test. Correlation between the average time of pedaling cycles (PT) and mean EMG (across subjects, cycles, and muscles) was computed for each resistance condition.
Result: The effect of speed change is significantly higher for hamstrings than for quadriceps (quadriceps ratio: 1,307; hamstrings ratio: 1,477; p=0,0264) when cycling with medium resistance (gear 5). Correlation data shows that if pedal resistance is increasing than the correlation between mean PT and mean EMG values is stronger (gear 1: r=-0,297; gear 5: r=-0,504; gear8: r=-0,495).
Conclusion: The cycling movement is mainly the result of quadriceps' activity. The hamstrings is responsible for fine control. As the speed increases the activity of both muscle groups is increasing but the activity of the hamstrings is increasing relatively more than that of the quadriceps. We assume that while faster motion needs more muscle force it requires enhanced neural control. To increase pedaling velocity one must increase both muscle groups' stimulation level, but at different rate. We suppose that as a reflex process the neural stimulation of the hamstrings increases more to ensure proper regulation of cycling movements.

Disclosures: J. Laczko: None. **P. Katona:** None.

Poster

886. Reflexes and Reflex Modulation II

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Topic: D.12. Kinematics and EMG

Support: NSF CRCNS IIS 0827684

Title: The effects of intrinsic musculoskeletal properties of the frog-hind limb during a spinal wipe reflex under varying force fields: A simulation study

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Abstract: We believe that the way the spinal cord constructs flexible reflex actions is by using a limited set of motor primitives. Using sensory information and central estimation of state, we believe that these motor primitives are modulated in amplitude and time to achieve the required motor function. Motor primitives and muscle synergies have been identified in many studies using animal and human subjects.
An adult spinalized bullfrog (Rana catesbeiana) can execute a targeted hind-limb wipe reflex in response to noxious stimuli. This movement is composed of feed-forward and feed-back aspects that enable the frog to cope with multitude of on-line (during motion) interactions with the environment like a physical obstacle, viscous liquid, etc. We reported that there is a fine tuning of motor patterns during a wipe based on the initial limb configuration in the workspace and collisions (Kargo 2000, 2008, 2010). However, in Richardson, 2005, robotic perturbations of the frog hindlimb during a wipe failed to elicit any motor pattern change and the limb reached the target zone after a brief deviation in its path. This was attributed to the stabilizing role of intrinsic musculoskeletal properties. What are the limits of this 'preflex'?
In this study, we explore the limits of correction with intrinsic musculoskeletal properties under different physical conditions. We use a 3-dimensional 5 degree of freedom, 21 muscle actuated frog hindlimb model in OpenSim (Stanford, CA). Simulations reproduce a typical wipe reflex when driven by a primitive based framework for muscle activations. We have also incorporated a feedback layer that can reorganize motor commands (but not employed here). We first explore force perturbation experiments. We iterate force pulses in wipes from different starting positions in the workspace. Using the primitive-based wipe as a drive template, we subjected the limb to low and high force perturbations during the initial period of the wipe, driven feedforward (with no corrections as seen in Richardson). The behavior of the model solely depends on the active muscle contractions and the viscoelastic properties of the system. The simulated trajectories suggest that although the ankle trajectories tend to converge towards the target portion from a few points in the workspace, they fail to converge from a majority of the workspace points. This fit with our initial expectation that the behavior of a wipe is different at different starting limb configuration and this is most likely due to the fine-tuning of the motor commands using the feedback.

Disclosures: A. ramakrishnan: None. **S.F. Giszter:** None.

Poster

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Topic: D.12. Kinematics and EMG

Support: Jeffress Foundadtion

Title: Nociceptive withdrawal response in intact, unanaesthetized rats

Authors: C. FRIDLEY, M. SEAMON, L. WYATT, B. RIFE, *C. L. CLELAND
Biol., James Madison Univ., HARRISONBURG, VA

Abstract: The nociceptive withdrawal reflex (NWR) results in an animal withdrawing a limb from a potentially tissue damaging stimulus. Previous studies revealed that withdrawal responses follow one of two alternative patterns for spatial integration--the continuous or categorical patterns. The continuous model corresponds to a continuous change in response direction with a continuous change in stimulus location, allowing for a more appropriate movement away from a stimulus. In contrast, the categorical model corresponds to a movement that occurs in a limited number of preferred directions. In spinalized animals, previous research on the tail and hind leg in our laboratory supported the categorical model. While research involving spinalized mammals provides important information about spatial integration in the spinal cord, it fails to address the influence of supraspinal pathways. However, there have been few studies of the NWR in intact animals and none that evaluated the NWR of the foot in intact, unanesthetized animals. The goal of this study was to explore the spatial integration of the NWR in the hind-limb of intact, unanaesthetized rats. Rats were gently restrained horizontally in an acrylic box placed on glass. Heat stimuli were applied to 8 diverse locations on the foot with a laser. The resulting movement of the hind-limb was recorded with one or two, high speed (500 fps), video cameras. The rat typically responded with extension of the lower leg that lifted the body upward. Subsequently the rat rapidly moved its foot upward in either the anterior or postererior direction. Most commonly the initial response was lateral-posterior but then rapidly (<20ms) transformed into an anterior movement. Neither response direction nor response magnitude depended on stimulus location. These preliminary results suggest that the rat chooses between only two movements, although up to nine different locations were stimulated., supporting a categorical strategy for the foot NWR in intact, unanesthetized rats.

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Poster

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Topic: D.12. Kinematics and EMG

Support: NIH Grant HD32571

Title: Task dependent changes to hindlimb impedance in the decerebrate cat

Authors: *C. TUTHILL[1], T. NICHOLS[2]
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Abstract: Prior investigations have shown that the patterns of heterogenic force feedback are altered in a task dependent fashion in the decerebrate cat (Tuthill and Nichols, SFN 2009). We found that inhibitory feedback between gastrocnemius and flexor hallucis longus was increased during downhill tasks. Our initial study to determine the impact of these changes on total limb impedance suggested that the directional organization of stiffness was altered rather than overall limb stiffness (Tuthill et al., SFN 2010). In an effort to reconcile these findings we have focused our attention to the modulation of limb impedance along the major axis of the leg.
Precollicular decerebrate cats were implanted with EMG electrodes and given a bilateral labyrinthectomy. The cats were mounted in a stereotaxic frame and secured at the pelvis such that the hip joint was allowed to rotate but not translate. The hindlimbs of were attached to robotic manipulators equipped with 6-axis force/torque sensors. These robots provided 1 cm perturbations directed along the hip-toe axis. Background force was modulated by electrically stimulating the contralateral tibial nerve evoking a cross-extension reflex response. Head pitches of +/- 20 degrees were used to simulate downhill and uphill conditions (Gottschall and Nichols, Phil. Trans. R. Soc. B 2011).
With this technique of measuring limb impedance, we found that stiffness about the major axis of the leg is decreased in the downhill condition as compared to level. This finding is statically significant when the results are grouped by background force in 5 N bins; however, this difference is lost when using the mean over the entire range of background forces which accounts for the contradiction between this study and the results presented previously.

Disclosures: C. Tuthill: None. **T. Nichols:** None.

Poster

886. Reflexes and Reflex Modulation II

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Topic: D.12. Kinematics and EMG

Support: NIH Grant NS035032

Title: Is motor equivalence during reaching observable at the muscle level?

Authors: D. J. S. MATTOS[1], *J. P. SCHOLZ[2], J. KUHL[3], M. L. LATASH[4]
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Abstract: Motor equivalence is defined as the ability to accomplish the same task using different effectors or in the presence of a perturbation. This idea reinforces the notion of synergy where elemental variables at different levels of analysis (e.g. muscles, joints) are organized to stabilize a performance variable of interest. A previous study showed that successful reaches where achieved by using different, motor equivalent joint configurations when the elbow joint was perturbed. The current experiment investigated whether a similar signature of motor equivalence was evident at the level of muscle synergies and whether motor equivalence differed during different phases of the reach.
Electromyography (EMG) of 15 arm/scapula muscles were acquired in 10 healthy individuals who reached to insert a pointer into a cylindrical target requiring position and orientation control. Arm perturbations were continuously provided by an elastic band placed across the elbow on trials randomized with non-perturbed reaches. Subjects were unable to predict whether a trial was to be perturbed. A smaller number of couplings (M-modes) of the 15 muscles were identified using principal component analysis of similar arm movements in a different task. EMGs from the main task were converted to M-modes and the relationship between small changes in M-modes and changes in pointer position/orientation (i.e., Jacobian) were estimated using regression analysis. Differences in the 5 M-mode configurations between perturbed and non-perturbed reaches (ΔM-mode) were projected into the null space (ME component) and range space (Non-ME component) of the Jacobian, and their lengths of projection computed. A repeated measures ANOVA tested the effect of performance variable (position *vs.* orientation), phase (pre-perturbation, 50-ms post-perturbation, 51-100-ms Post-Pert, and >100-ms Post-Pert), and component (ME *vs.* Non-ME)**.**
The ME component was significantly greater than the Non-ME component at all phases ($p<0.05$) and increased more than Non-ME during the first 100-ms post-perturbation ($p<0.05$), regardless of the performance variable ($p>0.28$).
The similarity of the effects of the perturbation on the relative amount of ME and Non-ME is not a trivial result. It suggests that muscle activations are organized into a synergy stabilizing not individual joint rotations but the endpoint trajectory. This result questions the idea of a hierarchy of synergies. It is more compatible with the view that motor elements at all levels of analysis are united into synergies stabilizing the ultimate task-related variables, not intermediate variables within the assumed hierarchy.

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Poster

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Topic: D.12. Kinematics and EMG

Support: UPMC (Univ Paris 06, Er6)

FRM

INSERM

APHP

ANR

IRME

Title: Impaired spinal presynaptic mechanisms in patients with pure hereditary spastic paraparesis

Authors: *J.-C. LAMY[1], D. MAZEVET[2], A. DURR[3], R. KATZ[2]
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Abstract: Pure (or "uncomplicated") hereditary spastic paraplegias are characterized by the appearance of progressive spasticity and paraparesis in the lower limbs with usually no spasticity in the upper extremities. In the present series of experiments, we focused on the study of presynaptic mechanisms acting at Ia fibre - alpha motoneurone synapse since a decrease of their activity would increase the stretch-induced Ia volley in firing motoneurones and might therefore be responsible for stretch reflex exaggeration. Presynaptic inhibition of Ia terminals and post-activation at Ia fibre - α motoneurone synapse were determined in both lower and upper extremities in 18 patients with pure hereditary spastic paraplegias (belonging to 17 different families), including 8 due to Spastin (SPG4) mutations and 18 age-matched healthy participants. All patients exhibited spasticity in the legs with no symptoms in the arms. In the lower limbs, both presynaptic Ia inhibition and post-activation depression of the soleus motoneurones were strongly reduced with respect to healthy subjects. In addition, in striking contrast with our expectations, we also found a significant reduction of both presynaptic Ia inhibition and post-activation depression of the Flexor Carpi Radialis motoneurones in patients in comparison with healthy participants despite the absence of clinical abnormalities in the arms. At both cervical and lumbar spinal cord, presynaptic mechanisms were less altered in SPG4 mutation carriers

with respect to non-SPG4 mutation carriers. Moreover, baclofen therapy administrated orally strongly depressed both soleus and FCR Hmax/Mmax ratios. These findings are in accordance with post-mortem pathological studies which have described the pattern of damage seen in hereditary spastic paraplegias as a 'dying-back' central axonopathy in which the ascending (sensory) and descending (corticospinal) tracts in the spinal cord degenerate progressively throughout the nevraxis, starting from their terminal portions and then proceeding towards the cell body. Interestingly, the intermediate pattern of SPG4 patients compared to others SPGs points out a different underlying mechanism.

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Poster

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Topic: D.12. Kinematics and EMG

Support: NSERC

CFI

Title: The effect of age on responses to superficial Peroneal cutaneous stimulation during walking

Authors: *R. BRODIE, D. COMMANDEUR, S. HUNDZA, T. GIBNEY, A. GAUR, M. KLIMSTRA
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Abstract: Age-related changes in human biomechanics and motor control quantifiably alter locomotor activities throughout the lifespan. For example, ageing is related to differences in the pattern of muscle activity and joint torque during walking as well as changes in reflex sensitivity during posture and locomotion. Combined with a reduction of muscle strength and power which occurs in a non-uniform manner throughout the body, these alterations make it important to determine if the underlying neural mechanisms which produce a stable gait are commensurately altered.
Studies of reflexes have proven invaluable for understanding functional responses to sensory stimulation during locomotion, as well as to characterize deficits in control. For example, in healthy young subjects, it has been observed that reflex responses result in coordinated neural and mechanical outcomes that serve important regulatory functions during human locomotion.

Specifically, reflexes originating from the cutaneous afferents of the superficial peroneal nerve (SPn) have been shown to assist with obstacle avoidance during the swing phase of walking. While there has been some research on muscle afferent reflexes in the elderly indicating changes in central control that result in altered neural and mechanical responses to perturbations, there is no known research quantifying neuromechanical reflex responses to cutaneous electrical stimulation in older adults.
In the present study, cutaneous reflexes were evoked across the gait cycle through stimulation of the superficial peroneal (SP) nerve. Reflex responses were compared between young adults (19-40) and older adults (70+) with subtracted, normalized EMG responses and alterations in kinematics of the lower limb. Overall, the study of reflex responses during locomotor activities in the elderly may be useful to establish mechanisms of age related neuromechanical changes and present potential markers for deterioration in functional ability related to fall risk.

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Poster

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Support: NIH EB012855

NS048844

Title: Modulation of muscular and interneuronal activity by afferent stimuli during locomotor activity in the spinal feline

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Abstract: Following spinal cord injury, the lumbar locomotor circuitry controls locomotor movements in the hindlimbs and its output is modulated by afferent input. It has been shown that transmission in the afferent pathways to the locomotor centers is altered with training following complete transection. We investigated gait-related modulation in the medial-plantar tibial nerve reflex, a polysynaptic cutaneous reflex from the branch of the nerve innervating the foot pad, in

two groups of animals with a complete thoracic transection: locomotor trained and untrained. The trained animals were followed for >20 weeks, and the modulation in the reflex during treadmill walking was measured. The untrained animals were used to measure changes in the activity of both lumbar interneurons and muscles with nerve stimulation applied during air-stepping in a terminal experiment.
In the trained group, we found that the reflex modulated with the phase of gait. Defining reflex activation/depression of the motor output as the region of EMG activity that fell outside the 95% C.I. of the background activity following single pulse stimulus delivered to the nerve, we found that flexor activity was enhanced by the reflex (10-35msec post-stimulus), while extensor activity could be both depressed (10-35msec) and enhanced (40-60msec). With further training, we found that tibial-evoked reflexes appeared during a larger percentage of the step cycle. We also found that stimulating the tibial nerve using long trains of stimuli (200 ms, 100Hz) evoked mainly flexor-related activity, rather than the mixed flexor and extensor reflex-evoked responses with single pulses.
In the acute experiments with untrained spinal animals, we found the same enhancement of the flexor activity and depression of the extensor activity following tibial stimulation. We observed single-unit activity that showed a phase-dependent increase or decrease in firing (10-25msec post-stimulus) following nerve stimulation. Our evidence confirms that gait-dependent modulation of muscle activity in response to afferent input is programmed at the level of the cord and that reflex circuits are potentiated following step-training.

Disclosures: D.P. Kowalski: None. **M.A. Lemay:** None.

Poster

886. Reflexes and Reflex Modulation II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:886.16/II3

Topic: D.12. Kinematics and EMG

Title: Motor point stimulation as a tool to investigate neural circuits

Authors: *K. MASANI, D. G. SAYENKO
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Abstract: Neuromuscular electrical stimulation (NMES) has been successfully used to investigate and/or modify neural circuits. When NMES is applied, the "motor point (MP)", which is the most sensitive location to the electrical stimulation and is supposed to represent the neuromuscular junction between a motor nerve and a muscle fibre, is stimulated. It is

hypothesized that, when the MP is stimulated, the motor axon is selectively activated without activating the Ia-afferents since the neuromuscular junction is connected only to the motor axon. Peripheral nerve stimulation is often used to investigate the spinal or central neural circuits (e.g., H-reflex test using the tibial posterior nerve stimulation), where selective stimulation of motor axons has been attempted to induce the antidromic firing of the motor axon by using a very short stimulation pulse width. However, even with a short stimulation pulse width, the Ia-afferent stimulation is inevitable especially with a high stimulation intensity. MP stimulation can be a good tool to achieve selective stimulation of the motor axon. The purpose of this study was to investigate whether the MP stimulation can successfully induce the antidromic firing of the motor axon without activating the Ia-afferent nerves. The subjects were seated on a chair with a torque sensor measuring the ankle plantarflexion torque. The MP of the soleus was stimulated using a pair of strap electrodes. As the result, with the stimulation intensity increment, the M-wave increased and became plateau at approximately 60 mA. There was no H-wave seen at the corresponding latency, while an F-wave occasionally appeared without changes its size during the increment of the stimulation intensity. This result suggests that the MP stimulation activates the motor axon while not activating the Ia-afferent nerve. Further, when tibial posterior nerve stimulation was applied at various inter-stimulus intervals, the H-wave was strongly inhibited with inter-stimulus interval below 200 ms. This suggests that the MP stimulation evoked the antidromic firing that caused the strong inhibition to the H-wave, although the mechanism of this inhibition is not clear. The recurrent inhibition induced by the antidromic firing is a candidate mechanism for this finding. However, the period of the recurrent inhibition is less than 50 ms and thus does not account for the long lasting inhibition to the H-wave. It is concluded that the MP stimulation selectively produces antidromic firing of the motor axon without activating the Ia-afferent nerve. As such, we believe that the MP stimulation can be a useful tool to investigate the neural circuits.

Disclosures: K. Masani: None. **D.G. Sayenko:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:887.01/II4

Topic: D.13. Motor Neurons and Muscle

Support: Research Council of Norway Grant 161341

Title: Characterization of motor unit activity in human lumbar multifidus: Evidence of self-sustained firing in motoneurons

Authors: L. R. LOTHE[1,2], T. J. L. RAVEN[1], *T. EKEN[1]
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Abstract: The underlying neurophysiology of low back pain is poorly understood. We have characterized motor unit firing activity in the lumbar multifidus muscle in healthy subjects as a reference before studying low back pain patients.
Three 50 μm diameter Teflon-insulated platinum/iridium wires were implanted in the deepest part of lumbar multifidus at L4 spinal level in 11 subjects (10 bilateral, 1 unilateral) using ultrasound guidance. EMG activity was recorded differentially during unhindered standing and during voluntary force production while standing in slight forward flexion. Individual motor units were identified using Spike2 software and analyzed with regard to instantaneous firing frequency, alternation of activity between units, and cross correlation between smoothed firing rates for concurrently active units. The common drive coefficient (CDC) was defined as the maximum value of the cross correlogram lying within 0±50 ms. Results are given as medians with 25th and 75th percentiles.
Total recording time in each subject was at least 20 minutes. Median firing frequency in 84 units from 9 subjects (7 males, 2 females; age 25 yrs (range 20-40)) was 7.4 Hz (6.2-8.7) for spontaneous activity, and 7.4 Hz (6.4-8.8) for voluntary activity. Cross correlograms from 97 unit pairs with median recording duration of 21.6 sec (range 4-793) showed a CDC in spontaneous activity of 0.48 (0.35-0.60) for ipsilateral units and 0.42 (0.22-0.55) for contralateral units. In voluntary activity, CDC was 0.28 (0.18-0.34) for ipsilateral units and 0.22 (0.16-0.31) for contralateral units. CDC in spontaneous activity was significantly higher than in voluntary activity ($P<0.005$; Wilcoxon rank-sum test) both ipsi- and contralaterally. In multi-unit recordings using one tonically active motor unit as a reference to monitor common synaptic drive, we found that slight postural changes could recruit new units to prolonged firing while the reference unit remained in its initial firing-frequency range. Similarly, after a period of concurrent firing one unit would stop firing while another simultaneously active unit continued to fire in its initial range.
We interpret our findings as evidence of self-sustained firing properties in motoneurons projecting to lumbar multifidus muscles in humans. Self-sustained firing would enable motoneurons to fire action potentials at a steady frequency for a sustained period of time, generating stable long-lasting muscle contractions. Further studies are needed in order to determine if disturbance in this activity might contribute to low back pain.

Disclosures: L.R. Lothe: None. **T.J.L. Raven:** None. **T. Eken:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:887.02/II5

Topic: D.13. Motor Neurons and Muscle

Support: HD 050111 from NCMRR/NICHD/NIH

Title: Error correction for EMG decomposition algorithms

Authors: *J. C. KLINE[1,2], S. H. NAWAB[1,3,2], C. J. DE LUCA[1,2,3,4]
[1]NeuroMuscular Res. Ctr., [2]Dept. of Biomed. Engin., [3]Dept. of Electrical and Computer Engin., [4]Dept. of Neurol., Boston Univ., Boston, MA

Abstract: The electromyographic (EMG) signal consists of motor unit action potentials superimposed with each other and varying amounts of noise. Decomposing this signal into individual trains of motor unit firings is a process inherently probabilistic - whether performed visually or with machine learning algorithms - and therefore subject to errors. We set out to elucidate the nature of these errors in more than 5,000 motor units decomposed from surface EMG signals collected during human voluntary contractions. Errors were identified from the decomposition of the EMG signal followed by repeated validations using the previously developed reconstruct-and-test approach. We classified two types of errors. Position errors were defined as variability in the temporal localization of the each motor unit firing. These errors were pseudo-normally distributed around zero. The standard deviation of the error ranged from 4 to 10 ms and was directly proportional to the temporal width of each motor unit action potential. Identification errors were found at longer latencies and consisted of false positives or missed detections of firings. These errors likely occurred from confusions between similarly shaped motor unit action potentials. While computations of mean motor unit firing rate were minimally (< 5%) affected by these errors, the values and distribution of inter-pulse intervals were affected to a greater extent. To mitigate these decomposition errors, we derived an algorithm to improve the estimate the firings of each motor unit. The original decomposition and validation results of each motor unit firing train were defined as independently obtained estimates or samples of the true motor unit firing times: containing a physiological deterministic component plus some error. Position errors were corrected by averaging the temporal locations of matched firings across estimates of the same motor unit. Identification errors were mitigated by retaining or discarding firings based on a decision criterion for the proportion of unmatched events to total number of estimates at each firing instance. This algorithm produces a new train representing the purest possible estimate of the true physiologic firings for each motor unit. Our algorithm works for any number of estimates of each motor unit firing train. Furthermore it is not limited to any specific decomposition strategy. Rather, this error mitigation technique can be implemented with a multitude of decomposition techniques ranging from visual template matching to machine learning algorithms.

Disclosures: J.C. Kline: None. **S.H. Nawab:** None. **C.J. De Luca:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:887.03/II6

Topic: D.13. Motor Neurons and Muscle

Support: FOC 82227

Title: Task specific relation between recruitment and firing rate of motor unit examined via the surface electromyogram

Authors: X. HU[1], H. SHIN[2], A. SURESH[1], *W. Z. RYMER[1,2], N. SURESH[1]
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Abstract: Voluntary muscle force control is accomplished by a combination of recruitment of motor units (MUs) and firing rate modulation of active MUs. There have been contradictory observations regarding the relation between peak firing rate and recruitment threshold of MUs. Namely, studies have shown that the earlier recruited MUs tend to reach lower peak firing rates than the later recruited MUs, that is there is positive correlation between peak firing rates and recruitment thresholds; whereas other studies have found inverse relation between peak firing rates and recruitment thresholds, termed ‘onion-skin principle’. In the current study, we hypothesize that the ‘onion-skin’ was specific to individual force contraction levels, and that the later recruited MUs have yet received excitatory drive that is high enough for these MUs to reach their peak firing rates within a specific contraction level. As a result, the lower plateaued firing rates observed in the high threshold MUs was an effect of plateaued excitatory drive.
Subjects performed an isometric ramp up-hold-relax abduction task of different contraction levels (ranged from 20% to 50% of the maximum voluntary contraction (MVC)) using their index finger. Surface electromyogram (EMG) of the first dorsal interosseous was recorded using a novel surface senor array. We obtained the firing activities of single MUs using a recently developed surface EMG decomposition technique. The peak firing rates were estimated from the initial plateau point of firing rates over time during ramp force. The plateaued firing rates of MUs occurred after the ramp force were also calculated.
Consistent with our prediction, our preliminary results show that 7.4, 63.1, 59.6, and 87.4 % of the analyzed MUs reached peak firing rate during force ramp at 20 to 50% MVC contractions, respectively. The MUs with similar MUAP sizes that showed plateaued firing rates after the ramp force at low contraction levels (low excitatory drives) showed higher peak firing rates

during the ramp force at high contraction levels (high excitatory drives). Additionally, the peak firing rates of MUs with common recruitment threshold across force levels showed no obvious association with the recruitment force, suggesting an absent or weak 'onion skin' across tasks.

Disclosures: X. Hu: None. **H. Shin:** None. **A. Suresh:** None. **W.Z. Rymer:** None. **N. Suresh:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:887.04/II7

Topic: D.13. Motor Neurons and Muscle

Support: BMBF Grant No. 01GQ0810

Title: Effects of visuomotor integration on motor unit coordination

Authors: *C. M. LAINE, F. NEGRO, D. FARINA
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Abstract: Muscle contractions require integration of command signals with afferent feedback in order to appropriately control force, time course, and precision. In laboratory settings, muscle contractions are often investigated in isometric conditions and subjects are given visual feedback of the muscle force output or of the level of EMG activity. The type of feedback may, however, alter the neural drive to the muscles. In this study, we address the influence of visuomotor integration on the behavior of motor units during voluntary muscle contractions. Subjects performed isometric contractions of the first dorsal interosseous (FDI) muscle while slowly and randomly varying the force level in the interval between 6 and 10 % MVC (this interval was shown through visual feedback of force) (no-target condition). To assess the impact of visuomotor integration, subjects repeated the same force fluctuations using a visual display of their previously generated force as a target (target-tracking condition). Task-related effects on motor unit coordination were assessed using coherence analysis. A total of 38 motor unit pairs were recorded from 2 subjects under both experimental conditions. No statistical differences were found between firing rates (mean(SD)=11.85(2.05) Hz vs. 11.69(1.38) Hz) or firing rate variability (CV of ISIs= 0.25(.05) vs. 0 .25(.05)) for no-target and target-tracking conditions, respectively. However, the coherence between motor unit pairs was significantly greater in the 5-10 Hz frequency band in the no-target than in the target-tracking condition, while high frequency coherence (15-40 Hz) was significantly increased in the target-tracking task. These data suggest

a frequency-specific modulation of common synaptic drive to motor neurons when visual input is used to guide force production.

Disclosures: C.M. Laine: None. **F. Negro:** None. **D. Farina:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

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Topic: D.13. Motor Neurons and Muscle

Support: Craig H. Nielsen Postdoctoral Fellowship

NIH Grant R01 NS077863-01

NIH Grant R01 AR053608

Title: Motor unit activity patterns in awake mice

Authors: *V. M. TYSSELING[1], L. RITTER[2], C. J. HECKMAN[1], M. C. TRESCH[2]
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Abstract: Recent work in anesthetized animals has suggested that modulation of muscle force in mice is primarily accomplished by varying the recruitment of motor units rather than varying the firing rate of already recruited units. Such recruitment based force modulation would be in contrast to findings in other animals that appear to use both recruitment and rate modulation. Further, individual motor units were often recruited at a very high rate in response to a slow depolarization. However, in the absence of motor unit recordings in behaving mice, it is unclear whether a similar recruitment based mechanism of force modulation takes place during natural behaviors. In this study, we successfully recorded motor units with good isolation via percutaneous electrodes in the lateral gastrocnemius of awake, quiescent mice. Sterilized 20 micron diameter stainless steel wires in a duplex configuration were used for the recording of single motor units. Gross EMGs were recorded simultaneously using 50 micron diameter stainless steel wires, also in a duplex configuration, with the recording surfaces separated by 2mm to record from a population of motor units. Candidate single motor units (SMUs) were detected by voltage threshold crossing then isolated using custom software written in Matlab to perform spike sorting and examination of individual waveforms. The quality of unit isolation was evaluated by examining distributions of interspike intervals and quantifying the L-ratio of

clusters (L-ratio < .05; Schmitzer-Torbert et al., 2005). Periods of steady firing ranging from 3 seconds to 14 minutes duration were included in subsequent analyses. We observed a wide distribution of firing rates across single motor units, including both low and high firing rates: modes for single unit frequency distributions ranged between 10-60Hz. Analysis of the relationship between these firing rate variations and the simultaneously recorded gross EMG will allow us to further examine the recruitment vs. firing rate modulation in the mouse. These recordings provide critical information about motor unit activations that can be compared to previous findings in reduced preparations, helping understand the contributions of synaptic inputs and intrinsic properties to MN activity during natural behavior.

Disclosures: V.M. Tysseling: None. **L. Ritter:** None. **C.J. Heckman:** None. **M.C. Tresch:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

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Topic: D.13. Motor Neurons and Muscle

Support: NCMRR/NICHD/NIH Grant HD 050111

Grant from the Italian Ministero dell'Università e della Ricerca

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Title: The control of motor units does not maximize force production

Authors: *P. CONTESSA[1,2], E. C. HOSTAGE[1], C. J. DE LUCA[1]
[1]Boston Univ., Boston, MA; [2]Univ. of Padova, Padova, Italy

Abstract: Early studies of the firing behavior of electrically stimulated animal motoneurons by Eccles (1958) and Kernell (1965, 2003) have generated the widely accepted hypothesis of a direct relationship between motoneuron firing rate and after-hyperpolarization. It has been posited that the after-hyperpolarization determines motoneuron firing rate, leading to greater firing rates in phasic (higher-threshold, larger-diameter) motoneurons and lower firing rates in tonic (lower-threshold, smaller-diameter) motoneurons. In this control scheme, the firing rate is matched to motor unit force twitch duration to provide fused tetani and maximizing force production.
We investigated motor unit firing behavior during voluntary isometric contractions in humans.

Surface electromyographic signals were recorded during the contractions and decomposed into the constituent motor unit action potential trains using the algorithm recently developed by De Luca et al. (2006) and Nawab et al. (2010). The mean firing rates of motor units was calculated from the firing trains and analyzed as a function of time and muscle force.
We found that firing rates increased as negative exponential functions with increasing muscle force; and that the time constant of the exponential functions was directly related to motor unit recruitment threshold. At any time and force level, including at recruitment, the firing rate values were inversely related to the recruitment threshold of the motor unit. Consequently, later-recruited motor units showed progressively lower firing rates than earlier-recruited motor units. This behavior was mathematically modeled in a set of equations named the "firing rate spectrum".
We believe that the disparate reports between our results on human subjects and the early studies on animal motoneurons stem from a different behavior of motoneurons when electrically vs. voluntarily activated. Larger-diameter later-recruited motoneurons may have the capacity of firing at greater rates than smaller-diameter earlier-recruited motor units, but they do not do so during voluntary isometric contractions. Instead, the higher-threshold, higher-amplitude and shorter-duration force-twitch motor units fire at lower rates and do not tetanize. This hierarchical control scheme does not enhance force. Instead, it enhances a combination of force and time duration by providing effective force generation for the earlier-recruited motor units and by reducing the fatigue of later-recruited motor units. The high-threshold motor units generally fatigue faster and could not sustain force if they were to fire at relatively greater rates.

Disclosures: P. Contessa: None. **E.C. Hostage:** None. **C.J. De Luca:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:887.07/II10

Topic: D.13. Motor Neurons and Muscle

Support: Falk Grant 0.82227

Title: Estimates of neural drive using motor unit action potential analysis

Authors: *N. L. SURESH[1], A. K. SURESH[2], X. HU[2], W. Z. RYMER[2,3]
[1]Sensory Motor Perf Prgm, Rehabil. of Chicago, CHICAGO, IL; [2]Sensory Motor Performance Program, Rehabil. Inst. of Chicago, Chicago, IL; [3]Biomed. Engin., Northwestern Univ., Evanston, IL

Abstract: *Abstract :*
INTRODUCTION Force production during voluntary isometric muscle contraction is generated through a combination of motor unit(MU) recruitment followed by increased firing of active motor units. MU recruitment order is a function of motoneuron size while the expected MU firing rate paradigm is such that the earlier, smaller recruited units fire at a slower rate than the units recruited later during the same force tasks. In this way the MU recruitment and firing rate pattern is optimized for maximal and efficient force production. During trapezoidal isometric force tasks, the reverse firing rate paradigm has routinely been observed. The objective of this study is to characterize and compare MUAP size as well as estimated 'neural drive' across varying levels of (trapezoidal) isometric force tasks. METHODS Surface EMG of the first dorsal interosseous(FDI) muscle was recorded from 3 healthy individuals. The subjects performed sustained isometric abductions at 4 different force levels (20%, 30%, 40%, and 50% of maximum voluntary contraction) each with 2 repetitions. Single MU firing activities were decomposed from the sEMG using the decomposition system (Nawab et al. 2010). A spike triggered average (STA) on the sEMG was performed to estimate the action potential (AP) shapes. The peak-to-peak amplitude of the STA MUAP was calculated as the estimated size of the motoneuron. The mean, median and range of the MUAP amplitudes for each trial and each subject were determined. Amplitude histograms of the MUAP amplitude were constructed using the number of firing times for each MU as the histogram count. The cumulative (discrete) integral of the amplitude histogram was performed. RESULTS All three subjects showed a progressively increasing MUAP amplitude range as a function of force contraction level. The cumulative integral exhibited a ramp increase followed by a plateau for a given trial and exhibited graded levels as function of task force, i.e., across trials. DISCUSSION Preliminary results from our study show that MU size increases across increasing force task levels and that the 'onion skin' firing rate pattern results in a 'neural drive' signal that is proportional to the required force for a trapezoidal force task with graded values as a function of force level.

Disclosures: N.L. Suresh: None. **A.K. Suresh:** None. **X. Hu:** None. **W.Z. Rymer:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:887.08/II11

Topic: D.13. Motor Neurons and Muscle

Title: The influence of the magnetic stimulation on characteristics of the motor system

Authors: *R. M. GORODNICHEV[1], A. BELYAEV[2]
[2]physiology, [1]Velikiye Luki State Acad. of, Velikiye Luki, Russian Federation

Abstract: The purpose of this research was to study the effects of the magnetic stimulation of skeletal muscles on maximum strength (maximal voluntary torque, MVT), amplitude H-reflex and M-wave.
18 subjects aged 19-22 performed 10 sets of concentric muscle contractions (plantar flexion in the dynamographic Biodex Multi-Joint System) daily during 10 days. These contractions made 80% of maximal voluntary contraction. 9 subjects of experimental group received magnetic stimulation with frequency 5 stimulus per second during each muscle contraction. Magnetic stimuli were applied to the gastrocnemius muscle by Magstim 200 magnetic stimulator.
It was revealed that after 10 days of the training MVT increased in subjects of the experimental group from 124.1 ± 8.8 Nm to 188.7 ± 12.2 Nm and amplitudes of the H-reflex m. gastrocnemius increased from 0.73 mV to 0.95 mV. MVT in control group subjects increased much less in comparison with the experimental group and the amplitude of the H-reflex did not change.
The present results suggest that magnetic stimulation of the muscles could change the performance of the motor command.

Disclosures: R.M. Gorodnichev: None. **A. Belyaev:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

Location: Hall F-J

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Program#/Poster#:887.09/II12

Topic: D.13. Motor Neurons and Muscle

Support: Faculty of Medicine and Dentistry, University of Alberta

Edmonton Civic Employee's Charitable Assistance Fund

Edmonton Orthopaedic Foundation

Edmonton Neurosurgical Associates

Title: Modulation of intermuscular coherence by functional electrical stimulation.

Authors: *J. A. NORTON[1], H. ADAMSON[2]
[1]Neurosci., Univ. of Alberta, Edmonton, Canada; [2]Neurosci., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Intermuscular coherence is known to be dependent upon feedback from the somatosensory system. Coherence between muscles has been proposed as a control source for Functional Electrical Stimulation. The purpose of this study was to examine what the effect of electrical stimulation on intermuscular coherence.
Fifteen neurologically intact subjects were recruited. Electromyographic activity was recorded from forearm extensors and finger extensors from the dominant hand. Electrical stimulation was applied to the same forearm extensors as were being recorded. Stimulation was applied at 20Hz at intensities that were ½ sensory threshold, sensory threshold, 2 times sensory threshold, motor threshold and 2 times motor threshold. During EMG recordings subjects maintained an isometric contraction. The order of stimulation was randomized.
EMG data was full wave rectified and coherence calculated between the two muscles using Neurospec software in Matlab. The peak frequency at which coherence was seen (above the 95% confidence limit and with appropriate phase, and lasting for at least 3 frequency bins contiguously) was recorded. We compared both individual and grouped responses using chi-squared measures of difference.
At low stimulus intensities (1/2 and 1 times sensory threshold) the stimulation there was no effect on the coherence in terms of either the peak frequency (excluding the stimulus artefact) or areas of coherence. At frequencies of 2 times sensory threshold or higher the peak frequency of coherence shifted away from the frequency of stimulation.
The lack of effect on intermuscular coherence of stimulation below two times sensory threshold, with a consistent effect above that level of stimulation suggests that Ia and/or Ib fibres may be responsible for providing at least a part of the sensory feedback portion of intermuscular coherence.
Further work is needed to elucidate more clearly these mechanisms.

Disclosures: J.A. Norton: None. **H. Adamson:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

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Topic: D.13. Motor Neurons and Muscle

Support: Fapesp Grant 2011/01519-4

CNPq Grant 302275/2011-8

CNPq Fellowship 130093/2011-4

Title: Dual coupling of β_2-adrenoceptors to G_s and G_i proteins in skeletal muscle involves extracellular cyclic AMP - adenosine pathway

Authors: *T. DUARTE[1], R. O. GODINHO[2]
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Abstract: The primary action of β_2-adrenoceptor (β_2-AR) agonists depends on activation of stimulatory G (Gs) protein and increased generation of cyclic AMP. However, in many tissues, β_2-AR-mediated responses are affected by promiscuous coupling of these receptors to other Gα subunits, especially the inhibitory Gαi subfamily. In skeletal muscle, activation of β_2-AR have been associated to cAMP-dependent potentiation of contraction force and reduced proteolysis. In the present study we investigate the possible dual coupling of β_2-AR to Gs and Gi proteins at skeletal muscle and the influence of the β_2-AR/Gs-Gi/cAMP signaling cascade on muscle contraction. Assuming that the increment of intracellular cAMP is followed by cAMP efflux and extracellular generation of adenosine, the contribution of the extracellular cAMP-adenosine pathway on the β_2-AR inotropic response was also addressed. The effects of clenbuterol (10-1000 nM; β_2-AR agonist), forskolin (1 μM; adenylyl cyclase activator) and cAMP was evaluated on the isometric contractility of adult mouse diaphragm muscle induced by transmural electrical stimulation (0.1 Hz, 2 ms duration). Clenbuterol and forskolin induced transient positive inotropic effects. The maximum clenbuterol effect, observed 30 min after stimulation onset, was followed by a negative inotropic effect, which was mimicked by either cAMP (10 μM) or adenosine (10 μM) and abolished by pre-incubation of diaphragm with pertussis toxin (PTX; Gi signaling inhibitor) or the organic anion transporter inhibitor probenecid. Remarkably, the PTX-sensitive β_2-AR inotropic effect was inhibited by the A_1 adenosine receptor antagonist DPCPX (50 μM) , indicating that β_2-AR coupling to Gi is indirect and dependent on A_1 adenosine receptor activation. The involvement of extracellular cAMP-adenosine pathway in the regulation of the β_2-AR inotropic response herein presented provides a negative feedback loop, which may limit Gs protein-coupled receptor response and possible harmful exacerbation of muscle contraction.

Disclosures: T. Duarte: None. **R.O. Godinho:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

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Topic: D.13. Motor Neurons and Muscle

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European Research Council (ERC) via the ERC Advanced Grant DEMOVE (No. 267888)

Title: Coherence between motor unit spike trains in human tibialis anterior muscle is influenced by ankle joint angle

Authors: *F. NEGRO, U. YAVUZ, D. FARINA
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Abstract: During sustained isometric contractions, the motor unit (MU) spike trains show a significant level of correlation [1] that is evident in the frequency domain as coherence peaks in the α-band (6-12 Hz) [2]. These α-band components have been demonstrated to be in phase with force oscillations [2]. The origin of this phenomenon is still not completely understood, but there is evidence [2] that suggest a role of the afferent loop. In this study, we investigate the possibility of modifying the common tremor components in the motor unit spike trains by changing the joint ankle angle and therefore the length of the muscle. Three men (age range 24-30 yrs) performed a 50 s isometric dorsiflexion at 5 and 10 % MVC with different joint ankle angle at 0° (maximal dorsiflexion angle), 20°, 40°, and 60°. Intramuscular EMG signals were recorded with two pairs of bipolar wire electrodes from the Tibialis Anterior (TA) muscle and analyzed with a decomposition algorithm [3]. The coherence function (epochs of 1 s) was calculated between all pairs of motor unit spike trains and averaged. Multiple spike trains were also combined in composite spike trains (CST. The frequency of the highest coherence peak in the α-band (6-15 Hz) was calculated for all contractions. Only motor units that were active from the beginning to the end of the contraction were analyzed. In total, 211 motor units (9 ± 4 per contraction) were used for the computation of the coherence measures. At 5 % MVC, the averaged significant coherence peaks between pairs of motor unit spike trains were 7.8 ± 0.1 Hz for 0° angle and 6.9 ± 0.3 Hz for 20°. At 10 % MVC, the averaged coherence peaks were 8.8 ± 0.4 Hz for 0° angle and 7.9 ± 1.0 Hz for 20°. The magnitude of these coherence peaks was on average 210 ± 53 % of the confidence level. For higher ankle joint angles, the magnitude of the coherence between pairs of motor units was lower compared with the other cases (on average, 110 ± 14 % of the confidence value). Therefore, we calculated the CST to identify better the locations of the peaks for these angles. At 5 % MVC, the averaged significant coherence peaks between pairs of MUs spike trains were 11.2 ± 0.8 Hz for 40° angle and 12.5 ± 0.5 Hz for 60° degrees. The results for the 10 % MVC were similar and not statistically different. In conclusion, our results demonstrated that the length of the muscle influences the tremor frequency components present in motor unit spike trains, which is supportive of the hypothesis that such components are originated by the afferent feedback loop.
[1] Nordstrom et al. J Physiol. 453: 547-574, 1992.

[2] Erimaki S & Christakos CN.. J Neurophysiology. 99(2):473-83. 2008.
[3] McGill KG et al. J Neurosci Methods. 149(2):121-33, 2005.

Disclosures: F. Negro: None. **U. Yavuz:** None. **D. Farina:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:887.12/II15

Topic: D.13. Motor Neurons and Muscle

Support: NSERC 46292-2008

Title: Octopamine and tyramine modulate synaptic properties and muscle contractions in *Drosophila*

Authors: K. G. ORMEROD[1], J. K. HADDEN[2], A. SCIBELLI[2], M. MILAKOVIC[1], A. J. MERCIER[1], *J. L. KRANS[2]
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Abstract: Octopamine (OA) and tyramine (Tyr) have been described as arthropod fight or flight hormones, although the mechanisms by which these biogenic amines act at the synapse and particularly on larval muscle force production in *D. melanogaster* are poorly understood. The present work utilizes identified muscle fibers in the body wall of 3rd instar *D. melanogaster* larvae to distinguish between OA- and Tyr-modulated changes at synapses and intramuscularly. To elucidate the effects of the biogenic amines on muscle fibers we initially characterized the effects of OA and Tyr on input resistance and basal tonus. OA elicited a dose-dependent decrease in input resistance between 10^{-8} and 10^{-4} M (2±2 and 17±8%, respectively). Tyr did not induce any changes in input resistance at 10^{-8} - 10^{-4} M. OA also induced a dose-dependent increase in basal tonus at 10^{-10} - 10^{-4} M (0.13±0.13 - 1.86±0.18 mN or 293±62 - 980±163% of resting tonus, respectively), while Tyr did not induce any significant changes in basal tonus. We next sought to characterize the roles of OA at the synapse by recording compound excitatory junctional potentials (EJPs) and larval body-wall muscle force production by synaptic and direct stimulation. OA (10^{-8} and 10^{-4} M) induced a dose-dependent increase in EJP amplitude (8±3.2 - 34±2.9%, respectively) and increased synaptically driven force production in a notably parallel manner between 10^{-8} and 10^{-4} M (125.7±23.2 - 496±30.3 uN or 13±4.6 to 32.6±6.1% of peak force, respectively). OA also significantly increased the decay constant of muscle relaxation (208±27 ms; 46.9±13.1% at 10^{-4}) compared to saline control trials. To further characterize the effects of OA on muscle fibers specifically, we activated muscle fibers directly to minimize or

eliminate contributions of the synapse. Results from direct stimulation of muscle parallel those of synaptic activation. The OA-induced changes in basal tonus far exceed those of evoked contractions and suggest involvement of a long-term change to an as yet unidentified intramuscular protein. We suspect that giant sarcomere associated proteins (gSAPs) are a major player in muscle plasticity. We are using UAS-RNAi to knock-down expression and investigate the role these proteins play in OA-mediated changes in EJPs, muscle input resistance, basal tonus, synaptically driven and directly stimulated muscle contractions. Our results indicate that there is little postsynaptic and muscle-specific action of tyramine. The action of octopamine, on the other hand, is profound and provides a useful experimental treatment for investigating muscle plasticity.
JKH and KGO contributed equally to this project.

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Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

Location: Hall F-J

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Program#/Poster#:887.13/II16

Topic: D.13. Motor Neurons and Muscle

Support: NIH DC 009587

Title: Genioglossus motor unit activities in quasi speech tasks

Authors: S. TAKANO[1], A. LACROSS[1], P. J. WATSON[2], *F. BAILEY[1]
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Abstract: Previous studies have characterized the single motor unit (SMU) activities of the genioglossus (GG) muscle in human subjects. This previous work has as its focus the respiratory-related activities of the GG and its role in preserving airway patency. In contrast, scant attention has been given to volitional movement of the tongue and the role that the GG plays in creating constrictions that modify the expired airstream for purposes of speech production. Here we used tungsten microelectrodes inserted into the GG muscle of healthy adults (3 men, 2 women; ages 22-25 years) to record SMU activities at rest and during articulation of the isolated open back and front vowels /a/ and /ae/, respectively. We simultaneously recorded motions of the rib cage and abdomen, the acoustic signal and single motor unit activities in the

following conditions: phonation, whisper, and electro-larynx to assess the separate and combined effects of respiratory, phonatory (laryngeal) and supra-laryngeal (oral-articulatory) subsystems, on MU activities. The range of GG MU firing rates recorded in these quasi speech tasks (range 9.1 - 22.4 Hz) is typically lower than for either rest breathing (range 19.8 - 22.5 Hz) but approximates firing rates previously reported during tongue protrusion (13.1 - 16.1 Hz) (Bailey et al 2007). Firing rate variability appears greater in quasi speech tasks than in either rest breathing or non-speech volitional protrusion. Minimum and maximum MU firing rates and firing rate variability are significantly greater for front versus back vowels. Such variability may reflect differences in the magnitude of displacement of the tongue from the rest condition. Moreover, greater variability in MU firing rates in speech production as compared to rest breathing or simple tongue protrusion are consistent with variations evident within phonemic categories (Liberman et al. 1957; Moore et al. 1988). These preliminary findings offer de novo insights into tongue muscle and motor unit activities as a function of the successive superimposition of respiratory, phonatory and articulatory subsystems.

Disclosures: **S. Takano:** Employment; The University of Arizona. **A. LaCross:** Employment; The University of Arizona. **P.J. Watson:** Employment; The University of Arizona. Research Grant; NIH/NIDCD 009587. **F. Bailey:** Employment; The University of Arizona. Research Grant; NIH/NIDCD 009587.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

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Program#/Poster#:887.14/II17

Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant 1R01NS072454-01A1

Title: Sensory evoked excitation of antagonistic motor groups

Authors: P. M. SONNER, *D. R. LADLE
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Abstract: Integration of sensory feedback with centrally generated motor commands is critical for adaptation of movement following perturbations from the environment. Sensory information influences motor neuron (MN) output directly via monosynaptic connections from Ia afferents and indirectly through various interneuron pathways in the spinal cord. A well characterized sensory-motor relationship is inhibition of flexor MNs of the knee joint (posterior biceps and semitendonous [PBST]) by activation of knee extensor Ia sensory afferents (Quadriceps [Quad]).

This phenomenon, known as reciprocal inhibition, is largely mediated by glycinergic Ia inhibitory interneurons (IaINs). We have characterized the efficacy of reciprocal inhibition during early postnatal development (P1-P7) in mice, when the animal begins to interact with the external environment. Interestingly, we have also found evidence for an excitatory interneuronal pathway between Quad afferents and PBST MNs at these early stages.
Inhibition of PBST MNs via Quad afferents was measured at P1 and P7 using a hemisected, isolated spinal cord preparation from wild-type mice. PBST MN excitability was measured indirectly by quantifying the area of the extracellular compound action potential (CAP) recorded from the PBST nerve via a suction electrode in the periphery evoked by stimulation of the L5 dorsal root (DRL5) at 0.1Hz. These “test” stimuli were alternated with “conditioning” stimuli in which the Quad nerve was stimulated at various intervals (0-50ms in 2ms increments) prior to stimulation of DRL5.
In all P1 and P7 preparations, conditioning with Quad stimulation resulted in reduction of the DRL5 to PBST test pulse CAP. As classically described for reciprocal inhibition, the greatest inhibition was observed at conditioning pulse intervals consistent with a disynaptic linkage between Quad afferents and PBST MNs. This inhibition was blocked by addition of 0.4μM strychnine. In the presence of strychnine, however, stimulation of Quad afferents evoked a CAP in PBST MNs, even in the absence of DRL5 stimulation. This was observed in 6/6 P7 preparations, but never observed in the absence of strychnine. Unlike the disynaptic reciprocal inhibition pathway, we found the latency of the Quad-PBST excitatory pathway was consistent with a trisynaptic linkage between Quad afferents and PBST MNs. Experiments in P1 mice suggest this excitatory pathway is also functional at the time of birth. Ongoing experiments aim to determine if normal levels of glycinergic inhibition act to suppress interneurons mediating this excitatory response on PBST MNs and whether this excitatory circuit persists during mature locomotion.

Disclosures: P.M. Sonner: None. **D.R. Ladle:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

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Program#/Poster#:887.15/II18

Topic: D.13. Motor Neurons and Muscle

Title: Passive length changes of human muscle with respect to tendon indentation and joint rotation - An ultrasound study

Authors: *M. K. CHARDON, W. Z. RYMER
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Abstract: In human muscle, most of the passive length changes have been measured with respect to joint rotation. On the other hand, passive length changes measurements relative to tendon indentation are still relatively unknown. In fact, background search has failed to provide any previous work. In this study we sought to quantify passive length changes of the biceps brachii with respect to its distal tendon indentation. We describe the use of a novel tendon indentation device used to indent the tendon, an arm rotation device used to rotate the lower arm around the elbow and the use of sagittal ultrasound images of the biceps brachii to measure length changes. Our preliminary result shows that a 20 mm indentation is equivalent to a 15 degree arm rotation. We expect that the result will confirm that tendon indentations are physiologically equivalent to a range of joint rotation and thus can be used as an input to muscle stimulation protocols.

Disclosures: M.K. Chardon: None. **W.Z. Rymer:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

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Program#/Poster#:887.16/II19

Topic: D.13. Motor Neurons and Muscle

Support: Barrow Neurological Foundation

Title: Reverse frequency adaptation and discharge properties of rat hindlimb motoneurons in the subprimary range

Authors: V. V. TURKIN[1], D. O'NEILL[2], *T. M. HAMM[3]
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Abstract: The frequency-current (fI) relation of rat hindlimb motoneurons has an initial high-gain subprimary range that likely exerts a substantial influence on rate modulation on force development (Turkin et al., 2010). We compared responses of rat hindlimb motoneurons to 10-second injected current steps and ramps to determine the dependence of discharge characteristics on excitation profile and characterize the responses to sustained levels of excitation within the subprimary range. Comparisons were made in 8 antidromically identified motoneurons. Six of the 8 motoneurons exhibited an initial reverse frequency adaptation at smaller injected current

steps and discharge rates. In these cases the rate of discharge increased during the first 1-2 seconds before reaching a plateau or adapting. Discharge during reverse adaptation was variable and associated with interspike intervals that often exceeded the duration of the hyperpolarizing trajectory of membrane potential following each action potential. Reverse adaptation was observed at discharge frequencies less than the transition frequency between the subprimary and primary ranges in ramp-generated fI plots. Typical adapting responses were observed at larger currents and frequencies. Frequency-current plots derived from step responses had subprimary ranges in some cases, but the narrow current window of the subprimary range combined with irregular discharge during reverse adaptation and adaptation sometimes prevented conclusive determinations. Discharge variability, assessed as the standard deviation of the interspike interval (ISI) increased with mean ISI. This relation included an inflection as observed in human motor unit and cat motoneuron discharge. This inflection occurred at ISIs which were considerably shorter than the afterhyperpolarization (AHP) produced by an injected current pulse at resting membrane potential, but which approximately matched the duration of the hyperpolarizing membrane potential trajectory during discharge. These trajectories were substantially shorter than the AHP at resting membrane potential. These results suggest that time- and activity-dependent changes in motoneuron intrinsic properties and excitability following the onset of activity contribute to the subprimary range and the near-threshold discharge characteristics of rat motoneurons.

Disclosures: V.V. Turkin: None. **D. O'Neill:** None. **T.M. Hamm:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

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Topic: D.13. Motor Neurons and Muscle

Support: PROMEP-SEP Grant UATLX-PTC-109

PAPIIT-UNAM Grant 228110

CONACyT predoctoral fellowship 265483

Title: Expression of myogenic markers in a pelvic and a perineal striated muscle of nulliparas and multiparas rabbits

Authors: K. LÓPEZ-GARCÍA[1], I. JIMÉNEZ[2], E. CUEVAS[3], *F. CASTELÁN[3], P. PACHECO[4], M. MARTÍNEZ-GÓMEZ[4]

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Abstract: Pelvic and perineal skeletal muscles are critical for the physiology of the lower urogenital tract in human and other mammal species. Vaginal delivery causes structural and functional changes in both muscles that in women are related with the onset of pelvic organ prolapses and urinary dysfunctions. By using female rabbits we have previously found that multiparity affects the EMG activity during micturition and contractility properties of the pubococcygeus (Pcm; a pelvic muscle) and the bulbospongiosus muscles (Bsm; a perineal muscle). Those effects coincide with histological alterations suggesting a process of skeletal muscle regeneration, which is mediated by activation and differentiation of satellite cells. At our present work, myogenic markers expressed in diferent stages of skeletal muscle regeneration (Pax7, MyoD, MyoG and desmin) were measured by immunohistochemistry in the Pcm and the Bsm of nulliparous and multiparous (4 consecutive deliveries) age-matched rabbits. Additionally, the activity of muscle β-glucuronidase was measured as a parameter of muscle damage. Results showed a similar expression of Pax7 in the Pcm and the Bsm of nulliparas and multiparas. At postpartum days 3 and 20, the expression of MyoD and MyoG remains unchanged in the Pcm whereas a higher expression of both was found in the Bsm only at pospartum day 20. The expression of desmin was increased in the Bsm of multiparas from postpartum day 3 to 20, whereas in the Bsm only was high at postpartum day 20. A high activity of β-glucuronidase from total protein extracts of the Pcm was measured at postpartum day 20. For the Bsm, the activity of β-glucuronidase was high at postpartum day 3, returning to the basal one at postpartum day 20. Collectively, our results suggest that the particular expression of myogenic markers in the Pcm and the Bsm might be related with the extent of damage for each. The later may depend on fiber composition, contraction exerted because of pregnancy and delivery, and hormonal factors related to reproductive experience.

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Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

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Program#/Poster#:887.18/JJ1

Topic: D.13. Motor Neurons and Muscle

Support: CIHR MOP-37765

Title: Consequences of the wide spread distribution of HCN channels on the intrinsic resonance properties of the dendritic tree of neck motoneurons

Authors: *P. ROSE[1], K. FENRICH[2], R. MARATTA[1], M. NEUBER-HESS[1], E. ZHAO[1]
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Abstract: Hyperpolarization-activated cation (HCN) channels act as resonating conductances. In motoneurons this feature leads to a subthreshold resonance with a frequency of 6 to 20 Hz at the soma. As a consequence, the input-output properties of motoneurons depend, in part, on the frequency of oscillations in synaptic activity. The goal of the present study was to extend the description of motoneuron intrinsic resonance properties to include the dendritic tree.
As a first step to meet this goal, we used a combination of immunocytochemical and image analysis techniques to quantitatively describe the distribution of one of the subunits that form HCN channels, HCN1, on the somatic and dendritic membranes of intracellularly stained neck motoneurons in the adult cat. HCN1 subunits were labeled with a specific antibody (NeuroMab clone N70/28). Analysis of HCN1 labelling was constrained to juxta-membrane regions. For each motoneuron, 30 to 90 dendritic segments located 100 to 1800 μm from the soma were examined.
For 7 of the 10 motoneurons examined, membrane-associated density of HCN1 was greater on distal, compared to proximal, dendritic segments. The increase was a linear function of distance such that density increased 40 to 110% every 1000 μm. For the other 3 cells, the slope was statistically not different from zero. There was a negative correlation between the slope and the HCN1 density of the most proximal dendrites such that HCN1 density was similar in 9 of 10 motoneurons, 800 to 1200 μm from the soma.
To determine the functional consequences of the distribution patterns of HCN1 on the intrinsic resonance properties at different regions of the dendritic tree, we constructed compartmental models of motoneurons with uniform and non-uniform distributions of HCN channels that matched the experimentally observed distribution of HCN1 subunits. Despite the differences in HCN channel distribution, both models had similar intrinsic resonance properties. The subthreshold resonance increased from approximately 6 Hz on proximal dendrites to 11 Hz at the distal dendrites. Thus, different regions of the dendritic tree are tuned to different frequencies of input oscillations. This increase in computational power was associated with a large decrease in the efficacy of current transmission to the soma for input oscillations with frequencies of less than 0.5 Hz. These signals were five times weaker than the same signals on models with no HCN channels. Thus, HCN channels on motoneuron dendrites may amplify local responses to synaptic activity in a location and frequency dependent fashion and filter the transmission of synaptic currents generated by slow oscillations in synaptic activity.

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Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

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Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant AG035147

Title: Visuomotor correction in older adults increases the variability in muscle force and motor unit discharge in tibialis anterior

Authors: *A. M. GROSSMAN, M. FRITZ, A. AMIN, M. TRABERT, A. JONES, R. PAXTON, B. TRACY
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Abstract: Force variability and motor unit discharge variability is often greater for older adults. This effect has been shown for small hand muscles and proximal leg muscles, and is more robust and consistent at low forces and high visual feedback gain. It has been suggested this is due to impaired visuomotor processing by older adults. However, the effects of visuomotor processing on the variability of force and motor neuron discharge have not been well characterized in the ankle muscles of older adults. The purpose was to determine the effect of high gain visual feedback on the variability of force and motor unit (MU) discharge in the dorsiflexor (DF) muscles of older adults. Older adults (79 ± 7yrs, 67-94yrs, n=37) performed force steadiness trials with the DF muscles while trains of single MU action potentials (AP) were measured via fine wire electrodes inserted into the proximal 1/3 of the tibialis anterior muscle. Trials were performed with (VIS) and without (NVIS) visual feedback of the force. MUAP's were discriminated via computerized algorithm (Spike 2); each discharge was manually confirmed. Recruitment thresholds (RT) were measured during slow ramp contractions. The steadiness trials were performed at a target force just above the RT of the motor unit. The discharge behavior of 59 single MU's (1-2 per subject) were recorded for VIS and NVIS conditions. The mean, SD, and CV of the interspike intervals (ISI) were calculated for 7-20s (13.2 ± 2.6s) of data per trial (119 ± 29 MU discharges per trial). The mean, SD, and CV of force was calculated from the same force epochs. RT's ranged from 0.2-5.5% MVC; 60% of the MU's had an RT under 3% MVC. Mean forces during the steadiness task were low (4.1 ± 2% MVC), therefore the visual gain of the force feedback was high. The CV of ISI was correlated with CV of force for VIS

(r=0.5, P<0.0001) and NVIS (r=0.28, P=0.03). The mean ISI was correlated with the SD of ISI (r=0.66, P<0.0001) for NVIS, such that the variability of discharge increased with slower discharge rates. For NVIS, the mean force exerted was correlated with the SD of force (r=0.4, P<0.01), indicating signal-dependent noise. The CV of force declined with target force (r=-0.28,P=0.03). The mean force and the SD of force were similar between VIS and NVIS. The CV of force was greater for VIS than NVIS (3.9 ± 3.6% vs. 3.1 ± 2.8%, P<0.01). The CV of ISI was also greater for VIS than NVIS (14.5 ± 4.1% vs. 13.4 ± 4.0%, P=0.02). The results indicate that the variability of motor unit discharge contributes to the variability of force, and that older adults exhibit greater variability in the descending command to dorsiflexor motor units when visuomotor processing is an important contributor to the control task.

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Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

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Program#/Poster#:887.20/JJ3

Topic: D.13. Motor Neurons and Muscle

Support: Det Obelske Familiefond

Spar Nord Fonden

Title: Extracting motor modules as a measure of interlimb coordination

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Abstract: The muscular activation symmetry between lower limbs during tasks such as walking can be severely impaired following lesions of the Central Nervous System (CNS), resulting in a limping gait pattern and reduced stability. While it is well know that, even if the injury is located within one cerebral hemisphere, both sides are affected, less is known about the possible role of spinal circuitry connecting the muscles of the lower limbs. A spinal pathway connecting muscles of the lower legs have recently been described (Stubbs & Mrachacz-Kersting, J Neurophysiol: 102, 2009); this pathway is significantly affected in stroke patients and may contribute to gait asymmetry .
While it is widely suggested that the CNS may reduce the complexity related to the execution of

movement and the large number of degrees of freedom by activation of a few motor modules, there is still the need of deepening our understanding of how the CNS controls lower interlimb coordination during walking. This may possibly lead to important implications for locomotor rehabilitation in patient populations.
The existence of central pattern generator (CPG) controlling each leg is a common interpretation; nevertheless, the presence of ipsilateral muscular activity through the whole gait cycle would suggest a complex bilateral control of motorneurons weighted according to the biomechanical demand.
The aim of the current study is to develop a method to investigate interlimb coordination during walking and assess the independence of motor modules of the two legs during such a task. The protocol requires subjects to perform three different walking tasks. The first is normal walking, where motor modules seem to be phase shifted for each leg. During the other two tasks the natural coupling between the two legs is disturbed slightly, by altering the speed of one belt of the treadmill (split-belt walking condition) or considerably, by changing the direction of one treadmill's belt (hybrid walking condition). Muscle activity of trunk and limbs is recorded bilaterally from 16 pairs of muscles (32 muscles in total). Motor modules extracted unilaterally are compared with motor modules extracted bilaterally and during the different walking tasks. Preliminary data showed a higher independence of motor modules of each leg during hybrid compared to normal walking. With this protocol, we are currently investigating lower interlimb coordination in a group of healthy subjects.

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Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

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Topic: D.13. Motor Neurons and Muscle

Title: To rectify or not to rectify··· An inquisition into the purpose behind rectifying electromyographic data

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Abstract: Over the past 25 years much research has gone into understanding the functional coupling between modulation in the sensory-motor pathways and muscle motor unit firing

behavior. In many of these studies oscillatory brain activity, captured using electroencephalography or magnetoencephalography, is correlated using measures such as coherence or cross-correlation with ongoing muscle activity (electromyography). Typically, electromyographic (EMG) signals are full wave rectified prior to their correlation with other signals and the reasoning for this preprocessing step has remained unclear (Boonstra 2010). Recently, researchers have questioned this practice which has elevated the subject of EMG rectification to a topic of much debate. Proponents for rectifying have suggested it accentuates EMG spike timing (Halliday et al. 2010;Yao et al. 2007) and envelope information whereas opponents have rallied against its non-linear distortion of data, suggesting it is an unnecessary and potentially inappropriate step in data pre-processing (McClelland et al. 2012; Neto at al. 2010).

Here we enter into this debate by examining the effects of rectification or applying the Hilbert transform (Myers et al. 2003), another method of signal envelope extraction, on the identification of vestibulo - muscle coupling. Electrical vestibular stimulation provides the ability to add a known input signal to the vestibular system which induces well-characterized changes in muscle firing behavior.

Methods: We re-analyzed data from previous studies in which twenty sinusoidal stimuli (1 to 20 Hz) or a single bandwidth stochastic vestibular stimulus (0-20 or 0-25 Hz) was provided and surface EMG recorded from muscles of the lower limbs. We compared several EMG pre-processing methods (rectification, no rectification and Hilbert transform) to determine which best identifies co-modulation between the stimulus and muscle activity.

Results: We found at low frequencies (< 20 Hz) EMG co-modulation with the stimulus is visually apparent but is not present in the coherence between the stimulus and the un-rectified EMG however it is evident in both the rectified and Hilbert transformed EMG.

Conclusion: While the Hilbert transform may avoid creating the discontinuities associated with rectification of EMG signals, in practice we observed similar SVS-EMG coupling between 0-25Hz for these two methods. We conclude that envelope extraction of EMG data prior to coherence analysis is a necessary step in the detection of coupling between vestibular stimuli and ongoing muscle activity.

Disclosures: C. Dakin: None. **B.L. Luu:** None. **J. Blouin:** None.

Poster

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Topic: D.13. Motor Neurons and Muscle

Support: LM205694

TA176300

Title: Characteristics of sensory interactions in pubococcygeus muscle motorneurons of receptive and non-receptive female rats

Authors: *M. A. LARA GARCIA[1], O. LARA-GARCIA[1], E. CUEVAS[2], A. TOVAR[1], P. PACHECO[1,3]
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Abstract: A dramatic transitory female behavioral change during sexual activity is represented by the receptivity period observed in rodents. In late proestrous this phenomenon derives from hormonal effects on neuronal circuits; however, this assumption still has not complete evidence. Here, hopping to provide a direct evidence of a neural mechanism involved during sexual behavioral changes we analyzed in pubococcygeus muscle (Pcm) motorneurons of diestrous and proestrous rats, the plastic properties of its neural circuitry. **Method:** In deep i.p. urethane anesthetized rats, the left Pcm was exposed and two stainless steel electrodes (0.1mm diameter) were inserted at the middle of muscle (3-5mm tip separation). Bipolar recordings, mechanical stimulation (pressure, taping, brushing and traction) of the perineal skin (including clitoral sheath) or/and cervix stimulation with a glass probe were performed. **Result**s: In comparison with diestrous, late proestrous rats showed a reflex activity of Pcm fibers characterized by biphasic potentials of high frequency (20Hz approximately), and uniform amplitude. This reflex activity was easily obtained by gentle skin mechanical stimulation. Continuous pressure or traction evoked continuous tonic high frequency activity that stops, when the stimuli cease or when the cervix was touch during the traction. An after discharge with a duration related to perineal skin traction duration was usually recorded. Diestrous rats did not present this Pcm reflex activity. **Discussion:** Apparently, our procedure allows recording of two or three single muscular fibers that correspond to its motorneuron activity. The
reflexive activity represents the motorneural sensory activation during the receptive period through a circuitry with one of the following characteristics: a) Appearance of spinal cord transitional interneuronal hyper-reactive on sensory inputs; b) Facilitatory activity of Renshaw cells from Pcm motorneurons; c) establishment of bistable properties on Pcm motorneurons.

Disclosures: M.A. Lara Garcia: None. **O. Lara-Garcia:** None. **E. Cuevas:** None. **A. Tovar:** None. **P. Pacheco:** None.

Poster

887. Motor System: Motor Units and Muscle Fiber Physiology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:887.23/JJ6

Topic: D.13. Motor Neurons and Muscle

Support: NSERC

OGS

Micheal Smith Foundation for Health Research

Title: Muscle-dependent MU recruitment behaviour of the elbow extensors

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Abstract: The majority of studies investigating the elbow extensors report neuromuscular measures from the lateral head of the triceps brachii. Although many properties of this whole muscle group have been described (i.e. torque distribution among the parts), few have investigated the motor unit (MU) properties of the four contributing muscles and their relationship to force production. Initial studies showed surface-detected electromyography of the long and lateral head of the triceps brachii increased linearly with force, but suggested the anconeus muscle reached an upper limit of recruitment at 30% of maximal isometric elbow extensor force. However, these studies did not investigate beyond 30% of maximal isometric contraction (MVC) force output. The purpose of our study was to determine MU recruitment behavior of the long and lateral heads of the triceps brachii, and the anconeus in relation to ramp isometric force production up to 75%MVC. It was hypothesized that MUs of the anconeus would be activated at lower relative forces compared with the other two synergistic muscles, but that anconeus MU recruitment would continue above 30%MVC. Ten healthy males performed three elbow extensor MVCs to determine the subsequent target forces (25, 50, and 75%MVC) used for the ramp isometric contractions. Subjects then performed three ramp isometric elbow extensions at each target force (rate of 5%MVC per second) with at least 2 minutes rest between efforts. Force was recorded with a custom elbow joint dynamometer and intramuscular electromyography was recorded via pairs of fine wire electrodes inserted into the muscle bellies of the lateral and long heads of the triceps brachii, and the anconeus. Preliminary results indicate ~50% higher average recruitment thresholds in the long head of the triceps brachii compared with the other two muscles, which did not differ from one another. Despite a majority of the anconeus MUs being recruited at low relative forces, anconeus MU recruitment thresholds were observed at forces exceeding 40%MVC. These results show differences in MU recruitment of the muscles contributing to elbow extension and challenge previous results that indicated MU

recruitment in the anconeus was complete at or below 30%MVC.
Supported by NSERC, OGS, and Michael Smith Foundation for Health Research.

Disclosures: B. Harwood: None. **B.H. Dalton:** None. **G.A. Power:** None. **C.L. Rice:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:888.01/JJ7

Topic: D.17. Voluntary movements

Support: NIOSH UIC ERC T42 OH008672

Title: Effect of vibrotactile stimulation on the response time to handle perturbation

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Abstract: Background: Falls from ladders are a major cause of disabling injuries among workers. Early detection of fall initiation may increase a person's ability to rapidly grasp a ladder with the hand and arrest the falling body. One of the fall detection medium is cutaneous sensation in the hand skin, sensing changes in contact pressure and alerting a person to respond to the fall initiation or perturbation. The objective of this study was to determine if vibrotactile stimulation applied to the upper extremity can enhance a person's cutaneous sensation and accelerate his/her muscular response to perturbation of the hand grip.
Methods: Eleven healthy young adults (age: 25±5 years) grasped an overhead handle with minimal effort at the beginning of a trial. A sudden upward load (20% of grip strength) was applied to the handle at a random time. Subjects were instructed to stop the handle from moving up as quickly as possible. Vibrotactile stimulation was applied to one of the 4 locations of the upper extremity (middle fingertip, thenar area, volar forearm, and dorsal forearm) using a vibration device that can generate stochastic resonance (C-3 Tactor, Engineering Acoustics, Inc., Casselberry, FL). The stimulation intensity was set at 50% of the sensory threshold. The muscle reaction times to handle perturbation were quantified as the time intervals between perturbation and increase in RMS EMG for 8 muscles (Flexor Digitorum Superficialis, Flexor Carpi Ulnaris, Extensor Digitorum Communis, Biceps Brachii, Triceps Brachii, Anterior Deltoid, Pectoralis Major, Latissimus Dorsi). The earliest reaction time among the 8 muscles was used for analysis. Four paired t-tests were used to determine the effect of stimulation on the earliest reaction times for the 4 stimulation locations.
Results: The earliest reaction time to handle perturbation significantly decreased by 9% (p=.008)

with the stimulation applied to the middle fingertip pad (from 93 ± 3 ms to 85 ± 3 ms). The earliest reaction time did not improve when vibrotactile stimulation was applied to other locations ($p>.05$).
Conclusion: Vibrotactile stimulation at the fingertip demonstrated a potential to accelerate people's muscular response to handle perturbation. The present study's finding suggests that the use of vibrotactile stimulation can enhance a person's reaction time to handle perturbation and may help reduce fall injuries from ladders or scaffolds. Future studies may investigate the efficacy of vibrotactile stimulation on motor function of people with reduced sensation such as neuropathy and stroke.

Disclosures: P. Hur: None. **Y. Wan:** None. **N.J. Seo:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:888.02/JJ8

Topic: D.17. Voluntary movements

Support: NIH Grant NS035032

Kligman Endowment Fellowship

Title: Effects of thumb and index finger fatigue on hierarchical control and synergies in prehension tasks

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Abstract: We investigated the effects of thumb and index finger fatigue on digit forces and points of their application across multiple repetitions of a static prehensile task. The purpose of this study was to explore the ability of the central nervous system (CNS) to reorganize synergies at two levels of a hypothetical control hierarchy involved in multi-digit prehension under fatigue of a digit. At the higher level of the hierarchy, the total force and moment of force produced on an object are distributed between the thumb (TH) and the virtual finger (VF, an imagined finger with mechanical output equal to the involved fingers of the hand), while at the lower level the VF action is distributed among the four fingers. One group performed a one-minute fatiguing exercise with the thumb (*group-thumb*) and the second group performed a fatiguing exercise with the index finger (*group-index*). Following the fatiguing exercise, the subjects repeated the static prehension tasks. Fatiguing exercises caused a significant drop in the normal force

($p<0.05$) and an increase in the vertical tangential force ($p<0.05$) of the exercised digit. The contribution of the normal and tangential forces to the total moment also changed during fatigue in a reciprocal way (*Fatigue*×Group, $p<0.001$) to keep the handle in static equilibrium. For group-thumb, the normal forces generated a larger counterclockwise moment and the tangential forces generated a larger clockwise moment during fatigue. This trend was reversed for group-index. The safety margin of the thumb decreased significantly for *group-thumb* ($p<0.001$). Changes in synergies stabilizing the total grip force were observed at the higher level of control hierarchy (TH-VF) but not at the lower level. The results suggest adaptive adjustments in both mechanical output of the digits and co-variation patterns in multi-digit prehension tasks during fatigue.

Disclosures: T. Singh: None. **V.M. Zatsiorsky:** None. **M.L. Latash:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:888.03/JJ9

Topic: D.17. Voluntary movements

Support: Natural Sciences and Engineering Research Council of Canada

Alberta Innovates Technology Futures

Title: Development of grip and moment control during a grasp, lift, and hold task

Authors: *J. W. ROBERTSON[1,2,3], J. A. JOHNSTON[1,2,3,4]
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Abstract: The superposition principle is a motor control theory in which commands are divided into multiple components, processed separately, and then summated to form complex actions. When applied to grasping, commands are typically broken into grip control - control of forces to prevent object slipping - and moment control - control of forces to maintain object orientation. We have previously examined superposition during an unconstrained static holding task in which moments were exerted outside anatomically-defined axes by changing external mass location. We found the principle to hold true under these conditions, suggesting that it is highly robust. We also found separate control of moments about each anatomical axis (i.e., Mx, My, and Mz), implying a further partitioning of moment control. The objective of the current study was to examine how the separation of control develops over the course of a grasping task, i.e., through

grasp, lift, and hold. We hypothesized that the separation of grip and moment control, and that of the moments about different anatomical axes, would first appear just prior to lift onset.
Ten subjects were asked to grasp, lift, and hold an object vertically under eighteen moment conditions. Force and moment data from all digits were analyzed using principal components analysis (PCA) during several time periods throughout the grasp: specifically, the early and late force rise and lift phases, defined as the periods prior to and during the lift, respectively.
During the early force rise phase, the PCA showed grip force and moment on separate components, however the variables related to Mx, My, and Mz did not appear together on the same principal component, often appearing comingled with one another (e.g., an Mx variable on the same component as an Mz variable). Over the course of the grasp, the complete pattern previously observed during hold phase does evolve: during late force rise phase, sources of moments about the same axis began to coalesce together onto the same components; by early lift phase, all sources of moment for each axis were on the same components, in the same pattern as observed previously in the static hold phase. Taken together, these results suggest that while grip force and moment control remain separately controlled throughout the grasp, the separation of moments about different anatomical axes do not develop until later.
Our findings indicate that separate grip force and moment control are implemented upon contact with the object in a feedforward fashion, whereas separation of moments about individual anatomical axes is developed over the course of the grasp, and may require some feedback control.

Disclosures: J.W. Robertson: None. **J.A. Johnston:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:888.04/JJ10

Topic: D.17. Voluntary movements

Support: European Commission in Seventh Framework Programme FP7-248587

Title: Haptic-motor transformations for the control of fingertip distance

Authors: *D. SHIBATA, J. Y. CHOI, J. C. LAITANO, M. SANTELLO
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Abstract: Dexterous manipulation relies on the ability to modulate digit forces as a function of digit placement. Although many studies have been devoted to understand the sense of hand or digit position, little is known about the sense of position of the fingertips relative to each other.

We addressed this question using psychophysics to quantify subjects' ability to sense and reproduce the vertical distance between the center of pressure (CoP) of the thumb and index finger pads (d_y) of the right hand ("reference" hand, R_{hand}) using the same or contralateral hand ("test" hand, T_{hand}). The R_{hand} digits were passively placed to three d_ys: thumb CoP 30 mm higher or lower than index finger CoP (d_y = 30 and -30 mm, respectively), and collinear CoP's (d_y = 0 mm). When T_{hand} was the contralateral hand, d_y matching was performed synchronously or with a 10-second delay, both of which required subjects to transfer the sensory information from the R_{hand} to generate d_y with T_{hand}. The matching task involving a delay required subjects to retrieve memory of perceived d_y. We also examined how accurately subjects reproduced d_y by using a different hand posture relative to the R_{hand}. We hypothesized that the matching absolute error (E_{abs}, absolute difference between R_{hand} and T_{hand} d_y) would be (a) greater in the collinear than non-collinear digits position, (b) greater when the posture of the R_{hand} is different than the posture of the T_{hand} hand, and (c) greater when subjects reproduced d_y using the contralateral hand. We also measured the relative error (E_{rel}) by taking into account the directionality of matching errors.

Subjects made larger E_{abs} when matching the collinear d_y (13.9 ± 5.7 mm: mean ± SD) than non-collinear d_y's (d_y = 30 mm: 9.8 ± 2.6 mm, d_y = −30 mm: 10.0 ± 2.3 mm; $P < 0.05$). Larger E_{abs} was produced when the posture of T_{hand} and R_{hand} differed (12.3± 3.4 mm; $P < 0.05$) and when T_{hand} was the contralateral hand (12.2 ± 3.5 mm; $P < 0.05$). E_{abs} was smallest when the task involved the ipsilateral hand with the delay and with congruent hand postures across T_{hand} and R_{hand} (8.7 ± 2.1 mm; $P < 0.05$). With regard to E_{rel}, positive error was produced with delay while negative error was produced with no delay. These findings indicate that the accuracy with which perceived fingertip distance is transferred across hands is lower than when it is reproduced with the same hand. Moreover, the 10-s delay changes the direction of matching error when the task is performed with the contralateral hand. We propose that the erroneous representation of fingertip distance, if not compensated for between contact and onset of manipulation, might lead to performance errors as digit forces have to be modulated to perceived digit placement.

Disclosures: D. Shibata: None. **J.Y. Choi:** None. **J.C. Laitano:** None. **M. Santello:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:888.05/JJ11

Topic: D.17. Voluntary movements

Support: PRODEX

European Space Agency

Interuniversity Attraction Poles (Belgium)

FNRS (BELGIUM)

FRSM (BELGIUM)

ARC (BELGIUM)

Title: Grip force adaptation to dynamic torques during object manipulation in weightlessness

Authors: *T. GIARD[1], J. MCINTYRE[2], F. CREVECOEUR[3], J.-L. THONNARD[4], P. LEFÈVRE[1]
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Abstract: In many situations, we manipulate objects grasped away from their center of mass, which induces torques applied tangentially to the grip surfaces when the object is accelerated. When the object configuration induces a tangential torque relative to the grip axis, we previously showed that the grip force exhibits a gradual and slow decrease as subjects learned to manipulate the unbalanced object (Crevecoeur et al. 2011). The specific contribution of a tangential torque in the sensorimotor control of grip force remains unexplored and very hard to dissociate from usual loads experienced during object manipulation.
To investigate this issue, we asked six naive human subjects to perform vertical point-to-point arm movements in the weightless conditions induced by parabolic flight. The weightless condition presents the advantage to remove gravitational forces, thereby isolating the effect of inertial forces in the control of grip force (GF). Participants were holding an instrumented object with an off-center precision grip (mass 360 g, including an 40 g mass placed 10 cm away from the grip), thus inducing tangential torques. They performed the task only during the micro-gravity phase (22 seconds) of 30 parabolas each. Four target LEDs were placed along a vertical structure. The program generated a random sequence of targets at a frequency of 1Hz. They were asked to move to and stabilize the object at the current target as fast as possible until the next target was illuminated.
Such movements typically presented static and dynamic phases that permit a distinction to be made between a static and dynamic component of the GF. The static component of the GF used during the five first parabolas was high (above 20 N). We observed a gradual decrease of this component across the parabolas with a stabilization between the fifth and the tenth parabolas. In spite of similar adaptation rate, the average static grip force across the five last parabolas (around 10 N) were much higher than during static phases of similar movements made in absence of a tangential torque (Crevecoeur et al. 2009). Hence, 0-g specific adaptation was unchanged across

conditions including or not the presence of a tangential torque. Furthermore, within each parabola, subjects seemed also to stabilize the static component of the GF after 5 movements. Finally, we observed a modulation of the dynamic component of the grip force that was scaled to the tangential torque loads. These results demonstrate that inertially-generated torque loads contribute to the higher level of static grip force that characterize manipulation of an unbalanced object.

Disclosures: T. Giard: None. **J. McIntyre:** None. **F. Crevecoeur:** None. **J. Thonnard:** None. **P. Lefèvre:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:888.06/JJ12

Topic: D.17. Voluntary movements

Support: PROgramme for the Development of Scientific EXperiments (PRODEX)

European Space Agency (ESA)

FNRS (BELGIUM)

ARC (BELGIUM)

Interuniversity Attraction Poles (BELGIUM)

Title: Movements should be slower on Moon than on Mars

Authors: *V. THEATE[1], P. LEFÈVRE[1], J.-L. THONNARD[2]
[1]ICTEAM / IONS, [2]IONS, Univ. Catholique De Louvain, Brussels, Belgium

Abstract: Slower movements in weightlessness and faster movements in hyper-gravity have already been observed. However, few studies have investigated the effect of partial gravity on human movements and especially on oscillatory movements. In this study, we investigated the influence of the gravity level on periodic movements and then we focused on the underlying motor control.
The dynamics of precision grip, the kinematics and the muscular activities of the upper limb have been investigated in six subjects performing oscillations of their right arm in Lunar gravity (0.16g), Martian gravity (0.38g), Microgravity (0g) and Hyper-gravity (1.6g). Subjects were asked to perform oscillations of the upper limb spontaneously at their most comfortable

frequency. These oscillations were executed between two LED targets with the arm extended (Figure 1). Results showed that for most of the subjects the grip force was better coordinated with the load force under Lunar and Martian gravity than under Microgravity. We also observed that the oscillation periods decreased as the level of gravity increased from 0.16g to 1.6g. Interestingly, it was not the case in Microgravity (Figure 2). In addition, time varying activities of the Deltoid and the Latissimus Dorsi muscles were observed through the different levels of gravity. The movement slowing down as well as the changes in muscular activity enable a decrease of the overall joint impedance when the gravity level decreases. The analysed process of the muscular activity emphasized that the Central Nervous System optimizes the control by modulating the frequency of the oscillations in function of gravity. Our results also showed that the case of weightlessness is singular, confirming previous results (White, 2008) supporting that a central pattern generator issued from the spine regulates the frequency of an oscillatory movement in the absence of gravity.

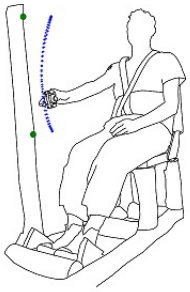


Figure 1 : Task illustration

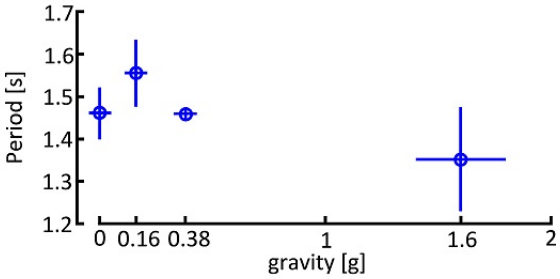


Figure 2 : Means and standard deviations of the oscillation periods across gravity for one subject

Disclosures: V. Theate: None. **P. Lefèvre:** None. **J. Thonnard:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:888.07/JJ13

Topic: D.17. Voluntary movements

Support: BrainGain

Title: Cortical electrophysiological representation of hand gestures: A human ECoG study decoding sensorimotor function

Authors: *M. BLEICHNER, Z. V. FREUDENBURG, M. J. VANSTEENSEL, E. J. AARNOUTSE, F. S. S. LEIJTEN, N. F. RAMSEY

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Abstract: From electrostimulation, lesion and imaging studies it is known that our hands and fingers are topographically represented on the motor and sensory cortex. Using electrocorticography (ECoG) it has been shown that it is possible to distinguish the movement of individual fingers. However, complex hand movements as they are used for example in sign languages have gained little attention so far. While much is known about the language aspect of sign language the motor representation of complex hand gestures has not been studied thoroughly.
Here, we address the question of how complex hand gestures are electrophysiologically represented on the sensorimotor cortex and investigate the possibility to differentiate hand gestures.
Five patients, undergoing epilepsy surgery and naïve to sign language, were implanted with high-density subdural electrode grids (32 electrodes, 1.3 mm exposed surface, inter electrode distance 3 mm center to center) for one week. The electrodes were placed over the central sulcus covering pre- and postcentral areas where the hand function was expected (based on preoperative fMRI). Patients were asked to perform four hand gestures taken from the American Sign Language alphabet. Each gesture was repeated 10 times in a randomized order. A data glove measuring the bending of each finger verified the correct execution of the gestures.
Data was epoched into individual trials and corrected for movement onset. The power in the 65Hz to 95Hz range was computed for all electrodes for a 2000ms window around movement onset. A matched filter leave-one-out classification was performed for each dataset. Classification accuracy varied between 65% and 95% (chance level 25%).
We conclude that the surface of the sensorimotor cortex shows a gesture-selective pattern of high-frequency-band activity.
The classification accuracy depended on the position of the electrodes: as expected the most informative electrodes were located on M1 hand knob. On average ten adjacent electrodes were sufficient to reach the best classification result.
We showed here that classification of complex hand gestures is possible using high-density grids located on the motor and sensory cortex. A very simple and straightforward classification procedure was sufficient to achieve optimal results in some cases. The information used for classification was comprised to a small area of cortex.
Our findings have important implications for the decodability of imagined/attempted gestures for multiclass brain computer interface (BCI) control in paralyzed people.

Disclosures: M. Bleichner: None. **Z.V. Freudenburg:** None. **M.J. Vansteensel:** None. **E.J. Aarnoutse:** None. **F.S.S. Leijten:** None. **N.F. Ramsey:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:888.08/JJ14

Topic: D.17. Voluntary movements

Support: 7th Framework Programme of the European Union, FP7-287888

Title: Anticipatory and reactive grip force modulation during object handover

Authors: *S. ENDO, A. M. WING
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Abstract: Passing an object to another person is a commonly performed motor task. During the transfer, the motor commands need to be scaled, not only with respect to the dynamics of the object, but also to those of the receiver. To achieve a smooth object transfer with limited time available for sensory feedback correction, learning and predicting the task partner's movement becomes important. The present study investigated contributions of interpersonal anticipatory and online control in transferring an object from one person to another.
Pairs of participants (passer and receiver) were instructed to transfer an object as they would naturally perform the task while sitting. In Standard trials, the receiver (a confederate of the experiment) accepted the object from the passer as naturally as possible. In Perturbation trials, the receiver lightly placed the index and middle fingers and the thumb on the object (< 1 N) but did not exert sufficient force to support the object alone. There were 180 Standard trials and 20 randomly occurring Perturbation trials. Grip force (normal to the grasp surfaces) and the interaction force of the pair (longitudinal force between the partners) were measured using three 6 DoF force/torque sensors embedded in a custom-made plastic housing. The total weight of the object was approximately 150 g.
The results showed concurrent grip force modulation by the passer and receiver. As the passer reduced grip force, the receiver increased the grip force, keeping the net grip force between the pair at a similar level in the Standard trials. The cross-correlation function further revealed a slight lead of the Passer in modulating grip force. In Perturbation trials, the passer successfully detected the lack of grasping by the receiver and readjusted the grip force within 150 ms after the first contact, highlighting the online motor correction by the passer to counteract a motor error of the receiver. Furthermore, detailed analysis showed a difference in the motor strategy before and after the perturbation such that the grip force modulations of the partners became more sequential with smaller force used by the passing partner at the next trial after perturbation. Nevertheless, there was quick learning and these measures returned to baseline within 3-4 trials.
These results indicate that the grip force modulations during object transfer involve both anticipatory and feedback control in case of unexpected event. Furthermore, unexpected grasping control by a receiver affects the subsequent grasping of the passer. In conclusion, the present

study highlights the role of feedforward and feedback mechanisms in grip force modulation during interpersonal object transfer.

Disclosures: S. Endo: None. **A.M. Wing:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

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Program#/Poster#:888.09/JJ15

Topic: D.17. Voluntary movements

Support: NIH Grant NS27484

Title: Hand muscle activity for digital coarticulation in pianists

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Abstract: Highly trained pianists are capable of individual finger movements that reflect their enhanced ability to restrict movement and force production of non-striking digits. Examinations of the kinematics of professional pianists have revealed that for individual keystrokes, the movement of the striking digit is consistent while the movement of non-striking digits varies depending on prior and subsequent keystrokes. With training, enhanced motor cortical excitability and plasticity has been observed during finger movements. However, at the neuromuscular level, the patterns of muscle activation that accompany these complex movement patterns are not known.

The purpose of this study was to characterize the temporal waveforms of multi-muscle activation patterns in highly trained pianists. Ten subjects (4 Male; 35±10 yrs) were included in the study. Each subject performed 14 different musical scores that required the use of the right hand. Prior to data recording for each new score, subjects were allowed to practice the musical score in order to play consistently and accurately. Each of the 14 scores was played for 10 successful trials at a normal tempo (125 ms ISI) provided by a metronome. Surface EMG was recorded from three portions of extrinsic finger muscles: a central portion of extensor digitorum (ED) and two portions of flexor digitorum superficialis, one closer to the middle finger (FDS) and the other closer to the ring finger (FDS2). Surface EMG was also recorded from two intrinsic thumb muscles: abductor pollicis brevis (APB) and flexor pollicis brevis (FPB), as well as two intrinsic finger muscles: first dorsal interosseus (FDI), and abductor digiti minimi (ADM).

Patterns of muscle activity were examined using Principal Component Analysis (PCA) and cluster analysis. Two to four PCs were required to account for 75% of the variance in the

patterns of muscle activity for an individual striking digit. For all subjects, thumb strikes required only two or three PCs. For individual finger strikes, the need for higher order PCs varied across subjects such that the professional pianists within our sample tended to require more PCs to account for the same amount of variance. Therefore, when performing the same keystrokes, professional pianists used more variable patterns of muscle activation, which were described by higher order PCs. Clustering patterns of muscle activity according to fingering sequences also revealed differences that may reflect the use of more complex patterns of coarticulation by the professional pianists.

Disclosures: S.A. Winges: None. **S. Furuya:** None. **M. Flanders:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:888.10/JJ16

Topic: D.17. Voluntary movements

Support: NIH Grant R01-NS050265

"THE Hand Embodied" European Commission with the Collaborative Project no. 248587

Title: Partial transfer of dexterous manipulation across hands reveals limited generalization of learned object dynamics

Authors: *J. Y. CHOI, M. JESUNATHADAS, M. SANTELLO
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Abstract: The extent to which learned dexterous manipulations can be transferred across hands is not well understood. We asked subjects to learn two-digit manipulation of an object with an asymmetrical CM (left or right CM) after which they either translated the object (Experiment 1), or translated and rotated the object (Experiment 2) to the contralateral hand. For Experiment 1, subjects had to exert T*com* in the same direction (object-frame of reference) with both hands, whereas for Experiment 2 they had to exert T*com* in opposite directions. Each experiment consisted of 8 consecutive trials followed by one trial where the object was either translated (T) or translated and rotated (T+R). This sequence was repeated four times. For both experiments, based on previous work on grasping at constrained contacts we hypothesized that initially subjects would be able to transfer the direction but not the magnitude of T*com* on the first post-T or –T+R trial. We further hypothesized that subjects would learn to transfer both T*com* magnitude and direction with further practice with object T and T+R.

We found that subjects failed to transfer learned manipulation on the first trial with the contralateral hand. Specifically, T*com* on the first post-T and post-T+R trial was significantly smaller (on average, 15% and 10%, respectively) then T*com* on the preceding trial ($P < 0.05$). However, for both experiments, subjects exerted T*com* in the direction appropriate to CM location (McNemar's test). Specifically, when comparing pre- vs. first post-T trial, direction of T*com* was not significantly different ($P > 0.05$) whereas pre-T+R and first post-T+R trial revealed a significantly different T*com* direction ($P < 0.05$). By the 4th T and T+R trial, the magnitude of T*com* was statistically indistinguishable from T*com* exerted at the end of the first sequence of consecutive trials ($P > 0.05$) and also exerted in the direction appropriate to CM location ($P > 0.05$, $P < 0.05$; Experiment 1 and 2, respectively).
These findings indicate that subjects correctly identified the object mass distribution but could not transfer the magnitude of T*com*. However, after further practice with object T and T+R, subjects might have been able to retrieve sensorimotor memory of previous manipulations and plan manipulation accordingly despite having to transfer or rotate T*com* direction, respectively.

Disclosures: J.Y. Choi: None. **M. Jesunathadas:** None. **M. Santello:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:888.11/KK1

Topic: D.17. Voluntary movements

Title: Sophistication of dexterous finger movements through daily piano practice

Authors: *A. NAKAMURA[1], T. GODA[1], H. MIWA[1], N. NAGATA[1], S. FURUYA[2]
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Abstract: Plasticity of the nervous system enables skilled hand movements following training and rehabilitation. Previous studies that compared pianists and non-musicians have demonstrated that extensive piano practice elicits structural and functional changes in motor-related brain regions, which endows dexterous finger movements. However, little has been understood about the causal relationship between piano practice and hand motor functions. The present longitudinal study aimed to straightforwardly determine effects of daily piano practice on hand motor functions such as speed, accuracy, and independence of finger movements. Six right-handed adult participants with no history of piano playing were asked to play a short tone sequence consisting of twelve strokes with the left hand synchronized with a metronome (two

strokes per second) for fifty trials per day over four successive days. Before and after four-days of practice, several tests to assess fundamental hand motor functions were carried out. Timing and velocity information of each keypress was collected from an electric piano. Thirteen high-speed cameras also recorded finger movements, and flexion/extension joint angles at the metacarpo-phalangeal, proximal-phalangeal, and distal-phalangeal joints were computed at the index, middle, ring and little fingers. Surface EMG was recorded from the extrinsic finger flexor and extensor muscles. Following the practice, the participants exhibited a significant decrease in temporal variability of keystrokes, indicating improved accuracy of finger movements. During playing the trained sequence as fast as possible, the maximum rate of keystrokes also increased after the practice, confirming enhancement of finger movement speed. The untrained right hand also showed improvement of both accuracy and speed following the left-hand practice, which suggests a transfer effect of uni-manual practice on the contra-lateral hand. A fastest finger tapping test, which required repetitive and fastest keystrokes by one finger with keeping the remaining digits depressing the adjacent keys, demonstrated an improvement in maximum movement rate following the practice at each of all fingers except for the ring finger. This suggests different plasticity across fingers in terms of independent control. A correlation analysis of movements across fingers supported for this view by demonstrating non-uniform improvements of movement independence across fingers following the practice. Finally, the finger muscular coactivation decreased with daily practice, presumably reflecting minimization process of energetic cost of movements.

Disclosures: A. Nakamura: None. **T. Goda:** None. **H. Miwa:** None. **N. Nagata:** None. **S. Furuya:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:888.12/KK2

Topic: D.17. Voluntary movements

Support: Fondazione del Monte di Bologna e Ravenna

Title: Role of online visual feedback on digit force-position coordination for dexterous manipulation.

Authors: A. BOSCO[1], Q. FU[2], N. GAW[2], *P. FATTORI[1], M. SANTELLO[2]
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Abstract: We studied the effect of online visual feedback on subjects' ability to perform dexterous manipulation. The manipulation consisted of grasping and lifting an object straight by counteracting an external torque caused by a mass added to the left or right of the object base. Three groups of subjects (group 1 = 6; group 2 = 9; group 3 = 10) were tested in one of three experimental conditions: (1) "No vision", (2) "No vision - 10 cm", and (3) "No vision - 20 cm". For conditions (2) and (3), vision of the hand and object during the reach was removed when the hand crossed a distance of 10 and 20 cm from the object, respectively. For condition (1), vision of hand and object was removed at reach onset. We quantified the effect of removing visual feedback on peak object roll, the torque exerted at object lift onset required to counter the external torque on the object (compensatory torque), vertical distance between thumb and index finger center of pressure, and grip and load forces. Subjects learned to minimize object roll in the "full vision" condition after 4 trials, after which peak object roll and compensatory torque remained relatively invariant. All subjects of group (2) and (3) were able to generate a suitable compensatory torque in both of these partial vision conditions. In contrast, group (1) presented a significantly higher compensatory torque in the "No vision" condition ($P<0.05$). No significant differences were found for the other variables for group (2), whereas group (3) exhibited larger grip and smaller load forces in no vision trials ($P < 0.05$). Lastly, no vision trials from groups (1)-(3) showed a significantly larger number of peaks in grip force derivative compared with the vision condition ($P<0.05$). This preliminary analysis suggests that online visual feedback is particularly important for the early phases of force development following contact. Nevertheless, tactile and proprioceptive feedback is sufficient to enable subsequent modulation of fingertip forces as a function of digit placement for object manipulation.

Disclosures: A. Bosco: None. **Q. Fu:** None. **N. Gaw:** None. **P. Fattori:** None. **M. Santello:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

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Program#/Poster#:888.13/KK3

Topic: D.17. Voluntary movements

Support: Prodex (contract nos. 90063, 90064, 90231, 90232)

NANOBIOTOUCH- project N°EU-FP7-NMP4-SL-2009-228844

Title: Frictional interactions in the contact zone of the finger pad before and during slipping.

Authors: *J.-L. THONNARD[1], B. DELHAYE[2], T. ANDRÉ[1], P. LEFÈVRE[2]
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Abstract: Grip function depends on the neurophysiological response to frictional interactions in the contact zone of the finger pad. The early adjustment to a new frictional condition, which may appear soon after the object is initially touched (ca. 0.1-0.2 s), depends on the vigorous responses of the mechanoreceptors. In order to prevent slip, the grip/load force ratio must exceed a minimal value determined by the coefficient of friction between the skin and the object, i.e. the critical ratio at which the slip occurs. The initial slip of a finger pad occurs by the propagation of an annulus of failure from the perimeter of the contact zone to its center, and this phenomenon, namely the incipient slip, is of importance in grip function. In this presentation, we will describe the complex skin movements observed during the transition from a stuck state to a fully developed slip, and the dependency of these events on skin hydration. Skin deformation inside the contact region was imaged with an optical apparatus that allowed us to quantify the relative sizes of the slipping and sticking regions. The ratio of the stuck skin area to the total contact area decreased from 1 to 0 when the tangential force component increased from 0 to a maximum. The slope of this relationship was inversely correlated to the normal force component. The skin hydration level dramatically affected the dynamics of the contact encapsulated in the course of evolution from sticking to slipping. The specific effect of moisture was to reduce the tendency of a contact to slip. In conclusion, a better knowledge of the fingertip skin deformation before and during slipping may contribute to understand how we can prevent the objects from slipping during their manipulation.

Disclosures: J. Thonnard: None. **B. Delhaye:** None. **T. André:** None. **P. Lefèvre:** None.

Poster

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Topic: D.17. Voluntary movements

Support: Japan Science and Technology Agency

Grant-in-Aid for Scientific Research

Title: Effect of visual feedback information on interpersonal force production

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Abstract: In human motor system, visual feedback plays an important role for updating the system and for maintaining the optimal performance. Previous studies on continuous force matching tasks demonstrated that the force was decreased exponentially within a few seconds after the removal of visual feedback information. This phenomenon was considered as reflecting a decay of visuomotor memory and was found both in unilateral and bimanual conditions. While these conditions involved intrapersonal organization of the performance, few studies have yet examined modulation of the force production in the interpersonal condition. This study examined how visual feedback information would affect a continuous force production task when the tasks were performed interpersonally.
Ten pairs (20 participants) performed continuous force production tasks, the goal of which was to match continuous forces exerted by the index finger on a given target force as accurately as possible for 20 seconds. Participants did this by observing two cursors representing each force (single condition) or one cursor representing the force averaged from the pair (joint condition). These tasks were performed under three visual feedback conditions: full visual feedback, no visual feedback, and pseudo visual feedback. In no visual feedback condition, visual feedback on the force level was withdrawn for the last 10 seconds of each trial. In pseudo visual feedback condition, the participants observed pseudo signals that were similar to their preceding signals under full visual feedback but the force was not reflected. The pseudo signals were created by surrogating methods in which the phases in frequency domain were randomized. Few participants recognized those as ‘pseudo’ signals in the experiment. The pairs performed four sets of the above conditions with a rest. Force modulation was evaluated by comparing the averaged force for the final two seconds with the force for two seconds before the visual conditions were imposed.
The results showed differential modulations of the force production among visual feedback conditions. Under no visual feedback condition, force decay was shown both in the single and joint condition and there was no significant difference between the two conditions. In contrast, pseudo signals induced increases of the force in the single condition and the degree was larger than that in the joint condition. These results suggest that the effect of visual feedback information on continuous force production depends on whether the production is performed individually or jointly.

Disclosures: M.O. Abe: None. **K. Watanabe:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

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Topic: D.17. Voluntary movements

Support: DLR grant 50WB0825

Title: Differences of manual grasping in a typical laboratory and in an everyday-like scenario

Authors: *O. L. BOCK, F. STEINBERG
German Sport Univ., Koln, Germany

Abstract: We have shown before that grasping movements from the same starting point to the same object of interest exhibit different kinematic and dynamic properties when they are executed in a typical laboratory context versus in an everyday-like scenario; those differences could be reduced to five orthogonal factors. The present experiments explore possible reasons for this observation.
In Exp. 1, we found that different grasping factors correlate with different cognitive abilities, namely, with overall psychomotor speed, attention focussing and a preference for controlled processing.. Exp. 2 yielded that withdrawing attention increased, and a higher task complexity decreased the context-dependence for one of the grasping factors.
The presence of factor-specific effects provides external validity to the view that contextual cues affect multiple sensorimotor processing stages. More specifically, the pattern of findings suggests that those stages are differentially sensitive to the speed, complexity and attentional load of a task. Possibly, these task characteristics modulate the distribution of sensorimotor processing between the ventral and the dorsal occipito-frontal stream.

Disclosures: O.L. Bock: None. **F. Steinberg:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

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Program#/Poster#:888.16/KK6

Topic: D.17. Voluntary movements

Title: Effect of cold-induced shivering on precision grip

Authors: *M.-A. IMBEAULT[1,2], R. BALASUBRAMANIAM[3], F. HAMAN[1,2]
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Métabolisme, Inst. de Recherche de l'Hôpital Montfort, Ottawa, ON, Canada; [3]Kinesiology, McMaster Univ., Hamilton, ON, Canada

Abstract: The aim of the study was to investigate the effects of cold-induced shivering intensity on precision grip. Using a liquid-conditioned suit, six non-acclimatized men had their whole body (except for hands, feet and face) exposed to cold (4ºC) for 90 minutes. Precision grip tasks (object manipulation) were performed before and after 90 min of cold exposure. In the object manipulation task, participants were asked to lift an object (250g) and either maintain it stationary or oscillate the object vertically for 20 sec. Shivering intensity (3.7 ± 0.5 %MVC) and oxygen consumption increased significantly (p= 0.0009) during cold exposures while core temperature remained stable throughout exposure. Skin and finger temperatures significantly decreased by 7 ± 0.2 ºC and 10 ± 0.7 ºC, respectively, during cold exposure compared to baseline ($p < 0.0001$). After cold exposure, grip force: load force ratio (GF:LF ratio) in both stationary and oscillatory tasks were 60 ± 22 % and 35 ± 11 % higher compared to baseline values (p= 0.05 and p= 0.04, respectively. No significant correlation was observed between shivering intensity and the increase of GF:LF ratio(%) but a strong correlation was observed between skin temperature and the % increase of GF:LF ratio in the oscillatory task ($r^2 = 0.77$, $p = 0.02$). These results suggest that despite no direct exposition of the hands, the sensorimotor control of precision grip was impaired after 90 min of cold exposure and skin temperature seems to be a good indicator of the alteration.

Disclosures: M. Imbeault: None. **R. Balasubramaniam:** None. **F. Haman:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

Location: Hall F-J

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Program#/Poster#:888.17/KK7

Topic: D.17. Voluntary movements

Title: Modulation of resting state and task-related activity by bihemispheric motor cortex stimulation in older adults

Authors: R. LINDENBERG[1], L. NACHTIGALL[1], M. MEINZER[1], *A. FLOEL[2]
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Abstract: Bihemispheric transcranial direct current stimulation (“dual” tDCS) of primary motor cortices has been described to enhance motor learning in healthy subjects and to facilitate motor recovery after stroke. In order to investigate the neural correlates of its mode of action, we

compared different tDCS montages in a group of healthy older adults in a cross-over design (‘dual’ vs. ‘anodal’ vs. ‘sham’).
20 subjects (mean age 68.7±4.7 years, all right-handed) underwent tDCS and simultaneous MRI at 3T, including resting state fMRI and a choice reaction time task. In the task, subjects were presented with different symbols in a randomized order and required to respond with button presses using either left or right index fingers. In both active stimulation conditions, the anode was placed over left primary motor cortex (M1). The cathode was positioned over right M1 (‘dual’) or the contralateral supraorbital region (‘anodal’). The current was constantly delivered during resting state and task-related fMRI. Task-related fMRI analysis was carried out with SPM8; resting state data were analyzed with Lipsia using low-frequency spectral Eigenvector Centrality Mapping (ECM).
In the task-specific fMRI analysis, an effect of stimulation condition became apparent: When subjects tapped with their right index finger, beta values of both left and right M1 were significantly higher in the ‘dual’ condition when compared to ‘anodal’ and ‘sham’; the latter conditions did not differ from one another. When subjects tapped with their left index finger, ‘dual’ yielded an elevation and ‘anodal’ yielded a reduction of beta values in bilateral M1 as compared to ‘sham’. In the resting state analysis, ECM values in the left dorsolateral prefrontal cortex (DLPFC) and posterior cingulate cortex were higher in the ‘dual’ condition as compared to ‘sham’; in contrast, ‘dual’ yielded significantly lower values in the right insula. ‘Anodal’ showed higher ECM values in the left DLPFC as well as left ventral premotor cortex when compared with ‘sham’; lower ECM values were found in the left precuneus (all $p<.001$, Monte Carlo-corrected).In conclusion, tDCS induced specific changes of M1 activation in the task-specific fMRI. As a complement, the resting state analysis demonstrated altered connectivity within a multimodal network including non-primary motor cortices as well as prefrontal and parietal regions, indicating that the previously documented stronger behavioral effects of dual as compared to anodal tDCS may not be merely mediated by a “simple” add-on effect of cathodal stimulation, but rather due to complex bihemispheric network modulations.

Disclosures: R. Lindenberg: None. **L. Nachtigall:** None. **M. Meinzer:** None. **A. Floel:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

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Program#/Poster#:888.18/KK8

Topic: D.17. Voluntary movements

Support: NSERC DG

U of L

Title: An evolutionary account of handedness

Authors: *C. L. GONZALEZ, T. RUTSCHMANN, J. W. FLINDALL
Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Many theories have been put forward to explain the origins of right-handedness in humans. Here we present evidence that this preference might have emerged from a right hand advantage in grasping objects for feeding. Fifteen participants were asked to pick up food items of 3 different sizes: Cheerios® (avg. 11 mm in diameter), Froot Loops® (avg. 15 mm), and Oatmeal Squares (avg. 21 mm). Participants used their right- and their left-hands in two separate blocks (50 trials each, starting order counterbalanced) to pick up the items. Within each block participants were asked to either eat the food item or to place it inside the pouch of a bib worn beneath their chin (25 trials of each, blocked design). The bib condition was included to ensure that the outward and inward trajectories were similar to the eating condition, but removing the final step of placing the food inside the mouth. Participants wore PLATO liquid crystal goggles that blocked their vision between trials. Each trial began with the goggles switching from opaque to clear. After 1000 ms of full vision, participants were cued to reach by an auditory cue (i.e., 'beep'). All trials were conducted in closed-loop with 5000 ms of vision before the goggles switched to opaque, at which time the experimenter placed a new food item for the next trial. Hand kinematics were recorded by an Optotrak Certus (NDI, Waterloo, ON, Canada), which tracked the three-dimensional position of three infrared light-emitting diodes attached separately to the index finger, thumb, and wrist. We found a hand (left/right) by action (eat/bib) interaction in the maximum grip aperture (MGA; the maximum distance between the index finger and thumb achieved in flight prior to the grasp). MGAs were smaller in the right hand but only when picking up the items with the intent to eat them. In fact, follow up t-test show that the right-hand/eat condition was significantly different from all other conditions which in turn did not differ among themselves. Because smaller grip apertures are typically associated with greater precision, our results demonstrate a right hand advantage for grasping objects to eat. From an evolutionary perspective it would make sense to resort to the hand that could grasp food with more precision maximizing the chances of successful retrieval, consumption, and thus, survival. If the right hand is better equipped for that task (as shown here), this could explain in part how we became right-handed.

Disclosures: C.L. Gonzalez: None. **T. Rutschmann:** None. **J.W. Flindall:** None.

Poster

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Topic: D.17. Voluntary movements

Support: FIRCA - (NIH, USA)

ANPCyT (Argentina)

Title: The time course of corticospinal excitability during action observation is consistent with a muscle, but not goal or kinematic based mechanism

Authors: *S. MC CABE[1], G. SAUNIER[2], J. I. VILLALTA[1], S. T. GRAFTON[3], V. DELLA-MAGGIORE[1]
[1]Physiol. of Action Lab- Sch. of Medicine- Univ. of Buenos Aires, Ciudad Autonoma De Buenos Aires, Argentina; [2]Ctr. de Ciências da Saúde- Lab. de Neurobiologia II, Univ. Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [3]Dept. of Psychology, Univ. of California, Santa Barbara, CA

Abstract: Passive observation of another person executing an action activates the motor system of the observer. This phenomenon, known as motor resonance, can be inferred based on the modulation of the corticospinal excitability (CSE) using transcranial magnetic stimulation. Previous studies have proposed that motor resonance reflects the activity of the muscles involved in the different phases of the action (e.g. Fadiga et al, 1995), its goal (e.g. Cattaneo et al, 2009) or its kinematics (e.g. Gangitano et al, 2001). It has also been postulated that changes in the time course of CSE reflect the prediction of future action phases (Urgesi et al, 2010). Distinguishing among these alternative accounts is difficult, since most of these studies used a unique goal or measured the level of CSE solely at the point of maximal muscle contraction or maximal aperture, and often during observation of static pictures. We aimed to further discern among these possible mechanisms by measuring the time course of CSE during the observation of grasping movements aimed at two targets of different sizes. We ran experiment 1 to have a precise description of the kinematics and muscle activity during normal execution of a grasping action. Extensor Indicis Proprius (EIP) was mostly active during hand aperture, whereas First Dorsal Interosseus (FDI) was mostly active during hand closure when approaching the target. ADM showed no significant temporal modulation. In experiment 2 we filmed videos of the same grasping movements with natural velocity (~1 sec) and selected 4 time points to characterize the time course of CSE during observation. To allow full prediction of the action to be observed, we indicated the target of the grasp action before each trial. Consistent with the muscular activity measured during execution, the observation related CSE for the FDI was maximal when the hand closed to grasp the object but minimal at maximal hand aperture. Observation CSE for the EIP correlated positively with hand aperture for the large object but showed no modulation for the small object. The ADM showed no temporal modulation. Our findings favor the hypothesis that motor resonance reflects muscle activity and not the movement's kinematics. In addition, the

temporal matching between CSE and muscle activity goes against the anticipatory simulation hypothesis, nor does it support the goal hypothesis since the goal of the action was known before movement onset. We conclude that when goal information is provided, motor resonance elicited by unperturbed actions reflects muscle activity. We are currently assessing the effect of goal uncertainty on the time course of motor resonance.

Disclosures: S. Mc Cabe: None. **G. Saunier:** None. **J.I. Villalta:** None. **S.T. Grafton:** None. **V. Della-Maggiore:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

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Program#/Poster#:888.20/KK10

Topic: D.17. Voluntary movements

Support: NSERC DG (U of L)

Title: Grasping without reaching: A kinematic analysis of vision, demand, and hand-use manipulations in right- and left-handers.

Authors: *J. W. FLINDALL, T. RUTSCHMANN, J. B. DOAN, C. L. R. GONZALEZ
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Abstract: Manual prehension can be defined in terms of two separate components; a reaching component, and a grasping component. Behavioural and imaging studies have shown that these components are programmed individually by separate neural circuits and then integrated into a single movement. While reaching kinematics have been described in depth, isolating the grasping component from the reach is more challenging; how does one grasp something without first reaching toward it? In the current study we attempted to separate the grasp from the reach. Participants were asked to grasp a glass of water located 4cm beneath a fixed starting position (a 1x1 cm rigid body suspended from a wire attached to a 25x25 cm wooden platform). With this manipulation all outward reaching motion normally accomplished via flexion/extension of the distal muscles of the forearm and wrist was replaced with minor flexion of the proximal muscles of the shoulder. Right- (n=12, 7 females) and left-handers (n=10, 5 females) grasped empty and full glasses of water, with right and left hands, under closed-loop, open-loop, and open-loop delay visual conditions. Kinematic analysis shows that both right and left-handers respond similarly to manipulations of vision (both groups demonstrated more accurate maximum grip apertures in closed-loop compared to open-loop and delay conditions, and time to grasp increases as visual feedback is reduced), demand (both groups complete grasps towards empty glasses

significantly quicker than grasps toward full glasses), and hand (reaction times towards empty glasses are significantly shorter than those towards full glasses, but only when participants used their right hands). Our results suggest that, like those for reaching and grasping, the neural processes responsible for planning and executing grasping alone are susceptible to manipulations of both visual feedback and task demand. Notably however, unlike those associated with reaching alone, the effects described herein appear to be independent of handedness.

Disclosures: J.W. Flindall: None. **T. Rutschmann:** None. **J.B. Doan:** None. **C.L.R. Gonzalez:** None.

Poster

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Program#/Poster#:888.21/KK11

Topic: D.17. Voluntary movements

Title: The effects of task and skill on sequentially produced grasp forces

Authors: K. A. DELAVE[1], J. M. LANGAN[2], K. L. KERN[1], *S. H. BROWN[1]
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Abstract: The control of grasp force is influenced by multiple factors such as task, skill, force level and the direction of force change - i.e. increasing or decreasing forces. Specifically, static force production has been shown to be more variable at higher force levels (Newell et al.,1984; Slifkin et al., 1995), and there is evidence that controlled force release is not only more rapid but also more variable compared to increasing force tasks (Masumoto & Unui, 2010). Practice/skill level reduces variability (Semmler & Nordstrom, 1998; Voelcker-Rehage & Alberts, 2005) and may be effective as a rehabilitation tool (Kim et al, 2010). In order to determine the applicability of a visual force feedback system for training purposes, this study examined the effects of task (unilateral vs bilateral), skill level (competitive Ultimate Frisbee players vs controls), and force direction, on whole-hand isometric grasp forces.
Using handheld dynamometers, grasp force was visually displayed as vertical bars on a computer screen. Target zones representing 5-20% of each participant's maximum voluntary contraction were superimposed upon the force display. Participants either increased or decreased force in a sequential "stair-stepping" pattern (0-5%, 5-10%, 10-15% 15-20%, 20-15%, 15-10%, 10-5%, and 5-0% MVC) as fast and accurately as possible and then held the force in the target zone for 3 s. The task was performed both unilaterally and bilaterally. The rate of force change (df/dt) was

higher during controlled force release compared to force generation ($p<.01$) but only during unilateral force tasks. No effect of force direction was observed in bilateral tasks. Changes in force level occurred more slowly in bilateral compared to unilateral tasks ($p<.01$). In contrast to predictions, skill level had no effect on df/dt in either task. Force variability was greatest following changes at the lowest force levels (0 to 5% or 10 to 5%), but, otherwise, was relatively constant. Variability was unaffected by task or direction of force change. As with df/dt, no differences related to skill level were observed.
The lack of skill-related differences was unexpected given the need for precise grasp force control in elite Frisbee players but may be related to the role visual feedback plays in controlling forces (Kennedy & Christou, 2011). It is possible that group differences may occur in tasks where target forces are established proprioceptively. The observation that variability was highest at the lowest force level contrasts with previous reports. To what extent these conflicting findings relate to differences in isometric force paradigms or the range of forces examined requires further clarification.

Disclosures: K.A. Delave: None. **J.M. Langan:** None. **K.L. Kern:** None. **S.H. Brown:** None.

Poster

888. Finger and Grasp Control: Normal Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:888.22/KK12

Topic: D.17. Voluntary movements

Support: Faculty Research Award Program II-Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University

Title: Sense of effort revisited: Relative contribution of sensory feedback and efference copy

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Abstract: We have shown that the sense of effort is asymmetric in the upper limbs and suggested that a difference in gain (input/output magnitude relationship) between each limb/hemisphere system may explain this asymmetry. The aim of this study was to investigate further the relative contribution of the efference copy and sensory feedback to the sense of effort, which is still being debated. Twenty- two (17 F and 5 M) right-handed, young healthy individuals performed grasp force matching tasks while seated in a standard posture with their forearms supported. Horizontal cylinders instrumented with strain gauges and placed next to each other at elbow level were grasped in pronation. The matching task consisted of reproducing

a 20% MVC reference, set by the right or left hand, with the same (ipsilatateral remembered, IR) or contralateral (contralateral remembered, CR) hand, with/without reference force visual feedback and with/without reference hand vibration (60Hz, applied to wrist flexor tendon). No feedback was provided for the matching hand. A marker controlled by the reference force signal, presented on a vertical scale displayed on a video screen, provided visual feedback. In the absence of this visual feedback the reference force was learned from verbal qualitative feedback. To minimize asymmetry issues the results focus on the absolute matching error (AE) expressed in %MVC. It was found that AE was greater without than with visual feedback. In the no visual reference condition, AE was greater without than with vibration, while vibration had no effect when the visual reference was provided. The results indicate that visual feedback was associated with the resultant grasp force, which represents the system output or summation of motor commands. This association was not observed in a similar but abbreviated experiment in which learning might not have occurred. The influence of vibration-induced alteration of sensory information observed only in the absence of visual feedback indicates that the utilization of sensory and /or efferent copy information is context dependent. The smaller AE observed when the reference hand is vibrated is assumed to be only an apparent error reduction resulting from the complex interaction of a TVR and the reweighting of agonist-antagonist commands used to reproduce the reference force output. Overall these results underline a major role of the efference copy in the sense of effort.

Disclosures: S. Scotland: None. **D.E. Adamo:** None. **B.J. Martin:** None.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.01/KK13

Topic: D.17. Voluntary movements

Support: NIH Grant R01NS076589-01

Title: The speed of voluntary movements modulates interhemispheric inhibitory interactions between primary motor cortices

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Abstract: Animal research has suggested that callosal neurons contribute to encode the time of behavioral events (Soteropoulos and Baker 2007). However, their role during human voluntary movement remains poorly understood. The aim of our study was to investigate the ipsilateral silent period (iSP, a measure of callosal interhemispheric inhibition) during movements of different speeds and durations. We used single pulse transcranial magnetic stimulation to elicit the iSP in an intrinsic finger and an elbow flexor muscle during continuous index finger abduction and adduction movements and during continuous elbow flexion and extension movements executed at fast (1 Hz) and slow (0.25 Hz) speeds. The left side completed the task while the contralateral side remained at rest (unimanual) or conducted a mirror-like (bimanual) movement. The iSP was measured as a transient suppression in electromyographic (EMG) activity in the left first dorsal interosseous and biceps brachii muscle, respectively. We found that the iSP was greater during fast compared to slow movements in all conditions tested in both muscles. We also examined the iSP during discrete index finger abducion and elbow flexion movements completed at fast (ballistic) and slow (self-paced) speeds. Here, we found that the iSP was increased during ballistic compared to self-paced movements in both muscles tested. Additional control experiments demonstrated that that the iSP remained similar at increasing levels of isometric voluntary contractions. Our findings demonstrate that the strength of interhemispheric inhibition between primary motor cortices is influenced by the speed of distal and proximal arm movements regardless of movement duration and background EMG activity. These observations suggest a role for callosal pathways in signaling the speed of a movement.

Disclosures: T. Tazoe: None. **M.A. Perez:** None.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.02/KK14

Topic: D.17. Voluntary movements

Support: NIH Grant R01NS076589-01

Title: Intermuscular coherence between hand and arm muscles during bilateral contractions in humans

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Abstract: Most of our daily activities involve coordinated muscle activation of hand and arm muscles. How corticospinal drive to muscles of the hand and arm interact during unilateral and bilateral voluntary contractions remains poorly understood. We examined coherence between electromyographic
(EMG) signals at frequencies between 15 to 35 Hz which is thought to indicate common drive to two muscles from corticospinal inputs. We recorded from the first dorsal interosseous (FDI), abductor pollicis brevis (APB), flexor carpis radialis (FCR), and biceps brachii (BB) muscles during unilateral and bilateral simultaneous elbow flexion and precision grip. Testing was completed when the non-dominant arm performed a precision grip and elbow flexion while the contralateral arm remained at rest or performed the same motor task. We found that during unilateral contractions mean coherence in the 15-30 Hz range was present to a largest extent between adjacent pairs of muscles such as FDI-APB, FDI-FCR and FCR-BB compared to distant muscle pairs such as FDI-BB and APB-BB. Mean coherence in the 15-30 Hz range between all muscle combinations tested was decreased (by 9% to 47%) during bilateral compared to unilateral contractions. In distant muscle pairs, the largest decrease in mean coherence was between APB-BB (by 41%) than FDI-BB (by 9%) during bilateral compared to unilateral contractions. These findings show task-dependent interactions between bilateral distal and proximal arm muscles during voluntary contractions in humans, and indicate that the decreased oscillatory muscle to muscle coupling may be part of the adaptations taking place to coordinate bilateral activation of different muscle groups.

Disclosures: D.S. Soteropoulos: None. **M.A. Perez:** None.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.03/KK15

Topic: D.17. Voluntary movements

Support: Grant-in-Aid for Scientific Research (C), JSPS

Title: Effects of unilateral voluntary movement on the firing rate of motor units on the contralateral side

Authors: H. OHTSUKA[1], T. NAKAJIMA[2], S. SUZUKI[3], G. FUTATSUBASHI[3], E. SHIMIZU[1], *T. KOMIYAMA[4]
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Abstract: Voluntary contraction of a muscle often generates electromyographic (EMG) activity in the homologous muscle on the opposite side (mirror-like activity), not only in pathological states but also in healthy adults. This is thought to be a default neural system which decreases with age. Interestingly, it was also reported that the amplitude of motor evoked potentials after transcranial magnetic stimulation were suppressed in concert with the onset of contraction of the contralateral homologous muscle. These findings suggest that the descending command responsible for mirror-like activity contains both excitatory and inhibitory effects on spinal motoneurons. Therefore, the purpose of the present study was to reveal the nature of descending inputs to motoneurons during mirror-like activity with a peri-movement time histogram of single motor unit (MU) activity.

Nine healthy volunteers participated in the experiment, and all gave written informed consent. Surface EMGs were recorded from the left and right first dorsal interosseous (FDI), and single MU activity was recorded with a fine needle electrode from the left FDI by weak isometric abduction of the index finger. Subjects performed phasic abduction of the right index finger during a visual reaction time task. The intensity of the right index isometric abduction was set at 2, 10, and 40% MVC; these values were displayed on an oscilloscope in front of the subject. At the same time, the subjects were instructed to maintain a given range (~8 - 10 Hz) of firing rate of MU in the left FDI with auditory and visual feedback. The peri-movement time histogram of MU discharge activity was constructed by calculating the number of discharges recorded during 30~50 trials with 10ms bins.

Modulation of the firing rate of MU activity in the left FDI was observed in conjunction with the onset of right index finger movement. However, changes in the firing rate of MU were strongly dependent on the exerted force level of the right index finger. An increase in the firing probability was more frequently observed during 40% MVC (15/18 units in 9 subjects) than 2% (5/18 units in 9 subjects) and 10% MVC (8/15 units in 9 subjects). In addition, although significant increases of the cumulative sum of MU firing (100 ms window) calculated from peri-movement time histograms were observed during 40% MVC, significant decreases in those were observed during 2% MVC. These findings led us to conclude that the spinal motoneurons receive both excitatory and inhibitory inputs depending on the force level of the contralateral finger.

Disclosures: H. Ohtsuka: None. **T. Nakajima:** None. **S. Suzuki:** None. **G. Futatsubashi:** None. **E. Shimizu:** None. **T. Komiyama:** None.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.04/KK16

Topic: D.17. Voluntary movements

Support: Faculty of Health Sciences Research Development Funds

Title: Interhemispheric differences in stimulus-response profiles for the contralateral and ipsilateral silent period in young healthy adults

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Abstract: The contralateral (cSP) and ispsilateral silent period and (iSP) derived from transcranial magnetic stimulation (TMS) can be used to probe the excitability of inhibitory circuits in the human motor cortex. Previous investigations have shown that the duration of both cSP and iSP are influenced by the intensity of stimulation (Chen, Yung, & Li, 2003, J Neurophysiol). However, few studies have compared hemispheric differences in cortical responsiveness to increasing TMS intensity using the cSP and iSP, as index of intra- and interhemispheric inhibition. In the present study, we investigated this issue in young healthy adults (n=6) who were strongly right lateralized. Participants underwent TMS of each hemisphere (order counterbalanced) using a Magstim 200 stimulator connected to a figure of eight coil. Motor evoked potentials (MEPs) were recorded from each hand with surface electrodes placed over the first dorsal interosseous (FDI). On each hemisphere, cSP and iSP were tested at 4 different intensities relative to the resting motor threshold (i.e., 110, 120,130 & 140% rMT). In each trial (3 s), TMS was delivered (2.5 s delay) to the hemisphere ispsilateral to the maximally contracting hand (iSP, squeezing a vigorimeter) while the opposite hand was lightly contracting (cSP, pinch-grip). The durations of the silent periods were determined by visual inspection of EMG traces on a trial by trial basis. A 2x2 repeated measures ANOVA found a large main effect ($p<0.001$) for “intensity” but no effect of “hand/hemisphere” for both cSP and iSP measurements. The intensity effect reflected the lengthening of both the iSP and cSP in response to increasing TMS intensity. Further post-test comparisons with pooled data from both hand/hemispheres revealed that cSP had significantly ($p=0.001$) steeper stimulus-response profile than iSP (mean slope, iSP, 0.44±0.01; cSP, 2.02±0.13). Our findings demonstrate that hemispheric dominance Is not a major factor in influencing iSP and cSP measurements while intensity relative to the RMT remains a critical parameter. Such findings have implications for studies examining the impact of exercises or interventions using silent periods as index of intra- and interhemispheric changes in motor excitability.

Disclosures: T. Davidson: None. **M. Gerguis:** None. **F. Tremblay:** None.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.05/KK17

Topic: D.17. Voluntary movements

Support: NSERC

Title: Enhancing excitability of the primary motor cortex: A TMS study exploring contributions from transcallosal connections and non-primary motor cortices

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Abstract: In primates, control of the distal muscles of the upper limb was thought to be regulated by the contralateral motor system and devoid of transcallosal connections between the individual primary motor cortical (M1) representations. More recent research with primates and humans has demonstrated increased ipsilateral motor cortical activity during unimanual movements. Transcranial magnetic stimulation (TMS) studies reveal increased corticospinal tract activation to the resting homologous muscle with contraction and rhythmical movement. A recent study in our lab demonstrated that extensor carpi radialis (ECR) M1 excitability was increased during a muscle contraction independent of the effector--i.e. homologous muscle contraction increased excitability to the same degree as contralateral dorsiflexion (DF). Within M1 there is no overlap of arm and leg representations, therefore there must be inputs from non-primary motor regions that contribute to this interaction. The purpose of this study was to investigate the non-M1 contributions to excitability of M1. We hypothesized that excitability of M1 ipsilateral to the contracting upper limb ($M1_{ipsi}$) would be enhanced during wrist-ankle movement and non-primary motor areas would influence this up-regulation. We delivered focal transcranial magnetic stimulation to healthy right-handed subjects targeting the M1 representation of the right ECR muscle during isometric and isotonic (rhythmical) contractions of the left ECR and flexor carpi radialis (FCR) and right and left DF and plantarflexors (PF) at 10% and 30% of their maximum voluntary contraction. Both isometric and isotonic conditions were performed for each of these conditions: (1) left wrist flexion/extension, (2) left wrist flexion/extension and left ankle DF/PF, and (3) left wrist flexion/extension and right ankle DF/PF. Motor evoked potentials (MEPs) were recorded using surface EMG from ECR, FCR, DF and PF bilaterally. Subjects were positioned with their left arm and left and right legs secured in a manipulandum that allowed for either full wrist flexion/extension or ankle PF/DF. They monitored their level of contraction on a computer screen placed in front of them during each

trial. Twenty MEPs were sampled over M1 during each condition. We sampled MEPs during the pre-movement phase as well as during the movements. Preliminary results suggest $M1_{ipsi}$ excitability is enhanced during isodirectional movements (i.e. ankle DF and wrist extension) when the left wrist is active with the right or left ankle simultaneously. These results suggest that the non-primary motor areas are responsible for this interaction, thereby influencing M1 excitability.

Disclosures: R.J. Ibey: None. **W.R. Staines:** None.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.06/KK18

Topic: D.17. Voluntary movements

Support: NSERC

Title: Continuous theta burst stimulation (cTBS) to the right primary motor cortex (rM1) and short-term bimanual training modulates the excitability of the left M1 (lM1).

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Abstract: Activity in cortical areas underlying motor preparation and execution is enhanced after a single session of bimanual visuomotor training. These adaptations occur specifically when training requires simultaneous activation of homologous muscles and is characterized by an increase in the cortical territory occupied by the trained muscles within the primary motor cortex (M1) [Neva et al, 2012 Neurosci Letts]. This modulation may include connectivity with both premotor regions and interhemispheric interactions between homologous muscle representations in M1. Continuous theta burst stimulation (cTBS) to M1 suppresses motor evoked potentials (MEPs) from the stimulated M1 [Huang et al., 2005 Neuron]. Few studies suggest that cTBS to rM1 increases corticospinal activity in lM1[Suppa et al., 2008 J Physiol]. Also, motor function of the affected limb improves in stroke patients after applying cTBS to the contralesional M1 or S1 in combination with movement training [Meehan et al., 2011 Neurosci Letts]. This study investigates the effects of cTBS to rM1 on wrist extensor representation in lM1, and its potential combined effects with short-term bimanual training. This experiment tests the hypothesis that cTBS to rM1 will increase excitability of the extensor carpi radialis (ECR) in lM1, and the addition of bimanual training will cause a greater increase. ECR representations in lM1 were

measured in 2 groups before and after (1) cTBS over rM1 and (2) cTBS over rM1 combined with bimanual training. Following cTBS over rM1 the lM1 representation of ECR was acquired over multiple time periods separated by ~30min. lM1 ECR representation was determined by acquiring MEPs (n=10) from several positions separated by 1cm centred over the hotspot. The borders of the representation were defined as the sites where MEPs were no longer observed. Neuronavigation using anatomical images was used to guide the TMS coil over the motor hotspot and ensure reproducibility of sampled sites. Single-pulse TMS was delivered at an intensity of 110% of resting motor threshold. cTBS was delivered to rM1 ECR representation at 80% of active motor threshold. Preliminary analysis indicates that ECR representation in lM1 is facilitated after cTBS over rM1. This facilitation post-cTBS to rM1 alone is observed in both central and global MEP amplitude. The combined effect of cTBS over rM1 with bimanual training on lM1 shows an increase in spatial extent with a modest increase in global MEP amplitude. These results suggest that modulation of one M1 may alleviate ongoing interhemispheric inhibition (or increase facilitation) to the opposite M1 in healthy individuals via transcallosal or subcortical connections.

Disclosures: J.L. Neva: None. **M. Vesia:** None. **J.S. Thaker:** None. **W.R. Staines:** None.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.07/KK19

Topic: D.17. Voluntary movements

Support: BBSRC: BB/G002355/1

Wellcome Trust

Title: Activity of spinal interneurones during movements with left or right hand.

Authors: *D. S. SOTEROPOULOS[1], S. BAKER[2]
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Abstract: Far from being a blind servant to cortical control, the spinal cord moulds descending and peripheral signals to drive motoneurones in the pattern required for a given movement. The spinal cord is bilaterally organised both in its descending inputs from cerebral cortex areas and brainstem but also in its peripheral inputs and local circuits (e.g. the spinal commissural system). Despite this bilateral anatomic connectivity and its importance for locomotory movements, it is unclear how primate spinal circuits on one side of the cord modulate their activity during goal

directed movements of the contralateral arm.
Extracellular neural recordings were made from spinal interneurones in an awake behaving macaque monkey performing a "reach to grasp" task with either hand. The monkey initiated the trials by placing each hand on a homepad; following a delay (~500ms) and a laterality cue (~750ms, tone), plastic flags occluding the task retracted for the cued manipulandum. The monkey then reached out and performed a precision grip with the index finger and thumb (auxotonic with left hand , isometric with right), while the non moving hand remained at rest on the homepad. Following a 1s holding period, a food reward was given. Ninety three interneurones were recorded from the intermediate laminae in the left and right lower cervical spinal cord during this task. Single pulse stimulation at these sites (bi-phasic, 0.2ms per phase, intensity 5 to 200 μA) primarily elicited finger and wrist movements.
Cell activity was quantified as mean firing rates during resting both hands on the home pads, the reach and squeeze phase (500ms window just before the grip hold period) and the 1s-long hold period. The majority of the neurones showed a significant firing rate modulation during movements of the ipsilateral arm compared to the home pad epoch (72/93 cells) - this was either during the reach and squeeze phase only (17/72 cells), hold phase only (12/72cells), or both phases (43/72). A large fraction (45/93) of cells also modulated their activity during movements of the contralateral arm (reach and squeeze:13, hold:8, both:24). Of those cells, eleven showed a significant modulation only with movements of the contralateral arm. Cells showed a rate increase (51/72) or decrease (21/72) relative to the homepad epoch for ipsilateral movements. In the case of cells modulating with both hands, the direction of rate change was similar between the two sides in 18/34 cells and different for 16/34.
Our results show that spinal circuits show a diverse modulation of activity during movements with the opposite arm. The relative contribution of descending, peripheral and local signals remains to be determined.

Disclosures: D.S. Soteropoulos: None. **S. Baker:** None.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.08/KK20

Topic: D.17. Voluntary movements

Support: Huffines Institute Student Research

Title: Rhythmical bimanual force production: 1:2 and 2:3 coordination patterns

Authors: *D. M. KENNEDY, J. B. BOYLE, C. H. SHEA
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Abstract: A large number of experiments over the last 40 years have isolated a coalition of constraints, including cortical and subcortical neural crosstalk, that influence the coordination of the two hands functioning together. Recent findings, however, have demonstrated that these constraints are eliminated or at least minimized when integrated feedback (e.g., Lissajous feedback) is used. Two experiments were designed to determine participants' ability to coordinate 1:2 (N=8, Experiment 1) and 2:3 (N=8, Experiment 2) rhythmical bimanual force production tasks. We hypothesized that neural crosstalk should be more easily detected and characterized in tasks where the forces required to produce the goal pattern of coordination are increased than in tasks where force requirements are minimal. The task was to rhythmically produce a pattern of isometric forces on a left side force transducer with the left arm that was coordinated with the pattern of isometric forces produced on a right sided force transducer with the right arm. The Lissajous display involved a goal template and a cursor indicating the forces produced with both limbs. The cursor moved from left to right as force was produced with the right arm and from bottom to top as force was produced by the right arm. The template illustrated the specific pattern of force requirements needed to produce the goal coordination pattern. The results indicated very effective temporal performance of the bimanual coordination patterns. This result is similar to that observed in our earlier work with reciprocal and circling motion, but is especially informative given that the increased forces required to produce the desired bimanual coordination pattern resulted in a consistent and identifiable distortion of the left limb forces that could be attributable to the production of right hand forces. We were not able to detect distortions of the forces produced by the right limb that could be attributable to the left limb. This type of right to left limb influence, which may be attributable to asymmetrical cortical and subcortical crosstalk, was not evident in our earlier work when the bimanual coordination tasks involved movements of the limbs in a relatively frictionless environment.

Disclosures: D.M. Kennedy: None. **J.B. Boyle:** None. **C.H. Shea:** None.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.09/LL1

Topic: D.17. Voluntary movements

Support: NSERC Grant

Title: Kinematic properties of the upper and lower arms during bimanual grasping

Authors: *A. LE[1], J. C. DESSING[2], J. D. CRAWFORD[2], M. NIEMEIER[1,2]
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Abstract: Grasping an object with two limbs is phylogenetically older than the hand, yet bimanual grasping is rarely studied. It is of particular interest because it involves lateralized visual object analysis in one hemisphere to program the grasp (Le et al., SfN, 2011) together with bimanual coordination of the two hands through the two hemispheres. To better understand the processes underlying this coordination, here we examined the kinematic rules of the two arms during bimanual grasping. In particular, the goal was to investigate how the two hands are coordinated, how the torsional demands of the two upper and lower arms interact, and whether the arms maintain Donders' Law. To this end we asked 15 subjects to use both hands to horizontally grasp a rectangular object that was presented in one of 15 locations (5 locations left to right of body midline, at 3 heights), and lift it off a pedestal. We found that as the object was placed more eccentrically, the two hands maintained a high correlation in trajectories. Nevertheless, the ipsilateral hand trajectories became more curved before object contact and the contralateral hand trajectories became more linear, as expected given that grasp trajectories have to avoid unwanted contact with the front face of the object. Perhaps as a consequence we found that the ensuing lift-off trajectories of the object were more variable for more eccentric object locations suggesting that the grasp was less stable at these locations. Another factor of reduced stability could be that eccentric object locations were biomechanically more challenging. However, this was not reflected in the 3D rotations of the two arms. That is, we found that the two arms did not show eccentricity effects in their torsional rotations and level of adherence to Donders' law. Consistent with this, we found that regardless of the eccentric location of the object, (a) the torsional rotations of two arms were always negatively correlated, (b) the lower arms had more torsional rotation than the upper arm (especially as the objects were more elevated), and thus (c) the upper arms adhered more closely to the Donders' Law than the lower arms. These observations suggest that although the grasp is less stable when bimanually grasping eccentrically located objects, the two hands and arms follow coordinated bimanual kinematic rules regardless of object's location, with the upper arms closely adhering to Donders' law and the lower arms accounting for most of the required torsional rotation. This research sheds new light on the coordinated kinematics of the two arms during bimanual grasping. Future studies will use these data to identify and monitor motor problems in patients with motor deficits.

Disclosures: A. Le: None. **J.C. Dessing:** None. **J.D. Crawford:** None. **M. Niemeier:** None.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.10/LL2

Topic: D.17. Voluntary movements

Title: Altered agonist muscle activation explains impaired endpoint accuracy during goal-directed movements of the ankle in children

Authors: *E. J. FOX, M. KWON, Y.-T. CHEN, M. COSTANZO, H. MORA, K. SOWALSKY, E. A. CHRISTOU
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Abstract: It is well-known that children exhibit impaired movement control compared with adults. Nonetheless, the ability of children to perform discrete goal-directed movements of the ankle is not well-understood. Accuracy during goal-directed movements of the ankle is a critical component of daily activities and is a requisite for mobility and gait adaptations. The purpose of this study was to compare movement accuracy and variability as well as the activation of agonist and antagonist muscles in young adults and children during goal-directed movements of the ankle. Ten young adults (20.0 ± 0.9 yrs, 4 males) and 7 children (9.7 ± 0.8 yrs, 5 males) participated in a single test session. Subjects attempted to accurately match the peak displacement of the foot to a spatiotemporal target by performing an ankle dorsiflexion movement. The targeted displacement (spatial) was 9° of ankle dorsiflexion and the targeted time (temporal) was 180 ms. Subjects received visual feedback for 5 s after each trial. Surface EMGs were recorded from the tibilis anterior (agonist) and soleus (antagonist) muscles. Endpoint accuracy was quantified as the overall error, which was calculated from the orthogonal relationship of the position and time errors (absolute deviation from the target). Endpoint variability was calculated as the coefficient of variation across trials for the peak displacement and time to peak displacement. Children exhibited greater overall error as well as greater temporal error and positional variability compared with young adults ($P < 0.05$). The amplitude of tibialis anterior muscle was greater in children ($P < 0.05$), but the duration of activation and the time to peak activation was shorter. The amplitude of soleus activation also was greater in children. The greater overall error and temporal error in children was predicted by the duration of the agonist muscle ($R2 = 0.72$ and 0.62, respectively). In conclusion, compared with young adults, children were less accurate during goal-directed movements of the ankle and differences were largely explained by altered activation of the agonist muscle. The muscle activation differences may be due to immaturity of the cortico-motor systems in children. Understanding motor control in healthy children is critical so that findings may be applied to the study of children with neuromuscular impairments.

Disclosures: E.J. Fox: None. **M. Kwon:** None. **Y. Chen:** None. **M. Costanzo:** None. **H. Mora:** None. **K. Sowalsky:** None. **E.A. Christou:** None.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.11/LL3

Topic: D.17. Voluntary movements

Title: In search of manual asymmetries in aging during complex tasks: Does upper limb performance become more symmetric with age?

Authors: T. LULIC, A. ENGELHARDT, C. R. DICKERSON, *E. A. ROY
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Abstract: Abstract: Handedness is defined as the tendency to prefer one arm to the other in performing daily motor tasks. Age-related reduction in hemispheric lateralization and simple motor tasks is known to occur in older adults (Cabeza et al., 2002; Przybyla et al., 2011). This study sought to identify potential reductions in manual asymmetries in older adults (OA) when performing more complex upper limb tasks, such in completing activities of daily living (ADLs). A secondary goal was to identify the influence of aging on dominant and non-dominant spatial and temporal aspects of movement during these tasks.
Methods: Upper limb and trunk movements of 8 healthy, older (67.25 ± 4.5; 2M, 6F) and 6 healthy, younger, right-handed adults (YA) (23.16 ± 0.93; 3M, 3F) were captured at 100 Hz via a motion capture system (Vicon, Oxford, UK). Forty-three reflective markers were placed on anatomical landmarks of the upper limbs and trunk. Participants performed two task types: 1) pantomiming a gesture (e.g. slicing an imaginary loaf of bread with imaginary knife) in 9 ADLs; and 2) gesturing while holding a physical tool in the nine ADLs. Tasks and ADLs were block randomized by limb, with each gesture performed three times by both limbs. The movement was divided into three phases: reach to grasp, tool use, and termination phase.
Results: The data analysis revealed no hand effects and no interaction of hand with age. There were, however, age effects on performance. Specifically, when looking at the entire movement cycle the OA demonstrated shorter cycle duration with no difference in extension/flexion at the elbow. When focusing just on the forward movement of the cycle, wrist amplitude was shorter for the OA and they demonstrated higher peak velocities and less time after peak velocity.
Conclusion: Overall, the current study found no differences in performance between limbs. However, there was an effect of age which suggested a difference in movement control. The time to complete the movement cycle was shorter for the OA which appeared due to a smaller movement amplitude, a higher PV and less time spent processing feedback in the deceleration phase of the movement. This pattern suggests that the OA may have been using a more ballistic

mode of control. Interestingly while movement amplitude was larger for the YA, the ROM at the elbow did not differ, suggesting that more of the movement was being controlled at the shoulder in this group. The implications of these findings for understanding movement disorders in stroke such as apraxia will be discussed.

Disclosures: T. Lulic: None. **A. Engelhardt:** None. **C.R. Dickerson:** None. **E.A. Roy:** Research Grant; Heart and Stroke Foundation.

Poster

889. Interlimb and Bimanual Control

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Program#/Poster#:889.12/LL4

Topic: D.17. Voluntary movements

Title: Method to redistribute effort during multi-limb rehabilitation exercise through implicit feedback

Authors: N. E. SKINNER[1], *A. D. KUO[2]
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Abstract: Humans sometimes exercise in a way that is easier in the short term but poorer for long-term goals. Those recovering from stroke or spinal cord injury may favor the limbs most needing exercise to prevent long-term disuse. One solution is to provide patients with explicit feedback of limb use during multi-limb exercise. This may, however, also add to cognitive demand.
We propose an alternative approach, termed implicit task weighting. We alter the power feedback normally displayed during exercise, by unequally weighting a targeted set of limbs. We hypothesize that humans determine their preferred distribution of limb effort based on relative ease. Here we test if implicit weightings, by distorting relative ease, can alter the distribution of limb effort. We also test whether this has lower cognitive burden compared to explicit feedback.
We compared implicit and explicit feedback during multi-limb exercise performed by young healthy adults (N = 16). We instrumented a NuStep recumbent stepper to measure the power produced by individual limbs, which all act against a single load. For implicit testing, we displayed a weighted power meter to subjects, without informing them of the weighting. To test explicit feedback, subjects viewed bar graphs separately displaying both the total power and the distribution of effort between arms and legs. We tested both conditions with and without a secondary cognitive task, a simple subtraction series.
Results showed that both implicit and explicit feedback can steer effort, altering a person's

preferred distribution by 25-30% toward either the arms or legs. With implicit feedback, subjects reached the power goal with less error compared to explicit feedback ($P<0.001$). This difference was not affected by the cognitive task ($P<0.001$). Subjects performed the cognitive task 5% slower when using explicit feedback compared to implicit ($P=0.03$).
Implicit task weighting may provide a simple means to encourage use of targeted limbs during exercise. It may also require less attention than explicit feedback.

Disclosures: N.E. Skinner: None. **A.D. Kuo:** None.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.13/LL5

Topic: D.17. Voluntary movements

Support: Human Frontiers Science Program

Title: Motor costs and control policies in the healthy and parkinsonian brains

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Abstract: There are often multiple ways to perform an action. The choices that people make are a window to how their brain evaluates costs of the task. In Parkinson's disease (PD), people exhibit bradykinesia in their affected limbs. A recent report suggests that this behavior is not due to an inability to accurately use the affected side, but rather due to an abnormally increased motor cost (Mazzoni et al. 2007). Here, we performed an experiment to quantify motor costs in PD. We considered an isometric task in which people held two force transducers, one in each hand. The goal was to use the left and right arms in such a way as to move a cursor toward targets distributed around a circle. The position of the cursor was the sum of the force produced by each arm. For each target the subjects chose how much force to produce with each arm. This choice was direction dependent, but remarkably consistent over repeated days. We hypothesized that the choice was due to a cost that had two components: a cost for variability (how well they can control each arm), and a cost for effort (strength of each arm). To measure variability, in a unimanual task we measured variance of force for each arm at each direction of force. To measure effort, we measured the maximum voluntary force for each arm and each direction.

Despite the fact that the choices that healthy (n=8) and PD patients (n=8) made in the bimanual task were fundamentally different, both choices were well described by the sum of variance and

effort costs. PD patients had increased variance for extensors on the affected side as compared to controls, and this accounted for the fact that in the bimanual task they tended to rely on the arm that had lower variance for directions that it tended to flex. Therefore, we found that the cost functions in PD and controls were similar: both avoided actions that produced higher variance. The main difference was that PD suffered from significantly increased variance on the affected side, mainly for actions that produced extension.
Next we applied anodal tDCS to M1 of the affected hemisphere of PD patients. This produced an immediate reduction in variance on the affected side in the unimanual task. Remarkably, this also made the choices of PD patients in the bimanual task more similar to healthy controls. In summary, we found that choices that PD patients make in their actions are due to a cost that has two components: variance, and effort. On the affected side, variance is significantly increased with respect to controls, and the choices of PD patients are generally consistent with this increased variance. We can improve unimanual variance of the PD patients using tDCS. This produces an immediate improvement in their actions in bimanual tasks.

Disclosures: R. Shadmehr: None. **Y. Salimpour:** None. **Z. Mari:** None.

Poster

889. Interlimb and Bimanual Control

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Program#/Poster#:889.14/LL6

Topic: D.17. Voluntary movements

Support: K23 HD049472

Title: Alternate-hand training for recovery of post-stroke dexterity

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Abstract: Post-stroke recovery is thought to occur due to re-organization of preserved neural substrate, and the unaffected hemisphere shows increased task-related activation after stroke. However it is not known if the unaffected hemisphere takes over, detracting from recovery of the affected hand, or if it can assist in its recovery through transfer of sensory-motor information across the hemispheres. We performed a pilot study to test the efficacy of alternate-hand training where the unaffected hand “teaches” the affected hand. Six subjects with post stroke hemipaersis participated in the study. Sensory-motor impairment, hand function and modulation of precision

grasp were measured prior to and following eight 45-minute sessions of alternate-hand training over 4 weeks. The training consisted of grasping and releasing objects alternatively, first with the non-involved hand and then with the involved hand, with the tasks progressing in difficulty across the sessions. The subjects consistently improved on upper limb joint proprioception, tests of higher order sensory integration (sterognosis and 2-Point discrimination) and pinch strength. Functional ability improved specifically on tasks which required precision grasp. Execution of precision grasp, measured with the preload phase duration, showed a consistent decrease across all subjects suggesting improved grasp stability and grip-load coordination. Subjects that showed impaired predictive control of fingertip forces and excessive grip forces also improved both on the affected and unaffected sides. Our results suggest that alternate-hand training can improve sensorimotor integration and hand function in subjects with chronic post stroke hemipaersis. The mechanisms by which this transfer of information occurs across hemispheres needs further investigation.

Disclosures: **V.M. Aluru:** Employment; NYU School of Medicine. Research Grant; K23 HD049472. **D. Geller:** Employment; NYU School of Medicine. Research Grant; K23 HD049472. **M. Wilson:** None. **S. Kastuar:** None. **P. Raghavan:** Employment; NYU School of Medicine. Research Grant; K23 HD049472.

Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.15/LL7

Topic: D.17. Voluntary movements

Support: Mount Sinai Technology Development Fund

Title: Simultaneous bimanual training for upper limb rehabilitation in patients with post-stroke hemiplegia

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Abstract: Few options exist for training arm movements in subjects with severe post-stroke hemiplegia. We designed a series of bimanual mechanical devices which link movements of the two sides so that movement of the unaffected side produces homologous movements on the

affected side. The utility of these devices lies in the ability of the patient to repeatedly move the affected body part without assistance from a therapist, providing the opportunity to maintain and/or increase range of motion and awareness in the affected limb, limit spasticity, contracture, and deformity, as well as gain a sense of control over their own rehabilitation. However, it is not known if movements of the unaffected side compete with or enhance movements on the affected side. We tested the efficacy of training with 4 devices which were designed to produce movements opposite to the typical post-stroke synergy patterns. Training involved (1) shoulder external rotation and elbow extension, (2) supination, (3) wrist extension and (4) finger extension either in a proximal-to-distal order or vice versa. Eight subjects with chronic post-stroke hemiplegia participated in the study. Subjects received 1.5 hours of training twice a week for 6 weeks. Motion analysis was used pre- and post-training to assess for increase or decrease in active range of motion (AROM). On average, patients showed improvements on the Fugl-Meyer (FM) score, although patients who trained distal-to-proximal showed greater improvements in wrist and hand FM scores. All subjects improved in one or more practiced directions on the affected side. Interestingly, although the unaffected side was the driver for the training sessions, there was some reduction in AROM on the unaffected side. The results suggest that simultaneous bimanual training can enhance active movement on the affected side even in individuals with chronic post-stroke hemiplegia, perhaps by recruiting redundant ipsilateral pathways.

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Poster

889. Interlimb and Bimanual Control

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:889.16/LL8

Topic: D.17. Voluntary movements

Title: Elucidating the basis of personalized protocols for post stroke rehabilitation

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Abstract: A stroke affecting the motor system leads to limited movement which can be due to varying degree of weakness, lack of selective control and spasticity. Decreased motivation to move may further impede movement. How these variables interact with each other to inform treatment is not known, but this information is critical to providing appropriate rehabilitation interventions. Here we had 21 subjects with post-stroke hemiparesis perform 18 trials of a wrist flexion-extension task for 15 seconds, where they performed 2 trials with both hands (bimanual) for every one trial with the affected hand (unimanual). We examined the learning effect of the bimanual trials on the unimanual trials in terms of wrist range-of-motion (ROM), and activity in the wrist flexors and extensors. We also examined the influence of auditory emotional (happy sounds), musical (up tempo self-selected music), and rhythmic (metronome beat at preferred rate) stimuli on learning. Based on their ROM, relative strength of wrist flexors (F) and extensors (E), and their co-activation (F/E ratio) at baseline trials, the subjects were clustered into those with (1) weakness (very low ROM, low extensor strength and high co-activation- F/E ratio>1), (2) spastic-co-contraction (low ROM, high extensor strength and high co-activation), (3) new learners (moderate ROM, high extensor strength and moderate co-activation-F/E ratio > 0.6), and (4) efficient learners (high ROM, high extensor strength and low co-activation). We found that only subjects with very low and low ROM (clusters 1 and 2) showed consistent bimanual-to-unimanual learning effect. Happy sounds increased ROM in all groups except the efficient learners (cluster 4), and the increase was correlated with individual emotional intelligence scores. Music increased ROM in the low and moderate ROM group (clusters 1 and 3), and the increase was correlated with baseline depression scores. Rhythm increased ROM variably, but particularly in those with spastic co-contraction (cluster 2), and the improvement was correlated with Fugl Meyer scores. The learning effect on ROM was mediated by differential effects of the stimuli on flexor and extensor activity in the various subject clusters. The results demonstrate that the treatment (bimanual) effect varies by extent of weakness and spasticity at the individual level, and that personalized auditory stimuli can modulate motor performance and learning. The results lay the foundation for personalized protocols for post-stroke rehabilitation.

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Poster

890. Cortical Planning II

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Topic: D.17. Voluntary movements

Support: Wings for Life WFL-AT-007/11

Vienna science and technology fund LS11-057

WTZ Austria/Slovenja: I 14/2011

Title: Modification of posterior root-muscle reflexes by volitional motor tasks

Authors: S. M. DANNER[1,2], N. SARABON[3], A. PANJAN[3], W. MAYR[2], U. S. HOFSTOETTER[2], K. MINASSIAN[2], M. KRENN[2], F. RATTAY[1], K. E. TANSEY[4,5,6], *M. R. DIMITRIJEVIC[7]

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Abstract: The conditioning effect of motor tasks upon lumbar spinal reflex activity in humans has been extensively studied using H-reflex activity and recurrent inhibition during antidromic motor volleys [1]. These methodologies test only one or two spinal cord segments while the conditioning motor tasks used with these reflexes are multisegmental events. The method of transcutaneously evoking posterior root-muscle (PRM) reflexes simultaneously in several segments can give insight into the motor control in humans with intact and disordered nervous system function [2-3].

We demonstrate the application of testing motor behavior expressed by multisegmental PRM reflex activity conditioned by volitional motor tasks in human subjects with an intact nervous system. Single- and multi-joint motor tasks of the lower limbs are performed in the supine, sitting and standing position in order to learn not only the conditioning effects on PRM reflexes but also the role of posture on the motor behavior of spinal reflex activity.

The excitability of spinal motor nuclei can be modified by the supraspinal motor control of spinal interneuronal circuits. Our hypothesis is that premotor spinal cord processing expresses the pattern of descending engagement of interneuronal pools. The pattern of distribution of segmental motor behavior during fundamentally different motor tasks (single- vs. multi-joint) suggests dynamic modifications of inhibitory and excitatory actions of spinal interneurons.

We are expanding our study to also test the interneuronal engagement by sustained electrical stimulation of peripheral nerves. Thus, we will be able to report on differences between spinal interneuronal processing features elicited by central motor tasks and by peripheral nerves inputs.

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3) Hofstoetter U et al., Artif Organs 2008;32:644-8.

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Poster

890. Cortical Planning II

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NSF #ENG-1137279 (EFRI M3C)

Title: ERP components during motor preparation predict subsequent reaching movement velocity and accuracy under risk and uncertainty

Authors: *M. PLANK[1], S. HILLYARD[3], J. SNIDER[1], H. C. HUANG[2], S. GEPSHTEIN[4], H. POIZNER[1]
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Abstract: Accurate reaching movements towards spatial targets require the proper interaction of sensory, motor, motivational and memory systems during both motor planning and execution. While brain areas involved in reach-related actions have been identified in humans and nonhuman primates, EEG dynamics associated with efficient and accurate reaching are only beginning to be examined. We studied the spatio-temporal EEG characteristics during reach planning of spatially targeted vs. non-targeted 3D hand and eye movements, and how EEG parameters associated with sensory processing and multimodal transformation were modulated by the amount of eye-hand coordination required for the subsequent reaching movement. Facing a vertical touch screen, healthy participants performed a total of 1100 trials of time-constrained reaching movements with their dominant hand from the screen center towards lateral targets. Hits, misses and time-outs (responses exceeding a time constraint) yielded monetary gains or losses. Subjects were allowed to move both hand and eyes or only their hand following a 500-

700ms delay interval during which the hand and gaze remained on a center fixation cross. An additional condition involved a non-spatially-directed hand “lift” movement from the screen. Synchronized 72-channel scalp EEG, eye tracking and optoelectronic finger/hand/arm motion recordings were obtained from 10 subjects. Extending previous research (Trommershauser et al., 2003), reaching accuracy, the 2D distance between hand endpoint and target center, decreased when subjects were required to fixate the screen center compared to when they were free to make saccades (10.0 ± 1.3 mm vs. 6.9 ± 1.2 mm, respectively; $p < .001$). Moreover, the amplitude of the P300, an ERP component associated with allocation of attentional resources, was markedly higher during the delay interval when subsequent eye/hand movements were directed towards a spatial target compared to non-targeted hand lifts. Furthermore, amplitude modulations of ERP components associated with early visuo-spatial processing (N170, P2) and attentional allocation (P300) significantly correlated with subsequent behavioral performance both in terms of reach accuracy and precision, and with kinematic parameters such as movement velocity and acceleration. The correlations were strongest over right parietal and bilateral frontal regions (r’s > 0.60, all p-values $< .05$). Thus, ERPs recorded over right parietal and bilateral frontal cortices within three hundred milliseconds following the onset of a spatial target predict subsequent movement accuracy and aspects of the upcoming kinematics.

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Poster

890. Cortical Planning II

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Topic: D.17. Voluntary movements

Support: ARC grant DP0770568

Title: Age-difference in the functional role of premotor and primary motor regions during response preparation

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Abstract: *Background.* The modulation of interhemispheric inhibition (IHI) between two motor regions during movement preparation is suggested to be instrumental for the production of unimanual responses by suppressing activation in the non-responding hand. Particularly, the left dorsal premotor cortex (PMd) appears to play a key role in the preparation of response of either

hand. Our preliminary work has indicated that older adults have a greater reliance on left PMd regions during the preparation of simple motor actions than younger adults. An association between a release of left PMd–right primary motor cortex (M1) inhibition and faster reaction times (RT) was evident in older adults in a simple RT task using the left index finger. Here we investigated the functional role of PMd and M1for both left (Exp1) and right (Exp2) hemispheres during response preparation in younger and older adults using double pulse transcranial magnetic stimulation (TMS) 'virtual-lesion' protocol (10 ms interstimulus interval, 110% resting motor threshold).
Methods. Younger and older adults performed a simple RT task abducting their left index finger as fast as possible in response to an imperative signal (IS) presented 500 ms following a warning signal (WS). TMS was applied to PMd and M1 at seven times during response preparation/execution. In Exp 1 TMS was delivered to the left hemisphere, while motor regions in the right hemisphere were stimulated in Exp 2. In each experiment, TMS was also delivered to the vertex (Cz) as a control.
Results. Both younger and older adults responded faster following PMd (left & right) and Cz TMS, particularly when delivered during the preparatory period, relative to baseline RT (obtained in the trials without TMS), indicating a non-specific intersensory facilitation effect. M1 TMS in the involved right cortex resulted in an overall RT delay in young adults, while non-involved left M1 disruption did not modulate RT. In older adults, the left M1 disruption during the preparatory period and right M1 disruption during response generation delayed RT, suggesting a differential time-course of functional involvement of left and right M1 during response preparation and generation in older adults.

Disclosures: H. Fujiyama: None. **M.R. Hinder:** None. **J.J. Summers:** None.

Poster

890. Cortical Planning II

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Program#/Poster#:890.04/LL12

Topic: D.17. Voluntary movements

Title: Corticomuscular coupling slows reaction time of ballistic new movement

Authors: *R. MATSUYA[1], J. USHIYAMA[1,2], J. USHIBA[3,2]
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Abstract: Oscillatory activity of the sensorimotor cortex shows coherence with muscle activity within the 15-35 Hz band (β-band) during tonic isometric voluntary contraction. Previous numerous cross-sectional studies have reported that the magnitude of the corticomuscular coherence can differ according to various factors, such as neurological disorders, sports experience, habitude, and aging. Recently, we have reported that magnitude of corticomuscular coherence varies among individuals even within healthy young adults. Especially in the subjects showing greater coherence, prominent grouped discharge is observed in the electromyogram (EMG) signals. We hypothesized that such difference in the extent of EMG oscillation due to the magnitude of corticomuscular coherence leads to variations of motor function. To test this hypothesis, the present study explored the potential effects of the β-band corticomuscular coupling on ballistic new movement as assessed by reaction time (RT). First, we calculated the coherence between electroencephalogram (EEG) over the sensorimotor cortex and rectified EMG from the tibialis anterior muscle during tonic isometric contraction in 15 healthy subjects. Based on the data, we classified the subjects into two groups; i.e., the subjects showing significant EEG-EMG coherence (COH+, n = 8) or the subjects showing no significant coherence (COH-, n = 7). Next, the subjects performed ballistic contractions in reaction to auditory signals from the preliminary sustained contractions for 100 times. RT was defined as an interval between the signals and the response onset. There was no inter-subject difference in RT between COH+ and COH-. However, when the trials performed by COH+ subjects were divided into two groups depending on whether clear grouped discharge was observed in EMG (GD+) or not (GD-) before reaction, RT was significantly longer in GD+ than in GD- trials. Pooled EEG-EMG coherence was significantly greater in GD+ than in GD- trials. These results indicate that the magnitude of corticomuscular coupling of COH+ subjects fluctuates from moment to moment, and a reaction of new movement is delayed when corticomuscular coupling is greater. We suggest that β-synchrony in the corticospinal system impairs the ability for a rapid modulation of the firing rate of cortical neuron population, and therefore slows reaction of ballistic new movement.

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Poster

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Topic: D.17. Voluntary movements

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Medical Research Council

Engineering and Physical Sciences Research Council

Title: Fractionation of muscle activity in the StartReact paradigm

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Abstract: Reaction times (RTs) of movements performed in response to loud, acoustically startling stimuli are around 80ms shorter than those following a quiet cue (Valls-Sole et al. 1995). Such 'StartReact' RTs may be too short for cortical involvement, and could reflect storage of a prepared movement in a sub-cortical structure, and rapid release of this motor programme by the startling sound. The reticular formation (RF) and its descending output via the reticulospinal tract play a well-established role in the acoustic startle reflex. The early phase of StartReact responses could be mediated via the reticulospinal tract, in contrast to a primarily corticospinal pathway underlying typical voluntary reactions.

Axons of the reticulospinal tract make divergent connections to multiple muscles, in contrast to the more focal termination pattern of single corticospinal axons, which each innervate only a small number of motoneuron pools. We hypothesised that muscle activity patterns would be less flexible and more stereotyped in the early part of StartReact responses compared to those following slower onset voluntary reactions.

To test this hypothesis, 5 subjects were asked to carry out 32 different movements in response to a quiet (ca. 80dB) or startling (ca. 115-120dB) acoustic stimulus. Example movements were gripping a variety of objects, pulling against elastic loads, and clicking a mouse button. Surface electromyograms (EMG) were recorded from 16 muscles in the proximal and distal upper limb. The single-trial reaction time was detected as the earliest EMG onset latency, and the mean activity in each muscle measured over the subsequent 50ms. These EMG activities were subjected to principal component analysis, to see how effectively muscle patterns could be represented by a low-dimensional space.

The proportion of the variance explained by successive principal components was not significantly different for short-latency responses to loud cues, and for longer latency responses to quite cues. This suggests that muscle activation patterns were similarly fractionated in the two response types, and are thus likely to be initiated by a descending tract with similar divergence to motoneuron pools. We thus propose that the corticospinal tract plays a key role in both StartReact and more usual voluntary reactions. It remains possible that the reticulospinal tract contributes to reaction time shortening by delivering an unfocussed input, raising the excitability of many motoneuron pools and allowing a rapid fractionated response to subsequent more focal corticospinal input.

Disclosures: L.R. Dean: None. **S.N. Baker:** None.

Poster

890. Cortical Planning II

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Topic: D.17. Voluntary movements

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Söderbergska Stiftelsen

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James S. McDonnell Foundation

Title: A moving motor homunculus? Dynamic representations of intrinsic hand muscles in two arm postures

Authors: *H. VAN DUINEN, C. BROZZOLI, H. H. EHRSSON
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Abstract: Background: The human primary motor cortex is somatotopically organized; different body parts are represented in different territories, and single muscles also have their representations. This study examined the assumption that such maps are stable across limb postures.
Methods: We measured corticospinal excitability using transcranial magnetic stimulation (TMS) over a grid of 25 stimulation sites (5x5 cm) centered over the left primary motor cortex (n=13). The corticomotor representation was mapped for two intrinsic hand muscles (first dorsal interosseus, FDI, and abductor digiti minimi, ADM), while the right arm was lying on a table in front of the subjects in two different postures, namely with the right hand at the right side of the body and with the right hand crossing over to the left. Each site was stimulated 8 times in both arm postures. All motor evoked potentials (MEPs) were expressed as a percentage of the maximal compound muscle action potential.
Preliminary results: Overall corticospinal excitability was higher for FDI than for ADM (averaged over all stimulation sites; $p=0.023$). Furthermore, the difference in corticospinal excitability between the two muscles changed between the two arm postures ($p=0.026$). FDI showed greater excitability when the right hand was placed to the left side and ADM greater

excitability with the right hand on the right. These changes occurred without any registered change in the peripheral muscle output. We also observed dynamic changes in the corticomotor maps with the peak response spatially shifting dependent on arm posture. Moreover, the corticospinal excitability in the site of best response for one arm posture was higher than the response at the same stimulation site with the arm in the other posture (FDI: $p<0.037$; ADM: $p<0.014$).
Conclusions: Changes in arm posture induced changes in the general corticospinal excitability recorded from intrinsic hand muscles. Furthermore, changes in arm posture cause changes in the location of the site of best response in the motor cortex. These findings indicate that the proprioceptive information related to arm posture dynamically modulates the representation of intrinsic hand muscles in the motor cortex. This dynamic representation can have important implications for the development of rehabilitation training programs after stroke.

Disclosures: H. Van Duinen: None. **C. Brozzoli:** None. **H.H. Ehrsson:** None.

Poster

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Support: A Grant-in-Aid for Young Scientists (B) (#23700683) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan

Title: Strength of beta-band corticomuscular coupling is modulated moment-by-moment during low-level prolonged contraction

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Abstract: Oscillatory neural activity of the sensorimotor cortex measured by electroencephalogram (EEG) shows coherence with electromyogram (EMG) activity of contralateral limb muscles within the 15-35-Hz frequency band (beta-band) when performing weak to moderate intensity of isometric voluntary contraction (e.g., Conway et al., 1995; Mima et al., 2000; Ushiyama et al., 2011). Recently, it has been suggested that this corticomuscular coupling is mediated by not only motor pathways, but also somatosensory pathways, and reflects the sonsorimotor integration processes (e.g., Riddle & Baker, 2006; Witham et al., 2011).

The present study was designed to examine whether the strength of the beta-band corticomuscular coupling is constant or fluctuates during low-level prolonged contraction, which does not induce prominent muscle fatigue. Seven healthy young adults (three males and four females, 21-35 years) performed low-level prolonged dorsiflexion at 2.5% of maximal voluntary contraction (MVC) for 60 min. During the task, we recorded EEG over the sensorimotor cortex and surface EMG from the right tibialis anterior muscle. We found that EMG activation patterns changed moment-by-moment during prolonged contraction; i.e., in one times the EMG signals showed prominent grouped discharges within the beta-band, while in other times it showed tonic activation patterns. When we compared EEG-EMG coherence between the period with clear grouped discharge and the one with no grouped discharge, the magnitude of beta-band EEG-EMG coherence was significantly greater in the former period. Additionally, as a result of time-frequency analyses of EEG-EMG coherence, we found that the magnitude of the beta-band EEG-EMG coherence fluctuated randomly. We speculate that by occasionally elevating the corticomuscular coupling during prolonged contraction, the central nervous system may promote a sensorimotor recalibration, providing updated information about the state of the semsorimotor system. In return, the increased oscillation in EMG would impair the stability of motor output in such periods.

Disclosures: J. Ushiyama: None. **M. Liu:** None. **J. Ushiba:** None.

Poster

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Topic: D.17. Voluntary movements

Support: ARO Grant W911NF-07-1-0415

ARO Grant W911NF-08-1-0216

Title: Decoding onset and direction of movements using electrocorticographic (ecog) signals in humans

Authors: *A. GUNDUZ[1,2,3], Z. WANG[4], P. BRUNNER[2,3], Q. JI[4], G. SCHALK[2,3,4,5]
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Abstract: Communication of intent usually requires motor function. This requirement can be limiting when a person is engaged in a task, or prohibitive for some people suffering from neuromuscular disorders. Determining a person's intent from brain signals rather than from muscles would have important applications in clinical or other domains. For example, detection of the onset and direction of intended movements may provide the basis for restoration of simple grasping function in people with chronic stroke, or could be used to optimize a user's interaction with the surrounding environment. In this study, we investigate whether electrocorticographic (ECoG) signals, collected from five human subjects engaged in an 8-target center-out task, hold information about the onset and direction of hand movements. In each trial, subjects were presented with a target, but had to wait for a cursor to appear (i.e., a “go” cue) before they could hit the target using a joystick. We characterized the neural mechanisms involved in intention and movement by determining ECoG amplitude changes in mu (8-12 Hz), beta (18-26 Hz), high gamma (70-170 Hz) bands, and in the moving average of the raw signals, also known as the local motor potential (LMP).
For the detection of movement onset, we used a support vector machine (SVM) that determines from 1 s of accumulated ECoG activity the probability that the subject would initiate a hand movement in the next time step. We then used a novel implementation of a time-varying dynamic Bayesian network (TVDBN), which was designed to take advantage of the spatio-temporal dynamics of ECoG features, to determine the direction of the intended movement using ECoG signals prior to the movement. Finally, we simulated a targeting application in which brain signals prior to the movement are combined with the actual movement signals. In this simulation, the time-to-target reduced by up to 150 ms when we use the directional information captured in ECoG signals. Overall, our results contribute to our understanding of the neural representation of intended movements and suggest that integrating information from brain signals and motor execution may eventually lead to systems that can improve a user's performance.
(Authors Gunduz and Wang contributed equally to this work.)

Disclosures: A. Gunduz: None. **Z. Wang:** None. **P. Brunner:** None. **Q. Ji:** None. **G. Schalk:** None.

Poster

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Topic: D.17. Voluntary movements

Support: ESRC Grant ECRP09

Title: A neural predictor of the voluntary choice to omit an action

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Abstract: The capacity to endogenously and intentionally inhibit actions that we have planned is crucial in human behaviour, but the way in which such a mechanism operates and what influences contribute to a decision to act or not act remain largely unexplored. Here, we use human EEG to examine the readiness potential (RP) during a continuous stream of actions, and to explore preparatory activity during omissions of actions in the sequence. We compare activity for omissions made based on a pre-specified rule, to omissions freely decided on by the participant. We find that RP amplitude is reduced for actions that immediately precede a voluntary omission, suggesting that the motor system is already primed to make an omission before it occurs. This is not found for rule-based omissions. When comparing the omissions themselves, there is also a divergence in preparatory activity slopes into opposite polarities, with rule-based omissions showing a pattern similar to a typical RP but voluntary omissions showing an opposite pattern, seemingly reflecting suppression of weak motor activity. The results suggest that spontaneously fluctuating activity in the motor system could bias voluntary action selection when options are closely competing and a decision must be made between them.

Disclosures: E. Misirlisoy: None. **P. Haggard:** None.

Poster

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Topic: D.17. Voluntary movements

Support: NIH Grant R01 HD053793

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Title: Can anodal tDCS stimulation over V1 alter visuomotor behavior?

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Abstract: Electrical stimulation of primary sensory cortex can induce changes in sensory perception. For example, anodal transcranial direct current stimulation (tDCS), a non-invasive technique for modulating cortical excitability via GABA and NMDA receptor activity, applied over the primary visual cortex (V1) results in changes in contrast sensitivity (Spiegel et al 2012, PLoS One). However, it is unclear to what degree tDCS-induced changes in primary visual cortex can affect sensorimotor behavior. If tDCS of primary sensory cortex can affect motor behavior and multisensory processing in addition to unisensory perception, it may be a potential tool in rehabilitation of individuals with sensory deficits such as low vision or proprioceptive loss. Here we address this question in healthy young adults, asking if anodal tDCS over V1 can decrease subject's variability in pointing to visual targets. In addition, we assess whether this intervention affects visuoproprioceptive weighting (how much the brain relies on vision vs. proprioception when estimating the hand's position) or realignment (how much the brain adjusts vision vs. proprioception when a visuoproprioceptive misalignment is experimentally introduced). Right-handed subjects sat at a reflected rear-projection setup with no vision of either hand. The tDCS anode was placed over the occipital pole and the cathode on the subject's right cheek. The behavioral task involved moving the right index finger from a start position to a series of visual (V), proprioceptive/left finger (P), or combined (VP) targets. After a baseline reaching period consisting of alternating V, P, and VP targets, the V component began to gradually shift away from the left finger's position such that by the end of the Adaptation block, subjects experienced a 7-cm misalignment between visual and proprioceptive information about their target finger's position on VP trials. 2mA of current was turned on halfway through the baseline period and remained on until the end of Adaptation (Real, N=10) or was turned off after 30s (Sham, N=14). Subjects who received real tDCS of V1 decreased their pointing variability on V targets relative to sham tDCS subjects ($p=0.020$), but pointing variability to P targets was unaffected. Visuoproprioceptive weighting and realignment also appear unaffected by V1 tDCS. These results suggest that anodal tDCS over V1 can improve movement precision during visuomotor behavior.

Disclosures: H.J. Block: None. **N.D. Pasricha:** None. **P. Celnik:** None.

Poster

890. Cortical Planning II

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Program#/Poster#:890.11/LL19

Topic: D.17. Voluntary movements

Title: Movement observation modulates interhemispheric inhibition: An ipsilateral silent period study.

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Abstract: While it has been shown that voluntary control of action involves fine neural interactions between both motor cortices, the question of such an interhemispheric communication during covert actions has not been fully studied (Giovannelli et al., 2009; Carson and Ruddy, 2012). Particularly, the implication of the transcallosal inhibitory circuits during the perception of other's movement is still unknown. Here, we wanted to know if movement observation could specifically affects interhemispheric inhibition. Thus, we quantified the ipsilateral silent period (ISP) in the left opponens pollicis (OP) in healthy subjects. The ISP is a short attenuation of an ongoing voluntary electromyographic (EMG) activity in a specific muscle, which is induced by focal transcranial magnetic stimulation (TMS) of the ipsilateral primary motor cortex. This paradigm is well suited to investigate interhemispheric inhibitory control of voluntary cortical motor output. We evaluated the ISP in the left OP from 17 right-handed adults while they were actively observing fine hand movements performed by an experimentalist in front of them (i.e. grasping and tapping movements executed with the right hand). The size of the ISP was assessed during different movement conditions: at rest, during the closing phase of grasping and tapping movements, and during the opening phase of the same motor tasks. Our data showed a clear increase of the ISP during movement observation (on average, 22.7 ms for grasping and 24.3 ms for tapping) compared to the rest (on average, 16.3 ms) condition. More interestingly, the increase of the ISP was specifically tuned to the kinematics of the observed finger movements (on average, 17.3 ms and 19 ms for the grasping and tapping opening phase, respectively). Although movement perception has been widely shown to facilitate the central motor circuits, here we bring novel data that point out the fact that movement perception has significantly influence on inhibitory interhemispheric circuits. Interestingly, this modulation seems to be tuned to the kinematic pattern of the observed movement. Our study therefore deepens the concept of mirroring, or motor resonance, and indicates that transcallosal structures that mediates inhibitory motor signals are significantly implicated during movement observation.
Carson RG, Ruddy KL (2012) J Neurosci. 32(2):646-52.
Giovannelli F, Borgheresi A, Balestrieri F, Zaccara G, Viggiano MP, Cincotta M, Ziemann U (2009) J Physiol. 587:5393-5410.

Disclosures: C. Papaxanthis: None. **N. Gueugneau:** None. **T. Pozzo:** None. **M. Bove:** None. **L. Avanzino:** None.

Poster

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Topic: D.17. Voluntary movements

Support: R01-EB000856-06

Title: Comparing the electrophysiology and decoding power associated with each hemisphere during direction specific movements using ECoG in humans

Authors: *M. SHARMA[1], C. M. GAONA[1], N. SZRAMA[1], J. D. BRESHEARS[4], J. ROLAND[2], Z. FREUDENBURG[3], K. Q. WEINBERGER[3], E. C. LEUTHARDT[2]
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Abstract: Numerous studies have shown the efficacy of electrocorticographic (ECoG) signal in both understanding the movement related electrophysiology and kinematic decoding. Although the importance of activity contralateral to the moving limb is extensively studied and well understood, the understanding behind the ipsilateral activity is still not well established. Many imaging studies have suggested that ipsilateral M1 cortical activity is a result of transcallosal inhibition to prevent the unwanted co-activation of the other limb. More recent evidence, however, suggests that ipsilateral cortical activity represents a unique physiologic process and is more associated with motor planning than its execution. Researchers have shown the existence of directional tuning on the hemisphere ipsilateral to the moving limb, which indicates that ipsilateral activity is, to some degree, analogous to the contralateral activity. In this study, we hypothesize that ipsilateral activity encodes kinematic information, which can be used to decode movement direction. We also provide a direct comparison to information decoded from contralateral hemisphere.
ECoG data was collected from five human subjects while they performed a center out joystick task with their hand either ipsilateral or contralateral to the grid. Both datasets were separately analyzed for power changes (features) during the planning, movement and reward phases of the task. Using well-established machine learning techniques, these features were used to decode the direction of movement. Our findings show that although in comparison to contralateral, ipsilateral decoding accuracies are lower, yet significantly above chance. It was also found that different features contribute differently depending on the hemisphere. Specifically, whereas contralateral accuracies peaked when primary motor areas were used for decoding; ipsilateral accuracies peaked when the features used were from pre-motor areas, suggesting its involvement

in planning rather than execution of movement.
The results of this study suggest that cortical activations during ipsilateral motor task contain more complex activation patterns, which can be used to decode kinematic information. Although the amount of information decoded from ipsilateral cortex is lower than contralateral, it is still significant. These results further support an understanding of the active role that ipsilateral cortex plays during motor activities. This also extends the notion that ipsilateral cortical activity could provide important information that can be utilized for control of Brain Computer Interfaces.

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Poster

890. Cortical Planning II

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Topic: D.17. Voluntary movements

Title: Whole-cell recordings from mouse forelimb motor cortex neurons during targeted reaching.

Authors: *B. C. VOIGT, J. F. A. POULET
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Abstract: Primary motor cortex (M1) controls voluntary movement. While a number of studies have documented extracellular recordings during motor tasks, there is very little data available on the synaptic activity underlying motor processing in M1. Here we present whole-cell recordings from forelimb primary motor cortex (fM1) in mice trained to perform a forelimb reach and touch task. To locate fM1 we performed electrical microstimulation in anaesthetised mice and, in good agreement with previous studies, found fM1 centered 0.3 mm anterior and 1.6 mm lateral to bregma. Up to 6 head-restrained mice were trained in parallel within 6-10 days to reach and touch a sensor. Pharmacological inactivation experiments with cortical microinjections of the GABA-A receptor agonist muscimol confirmed that the behaviour was dependent on fM1 activity. Whole-cell recordings from fM1 in awake, behaving mice revealed strong state-dependent changes in subthreshold activity. When mice were sitting still, large amplitude, slow oscillations were observed in fM1 neurons. During reaching, by contrast, the membrane potential was depolarised and smaller amplitude, higher frequency events occurred. This confirms that state-dependent changes in cortical activity are present in alert, trained mice performing

cortically dependent behaviour. Here we have developed an M1 dependent motor task that will allow investigations into the cellular, synaptic and network mechanisms underlying cortical motor control.

Disclosures: B.C. Voigt: None. **J.F.A. Poulet:** None.

Poster

890. Cortical Planning II

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Program#/Poster#:890.14/MM2

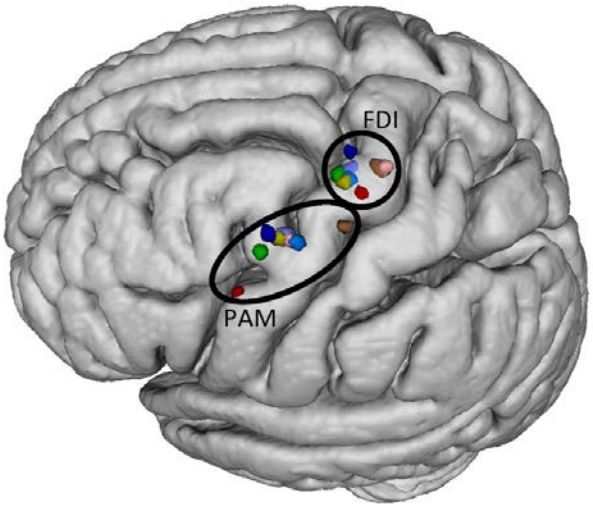
Topic: D.17. Voluntary movements

Title: Motor cortical representation of human auricular muscles: A robot-assisted and image-guided TMS mapping study

Authors: *J. MEINCKE, M. HEWITT, D. LIEBETANZ
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Abstract: In non-human species the auricular muscles play an important role during locating of acoustical sources. Besides neuroanatomical track tracing studies in primates, only sparse information exists concerning the neuroanatomy or function of the human auricular muscles, which are usually considered as rudimentary. To reveal the neuroanatomical structures involved in the function of these muscles, we aimed at determining a possible motor cortical representation of the auricular muscles in humans.
We performed a transcranial magnetic stimulation (TMS) mapping procedure on 8 healthy subjects (6 male; mean age 26,5 ± 5,0 years) using a novel robot-assisted and image-guided technology. A robotic arm (Adept Viper s850), linked to individual MRI data, ensured an exact positioning of the coil over the grid points with respect to the 6 degrees of freedom of the coil. For neuroanatomical mapping 12 TMS pulses were delivered over each point of a 7 x 7 cm grid with 1 cm spacing in a random sequence. EMG was simultaneously recorded from the contralateral posterior auricular muscle (PAM) using fine wire electrodes. Individual centers of gravity (COGs) were calculated and a TMS recruitment curve was recorded at this position.
In all subjects TMS over the primary motor cortex induced MEPs in the pre-innervated PAM with a mean onset latency of 9,2 ms (SD: 0,9). The hotspot was located on the precentral gyrus lateral to the first dorsal interosseus (FDI) hotspot, which was defined in a control experiment. The inter-COG distance between both muscles was 23,8 mm (SD: 5,1). In addition, MEP amplitudes in PAM depended on the intensity of stimulator output.
Our mapping results identify for the first time a specific motor cortical representation area of a

human auricular muscle which is located in short distance to the hand muscle area. The involvement of the primary motor cortex in the connectivity of auricular muscles may represent a neuroanatomical correlate of voluntary auricular muscle activation. Our results also point to a specific function of these muscles in humans, which still has to be



Positions of the centers of gravity (COGs) of the first dorsal interosseus muscle (FDI) and posterior auricular muscle (PAM) as determined by TMS mapping in 8 subjects and registered to the MNI152 T1 template. All COGs of PAM and FDI are located on the precentral gyrus.

elucidated.

Disclosures: J. Meincke: None. **M. Hewitt:** None. **D. Liebetanz:** None.

Poster

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Topic: D.17. Voluntary movements

Support: Wellcome Trust 086120/Z08/Z

European Research Council (ERC; ActSelectContext)

Biotechnology and Biological Sciences Research Council (BBSRC)

Title: Variability of cortico-spinal excitability tracks the preparatory state of human motor cortex

Authors: *M. C. KLEIN-FLÜGGE[1], D. NOBBS[1], J. B. PITCHER[2], S. BESTMANN[1]
[1]Sobell Department, UCL, London, United Kingdom; [2]Sch. of Paediatrics and Reproductive Health, Res. Ctr. for the Early Origins of Hlth. and Disease, Univ. of Adelaide, Adelaide, Australia

Abstract: Introduction: Task-evoked trial-to-trial variability is a ubiquitous property of neural responses, yet its functional role remains largely unclear. Recent work in non-human primates shows that the temporal structure of across-trial neural variability in several brain regions is task-related. For example, neural variability in pre-motor cortex tracks motor preparation, with increasingly consistent firing rates and thus a decline in variability prior to movement onset (Churchland et al., JNeurosci, 2006). However, whether non-invasive read-outs of the variability of population activity available from humans can also track the preparation of actions remains unknown.
Methods: We tested this by measuring cortico-spinal excitability (CSE) at different times during action preparation, using single-pulse transcranial magnetic stimulation (TMS) to the hand representation of human primary motor cortex (M1). Specifically, we sought to examine whether changes in variability prior to a movement differ a) between chosen and unchosen actions and b) processes involved in decisions-for-actions versus instructed action selection. First, we established the basic properties of intrinsic CSE variability at rest (n=46) which revealed a logarithmic relationship between CSE and variability. During the task (n=15), responses (left/right button press) were then either directly instructed (no-choice), or resulted from a value-decision (choice).
Results: Compared to rest, CSE variability during task performance was significantly increased throughout the trial. Notably, there was a further task-related decline in variability from about 230ms prior to movement onset in the responding hand which was more pronounced on fast compared to slow response trials, consistent with data in non-human primates. Critically, this effect occurred over and above that trivially expected based on the concurrent rise in CSE during motor preparation. Finally, variability showed a decrease for the unselected hand on choice trials only, possibly reflecting choice-specific suppression of unselected actions.
Conclusion: Measures of human CSE variability thus track the preparatory state of motor cortex, analogous to changes in firing rate variability during action preparation in monkeys that reflect the optimization of preparatory activity. This provides novel ways in humans to study the dynamics of action preparation but also more complex processes such as choice-to-action transformations.

Disclosures: M.C. Klein-Flügge: None. **D. Nobbs:** None. **J.B. Pitcher:** None. **S. Bestmann:** None.

Poster

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Program#/Poster#:890.16/MM4

Topic: D.17. Voluntary movements

Title: Variability of corticomotor excitability as an indicator of imagery ability

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Abstract: Motor imagery (MI) is the mental representation of an action without any concomitant motor output. Corticomotor excitability, assessed by motor evoked potentials (MEPs) from transcranial magnetic stimulation (TMS) of primary motor cortex is facilitated during MI and correlated to imagery ability (Lebon et al., 2012). In the present research, we examined the relationship between variability of MEPs and MI ability. Nineteen healthy adults (10 females; mean age = 26 ± 10) were assessed using the Motor Imagery Index (Collet et al., 2011) which is based on 6 sub-indicators: 1) Level of skin conductance (SC) , 2) Ohmic perturbation duration of skin conductance (OPD), 3) Respiratory sinus arrhythmia (RSA), 4) Mental chronometry (MC), 5) Self-estimation of imagery quality (SE), and 6) the revised version of movement imagery questionnaire (MIQ-R). Single-pulse TMS was delivered to the left primary motor cortex to elicit MEPs in the right opponens pollicis, when participants imagined a thumb tapping movement with their right hand. The intensity of TMS pulse was set to elicit 1 mV MEP amplitude at rest. The coefficient of variation (CV) of MEP amplitude from 12 MEPS collected during MI was determined. We performed a Least Squares Linear Regression, with CV as dependent variables (n = 19) and the 6 indicators of imagery quality as independent variables. The average CV was 0.46 during MI, and the linear regression was statistically significant ($R2 = 0.662$, $F = 3.915$, $P = 0.021$) for predictor variables SC, OPD, SE and MIQ-R . The variability of MEP amplitudes during MI was inversely correlated to imagery ability: the smaller the variability, the better the imager. In addition to the facilitation of corticomotor excitability during MI, the variability of responses provides relevant information to assess imagery ability.
Collet C, Guillot A, Lebon F, MacIntyre T, & Moran A (2011) Measuring Motor Imagery using Psychometric, Behavioral and Psychophysiological Tools. Exerc Sport Sci Rev. 39;85-92.
Lebon F, Byblow WD, Collet C, Guillot A & Stinear CM (2012) The modulation of motor cortex excitability during motor imagery depends on imagery quality. Eur J Neurosci. 35;323-31.

Disclosures: F. Lebon: None. **C. Stinear:** None. **W. Byblow:** None. **C. Papaxanthis:** None.

Poster

890. Cortical Planning II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:890.17/MM5

Topic: D.17. Voluntary movements

Support: NSERC

Title: Influence of an acute bout of aerobic exercise on cortical contributions to motor planning and execution

Authors: *J. S. THACKER, W. R. STAINES
Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Increasing evidence supports the use of physical activity for modifying brain activity and overall neurological health[1]. Specifically, aerobic exercise appears to improve cognitive efficacy with regards to decisional oddball tasks shown through the P300, whose amplitude and latency is augmented[2]. Furthermore, the effects of an acute bout of aerobic exercise on cardiovascular function are well established and are sustained following exercise cessation. Based on these findings, we proposed that (1) an acute bout of exercise may modulate movement-related cortical excitability within motor areas and (2) that transient effects would be sustained as long as heart rate (HR) remained elevated. Subjects (n=11) were placed in a soundproof booth and instructed to perform a self-paced unimanual ballistic wrist extension every 3-6 seconds of the right wrist while holding a moveable handle. The motion involved a brisk contraction followed by relaxation and positional reset, collected continuously for approximately 8 minutes. Electroencephalography was used to measure movement-related cortical activity using the Bereitschaftspotential (BP) time-locked to onset of muscle activity associated with movement. Following baseline measures, subjects performed 20 minutes of aerobic exercise at a moderate intensity (70% of age-predicted maximum heart rate) on a recumbent cycle ergometer. After the cessation of exercise, BP measures were recorded at two timepoints: immediately post-exercise (Post) and following a return to baseline HR (Post[Rest]), which was, on average, 45 minutes after the cessation of exercise. Movement characteristics were measured using both a potentiometer imbedded in the handle and electromyography (EMG) over the extensor carpi radialis muscle. The BP was separated into two components: the early, motor preparatory (BP1) and late, motor execution (BP2) phases. BP1 onset appears to present earlier and with larger amplitude, trending in Post and becoming significant ($p<0.05$) in the Post(Rest) condition compared to pre-exercise baseline measures. However, peak BP2 amplitudes, latency and slope did not reveal any significant differences in Post and Post(Rest) conditions for any electrode sites. In addition, no significant differences were found in the EMG profiles between all time conditions. These data suggest that motor preparation is susceptible to modulation by aerobic exercise. In addition, this affect seems more prominent even after other physiological markers such as HR have returned to pre-exercise baseline measures.
1.Hillman et al., 2008. Nature reviews. Neuroscience, 9: 58-65.
2.Magnié et al., 2000. Psychophysio., 37: 369-377.

Disclosures: J.S. Thacker: None. **W.R. Staines:** None.

Poster

890. Cortical Planning II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:890.18/MM6

Topic: D.17. Voluntary movements

Support: FRS - FNRS

Title: Interaction between selective attention and response selection during motor decision making

Authors: *P.-A. KLEIN[1,2], Q. FAVRE[2], A. MOURAUX[2], S. NOZARADAN[2], A. ZENON[2], J. DUQUE[2]
[1]Univ. Catholique De Louvain, Brussels, Belgium; [2]Inst. of Neurosci., Univ. catholique de Louvain, Brussels, Belgium

Abstract: On most occasions, multiple stimuli in the environment call for attention and action. We continuously have to attend to and act towards stimuli which are the most compatible with our goals, while refraining from selecting less relevant, yet possibly appealing, options. As such, decision making involves at least two major interrelated processes. The first one, called "selective attention", biases competitive interactions between sensory stimuli by favoring processing of information that is relevant to specific goals while ignoring irrelevant stimuli. The second process, "response selection", is associated with the accumulation of activity in competing response representations so as to ensure that only motor activity related to the most beneficial action reaches threshold and is selected.
Here, we used electroencephalography to characterize the interactions between selective attention and response selection. Participants performed a version of the Flanker task in which they were asked to indicate by a left or right button-press the orientation of a briefly presented left- or right-facing central arrow, flanked by a set of two distractor arrows on each side which either pointed in the same (congruent trials) or in opposite -conflicting- directions (incongruent trials). The proportion of congruent and incongruent trials was manipulated to produce two different conditions. In one of them, most trials were incongruent (80%) and thus subjects expected to face conflict (high conflict blocks, HCBs); in the "low conflict blocks" (LCBs), most trials were congruent (80%). Selective attention and response selection processes were assessed in each trial of the two block types by recording steady-state visual evoked potentials (SSVEP, central and distractor arrows flicked at different frequencies) and lateralized readiness potentials (LRPs over motor areas), respectively.
Behavioural and EEG data were collected on a preliminary set of 6 participants. As expected,

response times and error rates were larger in incongruent compared to congruent trials. However, this difference was attenuated in the HCBs compared to the LCBs, probably because in HCBs, control mechanisms were recruited to help sharpen selective attention and response selection. Our preliminary EEG data are consistent with this hypothesis. SSVEP recordings indicate a more selective processing of the central arrow (compared to distractors) in the HCBs than the LCBs. This effect was associated with larger and earlier LRPs in incongruent trials of the HCBs than LCBs. More subjects are required to establish precisely the relationship between SSVEPs and LRPs measures, with respect to behavior.

Disclosures: P. Klein: None. **Q. Favre:** None. **A. Mouraux:** None. **S. Nozaradan:** None. **A. Zenon:** None. **J. Duque:** None.

Poster

890. Cortical Planning II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:890.19/MM7

Topic: D.17. Voluntary movements

Title: Modulation on motor inhibition control caused by transcranial magnetic stimulation

Authors: *O. HIWAKI, N. ISHIMARU, H. FUKUDA
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Abstract: The flexible voluntary motor control is achieved with the plan prior to the movement and on-going adjustment. The function of the motor cortex for dynamic control of the finger motor inhibition has not yet been well understood. In the present study, we investigated the property of the voluntary finger force control involving inhibitory alteration triggered by the stop signal. The transcranial magnetic stimulation (TMS) of the motor cortex was applied for dynamic interference with on-going motor control processed in the motor cortex. The series of the go-stop task of the index finger movement, which consisted of TMS trials and sham TMS trials, was conducted. A clock whose hand completed a full rotation for four seconds was indicated on the PC monitor. The subject was instructed to start pushing a force sensor with an index finger at the moment the clock hand passed the line at three o'clock position. The subject controlled finger force increasing toward 2 N with watching the force indicator displayed on the monitor after the movement initiation. The subject was also asked to abandon the finger push movement when the stop signal with a red LED was indicated at the start time of the movement, that is, the time the clock hand passed the line at three o'clock position. In the trials with TMS, the single pulse TMS of the motor cortex was conducted at -100 ms, -50 ms, 0 ms, 50 ms, 100

ms, 150 ms, 200 ms and 250 ms after the start time of the movement. In the sham TMS trials, the TMS coil located over the scalp made clicking sound without stimulation of the motor cortex at the same timings as the TMS trials. As a result, the TMS prior to the stop signal inhibited the voluntary finger movement, whereas the TMS conducted at 100 ms and 150 ms after the stop signal enhanced the voluntary finger movement. The result suggests that the TMS of the motor cortex modulates the motor inhibition control of the voluntary finger movement. The timing of the TMS is important for inhibition or enhancement effect of TMS on the inhibitory motor process.

Disclosures: O. Hiwaki: None. **N. Ishimaru:** None. **H. Fukuda:** None.

Poster

890. Cortical Planning II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:890.20/MM8

Topic: D.17. Voluntary movements

Support: NSERC

Title: The role of attention on early somatosensory processing in frontal areas involved in movement planning and preparation

Authors: *M. J. N. BROWN, W. R. STAINES
Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Short-latency somatosensory evoked potential (SEPs) in parietal and frontal areas represent the earliest input of ascending somatosensory information into cortical areas. Studies on parietal SEPs have found that increasing attention (i.e. counting peripheral stimulations) may enhance the amplitude of parietal SEPs possibly as early as the P27 component. The frontal N30, a SEP component maximal over frontocentral sites, is thought to represent early somatosensory input to non-primary motor areas for planning and preparation of movement. Although movement (or mental simulation) can modulate the frontal N30, it is unclear how early somatosensory input is influenced by attention when such afferent information is needed for stimulus detection or sensory-guided movement. The first study recorded SEPs (i.e. N30) using a 64-channel EEG cap in 10 healthy young adults (22-38yrs) time-locked to peripheral median nerve (MN) stimulation to the left or right wrist. Attention was modulated by having participants silently count the number of infrequent high and low targets embedded with frequent non-targets to either the left (AttL) or right (AttR) index finger provided by vibrotactile stimulation. In addition, control conditions included participants silently counting backwards by 7 (Count) or

sitting quietly (Rest) during MN and index finger stimulations. It was hypothesized that unlike parietal components, attention would not modulate the frontal N30 during tactile detection since this afferent information is irrelevant for planning a movement. Statistical analyses revealed no significant effects of attention on the peak amplitude of the frontal N30 or on the peak-to-peak amplitude of P20-N30 complex ($p>0.05$). As expected, these results suggest that attentional modulations do not influence early somatosensory input to non-primary motor areas during detection of tactile stimuli. Consequently, this supports that the movement afferents to frontal non-primary motor areas are not influenced by a global increase in activity in the attentional network. The second study used a similar experimental design to measure whether modulations of the frontal N30 are observed when participant's direct attention to tactile stimulations that are required to prepare and execute a movement (i.e. finger tapping sequence). Finger tapping sequences were matched to the infrequent low or high targets provided by vibrotactile stimulation to the index fingers. Results will be discussed on whether combining attention of tactile stimuli relevant for planning a movement modulates early somatosensory input to frontal areas.

Disclosures: M.J.N. Brown: None. **W.R. Staines:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:891.01/MM9

Topic: D.18. Brain-Machine Interface

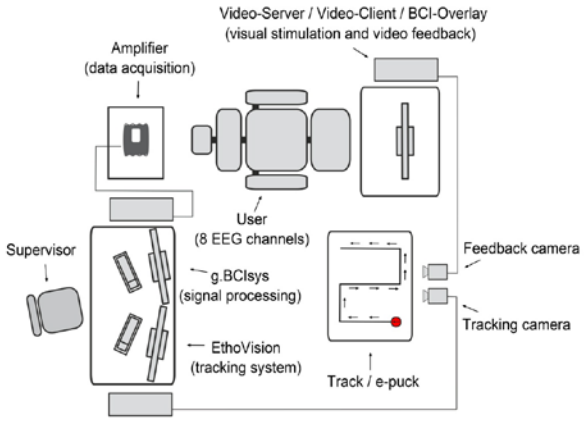
Support: European Union FP7 Integrated Project VERE (No. 257695)

Title: SSVEP based BCI system as general control interface for computer programs using a new overlay technique

Authors: *C. KAPELLER[1], C. HINTERMÜLLER[1], M. ABU-ALQUMSAN[2], B. GROßWINDHAGER[1], R. PRÜCKL[1], A. PEER[2], B. ALLISON[3], C. GUGER[1]
[1]Guger Technologies OG, Schiedlberg, Austria; [2]Tech. Univ. of Munich, Munich, Germany; [3]Univ. of California at San Diego, La Jolla, CA

Abstract: A brain-computer-interface (BCI) allows the user to control a device or software with brain activity. A BCI system can help disabled users communicate with their environment. Many BCIs rely on visual stimuli with constant stimulation cycles that elicit steady-state visual evoked potential (SSVEP) activity in the electroencephalogram (EEG). The SSVEP response can be generated with (i) a computer screen flashing with a constant frequency (f-VEP) or with (ii)

pseudo-random stimulation frequencies of a computer screen (c-VEP). This study compared these BCI methods in a new type of SSVEP BCI that used streaming video to help control a robotic device through tele-presence (Figure 1). The BCI controls were overlaid on a video image that showed a robot performing tasks in a remote environment. The designed stimulation module allows the integration of BCI controls in any OpenGL based graphics application. 11 healthy subjects used the BCI to drive a robot along a certain path as quickly and accurately as possible. The subjects used 4 BCI controls (forward, left, right, backward) to steer the robot based only on the video image of the robot. Results showed that the new video BCI overlay method was very robust and that the simultaneous video did not impair performance. The maximum online accuracy was 91.36 % (i), and 98.18 % (ii). The average time to complete the tasks was 573.43 s (i), and 222.57 s (ii). Subjects needed 93.29 s to complete the task with keyboard. Interestingly, all of the subjects achieved accuracy above 90 % in the c-VEP configuration, while three subjects were below 90 % using the f-VEP configuration. Therefore the c-VEP based BCI seems to be the fastest, most accurate, and most universal BCI in this study. Also, all of the subjects were comfortable with the video BCI overlay implementation that combined video images and BCI controls on one screen, which should increase usability and presence. This is especially noteworthy because both the control and feedback were continuous and not discrete, which is more realistic in many field settings but might have entailed greater distraction or confusion.



Disclosures: C. Kapeller: None. **C. Hintermüller:** None. **M. Abu-Alqumsan:** None. **B. Großwindhager:** None. **R. Prückl:** None. **A. Peer:** None. **B. Allison:** None. **C. Guger:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:891.02/MM10

Topic: D.18. Brain-Machine Interface

Support: ARO 54228-LS-MUR

Title: SSVEPs generated by unattended flickering fields can be used to predict the locus of covert spatial attention

Authors: *R. A. COLEMAN[1,2,3], A. LI[2], M. D'ZMURA[2]
[1]Cognitive Sci., Univ. of California, Irvine, Irvine, CA; [2]Univ. of California, Irvine, Dept. of Cognitive Sci., Irvine, CA; [3]Univ. of California, Irvine, Dept. of Statistics, Irvine, CA

Abstract: Users of current SSVEP BCIs attend to one of several discrete flickering fields to issue a command. We are studying the use of SSVEPs generated by peripheral, unattended, flickering fields. The user freely shifts covert visual attention throughout their visual field. SSVEP amplitudes are used to determine where they pay attention.
We performed an EEG experiment in which subjects were prompted to shift covert visual attention to locations along a horizontal axis while maintaining central fixation. On each trial, subjects attended to a location marked by a letter and used a keyboard to indicate letter identity. During the 2.5-sec trials, subfields at far left and at far right of the display provided contrast reversing flicker at different frequencies. 600 trials were collected per session, for eight sessions per subject, for eight subjects. Trials with incorrect key press responses were excluded from further analysis of EEG data, resulting in a mean of 4566 trials per subject.
EEG data were collected on 64-channel, ANT-NEURO ® EEG systems. Preprocessing generated both 1) autoregressive spectra and 2) canonical correlation analysis filter responses at fundamentals and relevant harmonics of each flicker. These features were used in preliminary analysis to classify trials according to whether attention was directed to the left or to the right of the central fixation point. Probabilistic linear discriminant analysis was conducted within subjects, across sessions, and with leave-one-out cross-validation. Classification resulted in a mean prediction rate of 61% across subjects with best and worst classification rates of 73% and 52%, respectively.
These results show that peripheral, unattended, flicker-induced SSVEPs can be used to predict the hemi-field in which the viewer is attending covertly. This motivates current work on whether the position of horizontal attention can be predicted continuously from SSVEPs. Such a BCI could be used alone or with other BCI methods to advance the state-of-the-art.

Disclosures: R.A. Coleman: Research Grant; ARO 54228-LA-MUR. **A. Li:** Research Grant; ARO 54228-LS-MUR. **M. D'Zmura:** Research Grant; ARO 54228-LS-MUR.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:891.03/MM11

Topic: D.18. Brain-Machine Interface

Support: DARPA REPAIR Project N66001-10-C-2008

Title: Application of hemodynamic prefrontal cortex desirability signals as reinforcers in machine learning

Authors: M. M. DISTASIO[1], *J. T. FRANCIS[2]
[1]Physiol., SUNY Downstate Med. Ctr., BROOKLYN, NY; [2]SUNY Downstate Med. Cent, BROOKLYN, NY

Abstract: Decision-making ability in the frontal lobe (among other brain structures) relies on the assignment of value to states of the organism and its environment. Then higher valued states can be pursued and lower valued (or negative) states avoided. The same principle forms the basis for computational reinforcement learning controllers, which have been fruitfully applied both as models of value estimation in the brain, and as artificial controllers in their own right. This work shows how state desirability signals decoded from frontal lobe hemodynamics, as measured with near-infrared spectroscopy (NIRS), can be applied as reinforcers to an adaptable artificial learning agent in order to guide its acquisition of skills. A set of experiments carried out on an alert macaque demonstrate that both oxy- and deoxyhemoglobin concentrations in the frontal lobe show differences in response to both primarily and secondarily desirable (versus undesirable) stimuli. This difference allows a NIRS signal classifier to serve successfully as a reinforcer for an adaptive controller performing a virtual tool-retrieval task. The agent's adaptability allows its performance to exceed the limits of the NIRS classifier decoding accuracy. We also show that decoding state desirabilities is more accurate when using relative concentrations of both oxyhemoglobin and deoxyhemoglobin, rather than either species alone. This suggests that studies of value-related brain function that infer patterns of neural activity based solely on deoxyhemoglobin concentration (as in fMRI) may yet be incomplete. This work is part of a continuing investigation into the use of reinforcement learning agents as controllers in brain-machine interfaces.

Disclosures: M.M. DiStasio: None. **J.T. Francis:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:891.04/MM12

Topic: D.18. Brain-Machine Interface

Support: Kinki University (No. 09-IV-14)

Title: Relationship between P300 latency and accuracy rate of auditory brain-computer interface

Authors: *N. YAMAWAKI, E. KATAYAMA, T. HAYASHI, S. SEKI
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Abstract: Patients who have difficulty moving their muscles freely due to amyotrophic lateral sclerosis (ALS) require support for various situations that occur in everyday life. Brain-computer interface (BCI) systems using motor imagery or event-related potential provide a means of communication for the patients. However, in later stages of ALS patients lose all voluntary muscular control, including voluntary control of gaze. Since the auditory BCI is suitable for the patients, we have developed a BCI based on auditory stimulation for the communication. P300 event-related potential for three-stimulus oddball paradigm (two target stimuli and one frequent stimulus (pink noise)) was used in the BCI. The frequency of one of two target stimuli was fixed to 3000 Hz, and the other frequency was changed from 300 Hz to 1000 Hz at interval of 100 Hz. Subjects were required to attend to a designated target and ignore the other target and the pink noises. Ten tasks were performed for each target frequency set, such as 3000 Hz and 500 Hz. The EEG signals recorded from Cz (sampling rate 500Hz) of international 10-20 system were used for the BCI. Twenty EEG signals for 512 ms in response to the target stimuli were added and the mean value of the added EEG (ERPs) was low-pass filtered at 4 Hz. The peak-to-peak values of the P300 amplitude for two target stimuli were compared and the stimulus observed larger peak-to-peak value was estimated as the attended target. As a result, the optimum frequency combination was a set of 3000Hz and 500Hz, and an average accuracy rate of 90% (subject A: 90%, B: 90%) was achieved. The P300 latency of low accuracy rate at another target frequency set shows a tendency of decrease.

Disclosures: N. Yamawaki: None. **E. Katayama:** None. **T. Hayashi:** None. **S. Seki:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:891.05/MM13

Topic: D.18. Brain-Machine Interface

Support: Air Force Office of Scientific Research

Title: The contribution of methodological variability to multi-day use of passive brain-computer interfaces

Authors: *J. R. ESTEPP, J. C. CHRISTENSEN
Air Force Res. Lab., WPAFB, OH

Abstract: The application of brain-computer interface (BCI) technology to healthy users is a promising avenue for enriching human-machine interaction. Passive BCI, which can be described as a paradigm of nonvolitional control derived from physiological and behavioral indices, may be especially useful in adapting systems to a user's moment-to-moment cognitive state. Recent work in this area has shown that passive BCI systems suffer from performance degradation over time, which is likely in part due to the underlying machine learning algorithm's inability to generalize to the nonstationary dynamics of a user. This can be ameliorated, to some extent, by the use of learning sets spanning long time courses. However, understanding the sources of variability that influence nonstationarity may lead to improved passive BCI system performance. In an effort to begin to understand what these sources may be, we investigated the most basic potential source of variability, removal and replacement of electrodes used for electrophysiological measurement, and its contribution to machine learning accuracy over the span of several hours. Two groups of participants (ten in each group) completed two blocks of two trials each of the Multi-Attribute Task Battery; task workload was the between-trial manipulation in each block. The blocks were explicitly separated by one hour. In the first group (the control group) the electrodes (vertical and horizontal electrooculography, electrocardiography and five channels of electroencephalography) used in the montage were left in place between blocks. In the second group (the test group) the electrodes were removed at the end of the first block and replaced prior to the start of the second block. The NASA Task Load Index (TLX) was used to assess the participant's subjective workload and electrode impedance was measured before and after each block. Accuracy of an artificial neural network (ANN) learning algorithm, with the learning set derived from the first block, was assessed using nested k-fold cross validation with test sets from both the first (within) and second (between) blocks. Results showed no significant differences in task performance, subjective workload or electrode impedance between groups or blocks. There was not a significant difference in test set accuracy between groups, although within-block accuracy was significantly higher than between-block accuracy. These results suggest that methodological variability, in the form of removal and replacement of electrodes, is not likely a contributor to the decline of the machine learning algorithm's accuracy over time, which was observed in this study as well as others prior.

Disclosures: J.R. Estepp: None. **J.C. Christensen:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:891.06/MM14

Topic: D.18. Brain-Machine Interface

Support: Grants-in-Aid for Scientific Research, base study: C, 23500151

Title: A new single-trial-EEG-based BCI using silent speech tasks

Authors: *T. YAMAZAKI[1], K. YAMAMOTO[2], K.-I. KAMIJO[3], T. YAMANOI[4]
[1]Kyushu Inst. of Technol., Fukuoka, Japan; [2]Biosci. and Bioinformatics, KIT, Iizuka, Japan; [3]NEC, Tokyo, Japan; [4]Hokkai Gakuen Univ., Sapporo, Japan

Abstract: We have proposed a new single-trial-EEG-based BCI using silent speech tasks. The framework consists of two phases. In the first phase (learning phase), single-trial EEGs and speech signals were recorded during overtly spoken janken tasks. The learning phase specifies a Kalman filter using both spectrograms of the speech signals and independent components (ICs) of the EEGs recorded during the actual janken task. The decoding phase predicts spectrograms for the silent janken by the Kalman filter with the EEGs recorded during the silent task. The present results demonstrated that when using the Kalman filter with the ICs whose dipole solutions were localized to the primary motor cortex, the premotor cortex and/or the Broca's area, covertly spoken vowels for "rock" and "paper" were all correctly recognized for ten subjects.

Disclosures: T. Yamazaki: None. **K. Yamamoto:** None. **K. Kamijo:** None. **T. Yamanoi:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:891.07/MM15

Topic: D.18. Brain-Machine Interface

Support: NINDS R01NS075889

University of Maryland-Baltimore's Pepper Pilot Study Award

Title: Decoding gait kinematics from scalp EEG of stroke patients: Preliminary comparison of Wiener and Kalman decoders.

Authors: *K. NATHAN[1], L. FORRESTER[2,3], R. GOODMAN[3], J. L. CONTRERAS-VIDAL[4]
[1]Univ. of Maryland, College Park, MD; [2]Dept. of Physical Therapy & Rehabil. Sci., Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Veteran Affairs Med. Ctr., Baltimore, MD; [4]Lab. for Noninvasive Brain-Machine Interface Systems, Univ. of Houston, Houston, TX

Abstract: Our group has recently shown the feasibility of decoding the kinematic parameters of controlled human locomotion from the lower frequency range of electroencephalographic (EEG) signals during a precision walking task (J Neurophysiol 2011; IEEE TNSRE 2012). Here, we turn our attention to stroke survivors who have had lesions resulting in chronic hemiparetic gait. In an identical experimental set-up based on our prior studies, we recorded the EEG of stroke recovery patients during rest, and normal and precision treadmill walking tasks while tracking bilaterally the kinematics of the hip, knee, and ankle. In offline analysis, we reduced the dimensionality of EEG channels and time lags with principal component analysis, and then passed the reduced data through a Wiener Filter and an unscented Kalman filter to predict estimates of the kinematic parameters. Here, we report on data from two stroke patients: one (S1) with left lateral medullary infarct, multiple bilateral cerebellar infarcts, small left occipital lobe tip infarct and right basal ganglia infarct, whereas the second patient (S2) shows both ischemic and hemorrhagic strokes on the right side. We found the performance results of our decoders to be asymmetric with respect to the body (R vs. L: 0.52(0.14) vs. 0.47(0.17) for S1 and 0.72(0.14) vs. 0.66(0.25) for S2), a reasonable expectation given the asymmetry in the impaired walking gait and lesions in the brain. Despite prior studies having shown better success with neural decoding using the unscented Kalman filter, we do not see noticeable improvement in accuracy (Wiener vs. Kalman: 0.51(0.14) vs. 0.50(0.17) for S1 and 0.68(0.22) vs. 0.67(0.22) for S2) except that the unscented Kalman filter's recursive algorithm may lend itself to be a better option for online decoding. With improved decoding of EEG signals from damaged brains, we hope to soon be able to correlate activity to more intentional and normal-form walking that can guide users of a prosthetic or exoskeleton.

Disclosures: K. Nathan: None. **L. Forrester:** None. **R. Goodman:** None. **J.L. Contreras-Vidal:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:891.08/MM16

Topic: D.18. Brain-Machine Interface

Support: The Hartwell Foundation

Title: Two-dimensional control of a mouse cursor achieved by contracting the Extensor Pollicus Longus with preselected frequency bands

Authors: *I.-M. SKAVHAUG[1], R. BOBELL[2], B. VERNON[2], A. BARSZAP[2], S. JOSHI[2]
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Abstract: We are developing a new class of Brain-Computer Interfaces (BCI) called a Brain-Muscle-Computer Interface (BMCI), which is based on electrical potentials recorded from one single surface muscle site to achieve multidimensional control of a mouse cursor. Participants learn to use this interface through an operant-conditioning paradigm based on visual feedback. We have previously demonstrated that participants can learn to navigate a cursor to targets by manipulating the surface ElectroMyoGram (sEMG) recorded from a muscle site located above the Auricularis Superior (AS; Perez-Maldonado, Wexler & Joshi, 2010). Control was achieved by simultaneously adjusting the power in two separate frequency bands selected to suit the individual participant. The AS muscle is responsible for re-orienting the ears independently of head movements, and are rarely used purposefully in humans. For this reason, and because the AS is usually accessible even to patients suffering severe neck-down paralysis, a BCI system based on sEMG from this muscle can enable individuals to control their physical environment without causing interruptions to other daily actions (e.g. speech and eye gaze). Here we report results from a newly developed portable android phone version of the BCI device (see Vernon & Joshi, 2011). We present evidence that healthy participants can use the interface by contracting the Extensor Pollicus Longus (a muscle located on the wrist that extends the thumb) - suggesting that muscles other than the AS can be used to navigate the cursor. Furthermore, because the frequency bands were preselected, and remained constant across participants, this demonstrates that each individual is able to adapt to parameters set by the device. The experimental protocol required participants to navigate the cursor to randomly presented target locations. Participants' accuracy scores significantly increased during the course of training and the cursor trajectories indicate that they moved the cursor with intentionality rather than at random. Future experiments will recruit disabled participants, focusing on the AS (or alternative accessible muscles), to establish the potential of the BMCI for this user group.

Disclosures: I. Skavhaug: None. **R. Bobell:** None. **B. Vernon:** None. **A. Barszap:** None. **S. Joshi:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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Program#/Poster#:891.09/MM17

Topic: D.18. Brain-Machine Interface

Support: Cerbomed GmbH Research Support

NIH - NIGMS 2R25 GM060826

Title: Activation of human vagus nerve afferent projections via electrical stimulation of external ear: fMRI evidence.

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Abstract: There is anatomical evidence that a branch of the vagus nerve provides sensory innervation of the external ear. More specifically, the auricular branch of the vagus innervates the concha, which is the concave region immediately superior to the external meatus of the ear. Premise: The question addressed in the present study is whether brain regions to which vagal afferents are known to project (e.g., solitary nucleus [NTS]) can be accessed via electrical stimulation of this peripheral branch of the vagus nerve in humans. Methods: We identified activated brain regions using functional MRI. We verified the location of the NTS in the medulla oblongata by administration of a gustatory stimulus (1 cc sauce of lime juice, sugar, salt and mustard). We localized and differentiated this nucleus from the laterally adjacent nucleus cuneatus by the subjects' tapping their fingers for 40sec. Electrical stimulation of the concha of the subjects' left ear was applied using the battery-driven NEMOS® device manufactured by Cerbomed. The stimulus intensity was increased from 0.1mA in 0.1mA increments until the subjects reported a "tingling" sensation that was below noxious intensity, typically below 1mA. The non-adjustable stimulation parameters of the device were continuous 0.25msec-duration monophasic square wave pulses at 25Hz. After a 20 sec baseline resting period, the stimulation was applied continuously for 7 min, followed by a 25 min rest period. The fMRI data were analyzed using FSL (FMRIB Software Library). Results (preliminary): Brain regions with clearly delineated landmarks activated by the electrical stimulation included: NTS, raphe nuclei, thalamic VPM, nucleus accumbens, amygdala, hippocampus, insula, and postcentral gyrus - paracentral lobule (genital) and thoracic homuncular zones. Activation was also observed in the regions of the anterior hypothalamus (paraventricular nucleus), substantia nigra, and locus coeruleus. Each of the 5 subjects tested spontaneously volunteered comments that they felt particularly relaxed after the stimulation period. Conclusion: There are clinical reports that stimulation of the left vagus trunk via electrodes implanted surgically in the neck provides therapeutic benefit against epilepsy, depression and pain. The present findings suggest that because electrical stimulation of the external ear activates the major projections of the vagus

nerve, the present method of vagal stimulation could provide a less invasive therapeutic alternative.

Disclosures: E. Frangos: Other Research Support; Cerbomed GmbH, Erlangen, Germany. Consultant/Advisory Board; Cerbomed GmbH, Erlangen, Germany. **J. Ellrich:** Employment; Cerbomed GmbH, Erlangen, Germany. **J. Dell'Italia:** Other Research Support; Cerbomed, GmbH, Erlangen, Germany. **N. Wise:** Other Research Support; Cerbomed GmbH, Erlangen, Germany. **B.R. Komisaruk:** Other Research Support; Cerbomed GmbH, Erlangen, Germany. Consultant/Advisory Board; Cerbomed GmbH, Erlangen, Germany.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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NIH grant EB00856

Title: Towards optimal design of a dichotic auditory streaming brain-computer interface

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Abstract: Most brain-computer interface (BCI) systems require users to modulate brain signals in response to visual stimuli. Thus they may not be useful to people whose paralysis (resulting for example from advanced ALS or severe Guillan-Barré) affects their eye muscles, resulting in limited vision. To address this problem, we are developing BCIs based on shifts of attention in non-visual modalities.
We used the online BCI framework described by Hill & Schölkopf (2012, J. Neural Eng.), which exploits attention-modulation of N1 event-related potentials in response to two or more

concurrent streams of periodic stimuli. We set out to optimize this system. Our principal contrast was between the original design (unequal periods in two dichotic streams of auditory stimuli), and a design that uses strict alternation between stimuli in the two ears (equal periods). Equal periods may yield cleaner brain signal features for classification, and would allow for a more even transfer of information over time, but unequal periods may provide an additional auditory feature by which subjects could group the stimuli of the attended stream.
To determine which design provides superior online performance, 16-channel EEG measurements were taken from 16 healthy subjects. Each trial lasted 5 sec and consisted of two dichotic streams. Each stream was a periodic sequence of 7-8 tone bursts. Most of these were amplitude-modulated at 40Hz (standard), but on each trial, 1-3 of the stimuli were presented without modulation (targets). Subjects counted the targets in one stream (chosen for them by computer) while ignoring the other stream. Successful modulation of auditory ERP amplitude was rewarded with feedback at the end of each trial.
The equal-periods condition was superior for 14 out of the 16 subjects: online binary BCI classification performance ranged from 60/100 to 100/100 correct across subjects (mean 79.9%), an increase of 7.8 percentage points relative to the unequal-periods condition. The difference was significant (Wilcoxon signed rank test, N=16, p=0.002).
Finally, we explored the potential of the system for real-time control/neurofeedback applications in one additional subject. After only 30 minutes of training, the subject was able to exert continuous control over the horizontal position of a paddle in a computer game by shifting attention between two auditory streams.
Our results demonstrate that ERP modulation via shifts of auditory attention can be harnessed to create an online BCI with as few as 16 EEG electrodes; that performance can be significantly improved by optimizing the stimulus timing parameters; and that the resulting approach supports continuous BCI control.

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Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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Topic: D.18. Brain-Machine Interface

Support: CIN Grant Pool-Project 2011-08

Title: Real-time fMRI-brain computer interfaces for rehabilitation of Parkinson’s disease patients

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Abstract: In Parkinson's disease (PD) medial motor areas are more impaired than those located on the lateral convexity (Goldberg, 1985). For example, relative reduction of activity in the supplementary motor area (SMA) during self-initiated hand movements has been reported (Tessa et al., 2010). In this pilot study, we investigated whether a Parkinson's disease patient could learn to voluntarily up-regulate the activity of SMA by means of real-time fMRI neurofeedback (RtfMRI-BCI).
The subject was a 52 year-old woman with mild, idiopathic Parkinson's Disease (right handed, disease duration= 39 month, akinesia type, left dominant, UPDRS I = 0, UPDRS II = 5, UPDRS III = 23, UPDRS IV = 0, Hoehn and Yahr = 2,5). After one functional localizer session, the subject underwent several sessions of real-time fMRI-BCI training to achieve voluntary control of the blood oxygenation level dependent (BOLD) signal from the bilateral SMA. During training, the subject was instructed to perform motor imagery in order to control the BOLD signal, while receiving contingent visual feedback in real-time. The influence of the learned enhanced SMA activity on motor performance was tested with interspersed blocks of a speeded, bimanual, button-pressing task (left index, right index, left middle, right middle). The subject successfully learned to control her SMA BOLD signal across RtfMRI sessions. Immediately following SMA up-regulation, response times for the initial button-press responses were elevated. These results suggest that self-regulation of SMA is possible with RtfMRI-BCI in Parkinson´s patients. However, it may be inferred that, to improve motor performance, down-regulation of SMA might be needed. To confirm these results, more subjects and longer training sessions are needed.
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Goldberg, G. (1985). Supplementary motor area structure and function: Reviews and hypotheses. Behavioral Brain Science, 8, 567-616.
Tessa C, Lucetti C, Diciotti S, Baldacci F, Paoli L, Cecchi P, Giannelli M, Ginestroni A, Del Dotto P, Ceravolo R, Vignali C, Bonuccelli U, Mascalchi M. (2010). Decreased and increased cortical activation coexist in de novo Parkinson's disease. Exp Neuro, Jul;224(1):299-306.

Disclosures: K. Buyukturkoglu: None. **S. Ruiz:** None. **M. Rana:** None. **S.R. Soekadar:** None. **T. Wächter:** None. **S.A. Hackley:** None. **N. Birbaumer:** None. **R. Sitaram:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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Support: WCU(World Class University) program (R31-100080)

National Research Foundation of Korea(NRF) (20110026502)

Title: Extraction of cross-frequency coupling features related to hand motor imagery for brain-computer interfaces

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Abstract: Non-invasive brain-computer interfaces (BCIs) based on electroencephalography (EEG) aim to provide novel communication channels for those who suffer from neurological disorders with paralysis such as amyotrophic lateral sclerosis. One of the prominent ways to build a non-invasive BCI is utilizing the modulation of sensorimotor rhythms (SMRs) in the EEG induced by motor imagery. SMRs generally exhibit event-related desynchronization and synchronization (ERD/ERS) during motor imagery, which have been widely used as key features for BCIs. However, the SMR features have major drawbacks including inter-subject variability and the limited spectral bands and topographical organization. To find alternative or supplementary features to ERD/ERS, we explored the properties of the brain oscillations during motor imagery using a cross-frequency coupling (CFC) analysis. CFC reflects interactions between underlying neural circuits represented by different oscillations during cognitive tasks. We hypothesized that CFC might provide useful features about motor imagery and help improve the performance to discriminate the hand motor imagery. To verify the hypothesis, we applied the CFC analysis to the EEG data recorded during the bilateral hand motor imagery tasks. The EEG data were filtered through a bank of five bandpass filters to extract the rhythms with different frequency bands, including theta (θ: 4-8 Hz), alpha (α: 8-12 Hz), low-beta (β_L: 12-20 Hz), high-beta (β_H: 20-30 Hz), and gamma (γ: 30-40 Hz). Phase-amplitude coupling between slow and fast rhythms was evaluated by analyzing how the amplitude of fast rhythms was modulated with the phase of slow rhythms. We selected seven slow-fast rhythm pairs including θ-α, θ-β_L, θ-β_H, θ-γ, α-β_L, α-β_H, and α-γ. We observed that phase-amplitude coupling was

modulated at the contralateral sensorimotor area during the hand motor imagery. Cross-frequency phase-amplitude coupling was quantified using the modulation index, which measured dissimilarity between a phase-coupled amplitude distribution and the uniform distribution using the Kullback-Leibler divergence. Then, we used these modulation index values as features for classifying the left and right hand motor imagery. We found that using the CFC modulation index features increased classification accuracy by approximately 10% compared to the case of using ERD/ERS features. This suggests that the CFC index may provide novel features from the scalp EEG signals about the hand motor imagery and subserve the further improvement of EEG-based BCIs.

Disclosures: M. Kim: None. **J. Kim:** None. **S. Kim:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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Topic: D.18. Brain-Machine Interface

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Title: Brain-computer interface design and performance standards desired by potential users with spinal cord injury

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Abstract: Brain-computer interfaces (BCIs) allow people to operate technology without physical movement. Development of an effective and practical BCI requires understanding the end users' needs and constraints. Our previous study [1] included 61 people with amyotrophic lateral sclerosis (ALS). Current participants were drawn from the University of Michigan Spinal Cord Injury (SCI) Registry with a paralysis level C1 - C4 that can result in significantly impaired arm and leg function. A telephone survey addressed current use of assistive technologies, interest in BCI, and preferences for BCI performance and training.
Preliminary data are from 15 people with SCI (n=6 women) . Participants expressed a range of interest in differing options for BCI set-up and use. While 93% reported a willingness to use dry electrodes, only 53% were willing to use non-invasive EEG gel electrodes versus 67% willing to

have electrodes surgically implanted. All respondents would invest in 2-5 training sessions to get a useful BCI. Additionally, 69% would accept daily setup times of 10 to 20 minutes (though the minimum time of less than 10 minutes was preferred).
Respondents were also asked to define the minimum BCI performance they would consider acceptable. An accuracy of 90% would satisfy 92% of respondents. Accidental exits from standby mode only once every 3 hours would satisfy 75% of respondents. In addition, text generation of 20-24 letters per minute would satisfy 67% of respondents (though the maximum speed of 25+ letters per minute was preferred).
BCI design features rated most important were: “accuracy of BCI use” (50% of respondents), “functions the BCI would provide” (33%), and “speed with which the BCI works” (17%). Most important functions were the ability to: “communicate with someone in case of emergency” (36%), “control the room temperature” (23%), “operate a power wheelchair” (23%), and “operate a computer” (21%).
People with SCI showed interest in a BCI to help manage impairments. A majority were willing to have surgery to get a BCI and would participate in sufficient training sessions. Gel electrodes were less acceptable to people with SCI than to people with ALS (84%). People with SCI wanted faster BCI speeds. Current BCIs do not yet approach the desired levels of performance. Factors like daily set-up time, stand-by mode reliability, accuracy, and speed require future development.
[1] Huggins JE, Wren PA, Gruis KL, What would brain-computer interface users want? Opinions and priorities of potential users with amyotrophic lateral sclerosis. Amyotroph Lateral Scler. 12(5):318-24, 2011.

Disclosures: A.A. Moinuddin: None. **P.A. Wren:** None. **A.E. Chiodo:** None. **J.E. Huggins:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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Support: Deutscher Akademischer Austauschdienst (DAAD)

Werner Reichardt Centre for Neuroscience (CIN), German Excellence Initiatives

Deutsche Forschungsgemeinschaft (DFG)

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ERC

EU-FP7

Title: Movement related slow cortical potentials change after neurorehabilitation in severely affected chronic stroke patients

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Abstract: Introduction: Recent studies on stroke correlated the increased activation of contra-lesional, intact hemisphere with poor recovery. On the other hand, slow cortical potentials (SCPs) have been suggested as an important tool in brain plasticity research as an indicator of motor recovery after brain damage and as a positive source of information for movement decoding. In this study we investigated movement related slow cortical potentials before and after a month of neurorehabilitation in severely affected 5 chronic stroke patients presenting hand Fugl Meyer Assessment (FMA) scores of 3.7 ± 2.4 before the intervention. The neurorehabilitation was based on linking ipsilesional rolandic brain oscillations during the intention of hand opening and closing with the same grasping movements of a hand orthosis attached to the paralyzed hand and an immediate goal oriented physiotherapy session of the same movements. We expected to find a more balanced SCP activity between hemispheres after the rehabilitation in patients presenting increase in hand FMA scores.
Methods: Patients were randomly presented with visual and auditory cues corresponding to three different conditions indicating to either relax, actively open and close the healthy hand or try to open and close the paretic hand on their own pace for 4 seconds. SCPs were measured using a 16 channels EEG acticap system. Electromyographic (EMG) data was acquired using 8 bipolar Ag/AgCl electrodes and placed on antagonistic muscle pairs; one close to the forearm extensor, other on the forearm flexor, other on the external head of the biceps and the last one placed on the external head of the triceps. EEG data were filtered between 0.1 to 5 Hz. EMG onset were detected rectifying and computing the mean and standard deviation of EMG at rest. EEG data were segmented with respect to the EMG onset.
Results: SCPs produced pre- and post-movement of healthy hand did not show any difference between pre and post-treatment as expected. However, during paretic hand pre-movement and movement significant amplitude and interhemispheric changes were observed. After the treatment intact hemisphere showed increased activity at pre-movement stage, while this activity was shifting towards ipsilesional hemisphere after the movement onset. Furthermore, these increase in the SCPs during paretic hand premovement and movement correlated with increase in hand FMA. These findings indicate SCPs as a valuable functional and neurophysiological motor recovery measure.

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Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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Topic: D.18. Brain-Machine Interface

Support: NRF grant 2010-0006135

Title: Neuro-physiological difference of default mode network (DMN) between BCI able and illiteracy subject groups

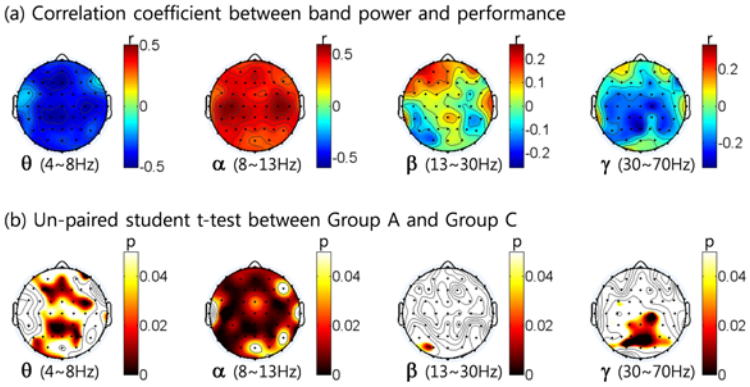
Authors: M. AHN, J. H. HONG, H. CHO, S. AHN, *S. C. JUN
GIST, Gwangju, Korea, Republic of

Abstract: * Introduction
In brain computer interface field (BCI), some subjects are not able to generate sufficiently large brain signal for controlling BCI system. The proportion of these subjects, named as BCI illiteracy [1, 2], is not negligibly small. In this study, we investigated the neuro-physiological difference in the default mode network (DMN) of resting state over subjects (classified by BCI performance).
* Methods & Materials
We analyzed EEG datasets from 52 subjects under left/right hand motor imagery (MI) experimental paradigm [3]. The classification accuracy was estimated using Common Spatial Pattern and Fishers Linear Discriminant Analysis and cross-validated. According to the BCI performance, subjects were categorized into three groups - 'A' (70% accuracy or above), 'B' (60% ~ 70%), and 'C' (lower than 60%). To investigate DMN, spontaneous EEG was acquired for one minute prior to MI experiment.
* Results & Discussion
In Figure (a), θ was correlated negatively with performance on overall region, while α was positively correlated [1]. β and γ seemed not to be highly correlated with the performance; however, γ showed weakly negative correlation on the centro-parietal area, which is relevant to the finding on the causality of γ on sensory motor rhythm [2].
Figure (b) describes the statistically significant areas between the poor and moderate performance groups. Except for β, these areas are quite distinct; the frontal and mid-parietal areas for θ, overall area for α, and the mid-parietal and near occipital for γ. Noticeably, θ behavior among our findings was never reported to our best knowledge. θ may play a critical role

in attention and memory processes [4]; therefore we expect that it may be deeply related to motor imagery since a person should recall body movement for sufficient kinesthetic imagination as close as real movement during MI experiment.

* References

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[3] H. Cho et al., TOBI 3rd workshop, 2012
[4] M. J Kahana et al., Curr. Opin. Neurobiol. 2001



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Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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Title: A multi-command spatial auditory BMI based on evoked EEG responses from real and virtual sound stimuli

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Abstract: One of the major topics in auditory neuroscience is spatial sound localization modeling, entailing spatial attention and “cocktail party effect.” While the movement imagery and visual paradigms are routinely used in the BMI (brain machine interface) paradigms, auditory modality is still under-explored, yet has a potential to give users a vastly enhanced interfacing experience. We propose a paradigm based on spatial sound oddball concept and introduce signal processing methodology optimizing the discriminability of the attended versus ignored directions evoked responses to real or virtual sound sources in the auditory space. We present a non-invasive multi-command auditory BMI paradigm which utilizes evoked brain responses to spatially distributed sound stimuli. The method optimizes full surround horizontal plane spatial sound sources, generated from real loudspeakers or virtual sound images created with an acoustic loudness panning approach proposed. EEG signals and especially ERP responses are non-stationary, yielding classical linear methods unsuitable. Our proposed EEG preprocessing and P300 related response identification method is data-driven and is capable of separating the information bearing sources. This is achieved by analyzing in the Hilbert-Huang domain the multichannel similarity of spectral domain ridges of the P300 evoked responses. This allows clustering of common oscillatory modes revealed in the amplitude and frequency ridges related to the propagation of evoked responses among the EEG electrodes. Next, correlations in amplitude and frequency of the spectral ridges, are used in the time-domain reconstruction. The identification of these components within each channel prevents information leakage. The spatial auditory stimuli are played directly from one of the octagonally placed loudspeakers or from locations generated virtually with the amplitude panning approach. For this purpose we developed an auditory loudness spatial panning algorithm allowing to maximize the EEG P300 responses in horizontal octagonal loudspeakers’ setting. Thanks to this, subjects are able to utilize a fully octagonal loudspeaker setup (including the front and back of the head sound locations) creating the eight-commands BMI. To prove the concept of usability of surround sound sources, we used the same pink and white noises for all directions in each trial. Further experiments with various sound timbres are planed to further extend the number of commands. The developed paradigm advances contemporary multi-command auditory BMI.

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Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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Program#/Poster#:891.17/NN5

Topic: D.18. Brain-Machine Interface

Title: Detection of motor intention by using single-trial readiness potentials.

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Abstract: The present study aimed at detecting motor intention of a human subject by using a single-trial readiness potential (RP). In the present experiment, two subjects carried out a simple self-paced button press task. The experiment consisted of 49 trials with inter-trial intervals of roughly 5 s. The electroencephalogram (EEG) was recorded from 5-channels (C_Z, C3, C4, F_Z, and P_Z). By averaging the data across trials, we confirmed that RP started at around -1.5 s prior to the movement onset. Then, movement (-2.0 to 0.5 s period; the time of the button press was set to 0 s) and non-movement data (-4.5 to -2.0 s) were extracted as positive and negative samples (49 samples each) of RPs. Forty training and 9 testing samples were chosen randomly for positive and negative data. This procedure was repeated 100 times, and a cross-validation method was used to estimate the rate of correct detection of RP in a single-trial basis. Feature parameters of a single-trial RP were calculated by using training data as follows. (1) The length of the time window for detecting a single-trial RP was determined as the duration from the time when the gradient of EEG waveforms falls steeper than the threshold that was determined by using the averaged-RP, to the time when the gradient becomes shallow. If there are several periods satisfying the above criteria, the one closer to the movement onset was chosen. (2) Upper and lower limits of the EEG amplitude during the time window were calculated. The upper limit was set to the value less than the average of the baseline (-2.0 to -1.8 s before the onset of the time window). The lower limit was the difference between the minimum value in the time window and the baseline. For testing, if the amplitudes are within limits and gradients are above the threshold throughout the time window, it is regarded as detection of a single-RP. The offset of the time window was defined as a “detection time” of a single-trial RP. If RP was not detected, the onset of the time window is shifted step by step. The results showed that the average of correct detection rate was 71.4±9.5% (Hit: 75.3%, Correct-Rejection: 67.6%) and 69.2±9.3% (Hit: 45.9%, Correct-Rejection: 92.4%) for Subject A and B, respectively. The average of the detection time was -740±180ms and -783±337ms. Considering the noise level in a single-trial EEG data, the correct detection rate of about 70% seems reasonable and adequately higher than the chance-level. It is interesting that a single trial RP was detected at about -750ms in our experiment, so that early detection of a single trial RP seems realizable.

Disclosures: S. Yamauchi: None. **S. Shimada:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

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Topic: D.18. Brain-Machine Interface

Support: KAKENHI 24700583

NEXT program

SRPBS

KAKENHI 24300051

Title: Simple method to remove skin blood flow artifact in functional near-infrared spectroscopy by multidistance probe measurements

Authors: T. SATO[1], K. TAKEDA[2], R. OSU[3], I. NAMBU[1], *Y. WADA[1]
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Abstract: Functional near-infrared spectroscopy (fNIRS) is a noninvasive functional neuroimaging technique used to obtain cerebral activations by monitoring the concentration changes in oxygenated and deoxygenated hemoglobin (oxy- and deoxy-Hb). It has some advantages over other techniques: portability, fewer physical restrictions, freeness from electrical-noise and simplicity of use. However, undesirable artifacts such as physical movements, head posture changes, and skin blood flow (SBF) are often observed in fNIRS measurements. Particularly, SBF is caused by systemic physiological changes from superficial tissues such as the scalp and muscles that cannot be avoided experimentally and cannot be removed with conventional filtering methods.

A recent study has shown that a major part of the task-related changes in the oxy-Hb in the forehead may be due to task-related changes in the SBF during a verbal fluency task (Takahashi et al., 2011). A study using a multidistance probe arrangement (source-detector distance of 30 mm and 20 mm) has shown that SBF can be eliminated by subtracting the signal of the short channel from the signal of the long channel (Yamada et al., 2009). However, this method can only measure a narrow area since it requires many measurement probes.

We propose a new method that only uses a few short channels (source-detector distance of 15 mm) to measure a wide area. In the proposed method, we assume that temporal pattern of SBF in fNIRS signals is common in all channels and a major part of the short channel signals is SBF from past studies. The common temporal pattern of the SBF is estimated from short channels using principal component analysis (PCA) and general linear model (GLM) is applied to estimate amplitudes of SBF and cerebral activation in each channel. The design matrix contains five components: hemodynamic response function (HRF), temporal and dispersion derivatives, SBF, and constant. Proposed method can estimate cerebral activations are not affected by SBF

from oxy-Hb.
We ran simulations to assess the performance of the proposed method on simple fNIRS signals. We simulated 43 long channels that contained cerebral blood flow, SBF, and white noise, and 4 short channels that contained only the SBF and white noise, and compared the proposed method with the standard GLM that doesn't contain SBF component. The proposed method estimated exactly the amplitudes of cerebral blood flow while the standard GLM method yielded estimates larger than the theoretical values. These results suggest that the proposed method is an effective technique for the removal of SBF artifacts.

Disclosures: T. Sato: None. **K. Takeda:** None. **R. Osu:** None. **I. Nambu:** None. **Y. Wada:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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Program#/Poster#:891.19/NN7

Topic: D.18. Brain-Machine Interface

Title: Naturalistic stimuli enhance detection time for steady-state visual evoked potential-based brain computer interface

Authors: *K. NG[1], A. P. BRADLEY[2], R. CUNNINGTON[1]
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Abstract: Neural activity in the visual cortex, recorded from EEG, can be decoded in real time to determine which stimulus on a computer display a user selects by attending. Steady-state visual-evoked potential brain-computer interface systems (SSVEP-BCI) use flickering visual stimuli, with each item on the screen tagged at a different frequency, and classification methods to determine which item on the screen is attended or selected by the user. Most SSVEP BCI applications use simple stimuli such as checkerboard reversal patterns or single spots of light (LEDs). These stimuli are effective to evoke visual responses but provide no contextual meaning that allows a user to relate what they intend to represent, such as the choice between an apple or water. Hence, we developed visual stimuli that are more intuitive and naturalistic, by performing band pass filtering on natural photographs (e.g. grey-scale pictures of an apple or glass of water) such that the images retain the structure and characteristics of the original image but also have high luminance contrasts along the contours of the image which should still elicit strong neural activity from the visual cortex (SSVEPs). We compared the classification speed; that is, how

quickly our support vector machine (SVM) classification could identify the choice made by participants from their EEG visual-evoked potentials when selecting items on the screen represented by classical checkerboard patterns compared with the naturalistic stimuli. Results indicate that the detection speed is significantly faster when using naturalistic stimuli compared to using plain simple checkerboard reversal patterns. We suggest that naturalistic stimuli are able to better draw and maintain the user's visual attention than the plain checkerboard stimuli. This provides evidence towards using naturalistic stimuli for SSVEP-BCI applications.

Disclosures: K. Ng: None. **A.P. Bradley:** None. **R. Cunnington:** None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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Program#/Poster#:891.20/NN8

Topic: D.18. Brain-Machine Interface

Title: Detection of directional movement control using fNIRS optical imaging of motor cortex

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Abstract: The present study uses functional near-infrared spectroscopy (fNIRS) optical imaging technique to decode directional arm movements based on the activation pattern of the motor cortex, as detected by the oxy-hemoglobin and deoxy-hemoglobin levels from the near-infrared signatures. Multi-channel fNIRS is used to localize the specific motor cortical activations that correspond to the motor command signals. The motor cortical activities are correlated with the direction of volitional motor execution in healthy human subjects to deduce the movement directions so that brain-derived motor command signals from optical imaging can be potentially used to control a wheelchair for quadriplegics.
The results showed that movement directions can be decoded by changes in all fNIRS signals (oxy-hemoglobin (oxyHb) signal, deoxy-hemoglobin (deoxyHb) signal, and sum of oxy- & deoxy-hemoglobin (oxyHb+deoxyHb) signals). The temporal changes in the oxyHb+deoxyHb signals from different optical sensors can be used to deduce the movement direction based on the differential changes to the near-infrared signals. This differential change in oxygen consumption at different locations within the motor cortex represents differential neural activation with respect to the movement direction. This provides a demonstration of detection of volitional motor activities using fNIRS optical imaging as a potential means to obtain noninvasive brain-derived optical signals to deduce intentional movement directions.

Disclosures: N.D. Tam: None.

Poster

891. Brain Machine Interfaces: Non-Invasive Methods

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Program#/Poster#:891.21/NN9

Topic: D.18. Brain-Machine Interface

Title: Time-course analysis of single-trial mu-suppression during finger opposition task

Authors: *K. KANEKASU[1], S. SHIMADA[1], Y. ONO[1], M. KAWASAKI[2]
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Abstract: The present study examined the time-course of mu-suppression in a single-trial basis in order to see in what time interval after the movement onset the mu-suppression is detected most sensitively. Electroencephalogram (EEG) signals were recorded continuously by 11 pin-type active electrodes (C3, FC3, C1, C5, C4, FC4, C2, C6, Cz, Fz and Pz). Bipolar electrooculogram (EOG) were also measured. The data were filtered (0.5-30Hz) and digitized at 512 Hz. Two subjects participated in the experiment (Subject A: left-handed, and B: right-handed). Each subject carried out the finger opposition task while a cue stimulus was presented for 2 s on the left or right side of the screen (20 times each) in a random order. EOG artifacts were removed from the recorded data by using the independent component analysis (ICA). The resulting data were transformed by a continuous Morlet wavelet transform. We confirmed that there was a significant decrease of the power of 10 to 14 Hz band when the subject performed the task with their left hand compared to the baseline (-3 to -2 s before the movement onset). Similar results were observed in relatively higher frequencies (14 to 20 Hz) for the right hand condition. Next, we examined in what time interval the mu-suppression can be detected most prominently in a single-trial basis. The data for each trial (from -1 to 2s; the movement onset was set to 0 s) were divided into 24 time intervals (every 125ms). For each interval, the decay rate compared to the baseline was calculated. We regarded the detection of mu-suppression if the decay rates of both N and N+1 intervals were above the predetermined threshold (20% and 30% for subject A and B, respectively). For subject A, the detection rate of mu-suppression was the highest at 0 to 250 ms interval in the left hand condition (75%) and at -500 to -250 ms in the right hand condition (70%), while those for the intervals before the movement onset (-1000 to -500 ms) was relatively low (22.5 and 27.5 % for the left and right hand, respectively). Subject B showed similar results with the highest detection rate at 250 to 500 ms interval in both hands (70 and 80 % for the left and right hand, respectively) while the detection rates in before-onset intervals were lower (38.8 and 35 %). Although there was variability among subjects in the most

sensitive time interval to detect mu-suppression, the present result suggests the possibility that the single-trial mu-suppression can be detected early after or before the movement onset.

Disclosures: K. Kanekasu: None. **S. Shimada:** None. **Y. Ono:** None. **M. Kawasaki:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

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Topic: D.18. Brain-Machine Interface

Support: NIH/NIDCD (4R33 DC010470-03)

NIH/NIDCD (1R15DC011002-01)

Title: Optimizing P300-based brain-Computer interface communication speed via error potentials

Authors: D. R. BERRY[1], K. A. COLWELL[2], *E. SELLERS[1]
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Abstract: To date, P300 BCI research has aimed to increase communication speed and accuracy. One possibility for providing faster information transfer rates is by decreasing the amount of selections required to produce a meaningful message. Previous work shows that single-trial classification of error potentials (ErrP), a unique event-related potential (ERP), can improve online performance by detecting and deleting erroneous item selections. However, nearly all of this research constructs ErrP classifiers via manual button presses on a keyboard; this method may not be translatable to clinical settings, particularly for people with severely limited motor control. Moreover, false alarms (erroneously deleting a correct selection) can be detrimental to performance, especially for users with high accuracy. Here, we propose a clinically translatable ErrP classification option by using sham feedback during a P300 BCI task. In addition, we develop an algorithm that optimizes ErrP classification, such that false alarms and the probability of correctly deleting an error are balanced to bolster information transfer rate.
Nineteen able-bodied subjects completed a P300 BCI spelling task. Stimuli were arranged in an 8x9 matrix of alphanumeric items on a computer monitor; subjects were instructed to attend to a predefined target item and mentally record each time the item ‘flashed’. Each character flashed within the matrix 2 times (125 ms SOA). Following each flash sequence sham feedback (20% error rate) was provided on a total of 300 item selections. Thirty-two EEG channels were recorded (right mastoid reference), and all of the electrode locations were used to develop a

SWLDA classifier.
Our manipulation provided distinct ERPs for correct and incorrect feedback, consistent with feedback error-related negativity. Five-fold cross-validation of the single-trial ErrPs were detected at a mean accuracy of 85.3% (AUC 0.85). After applying the optimized classification threshold, offline analyses revealed that subjects saved an average of 79 item selections (16.5%). These data suggest that ErrP classification can afford time savings (7.9 minutes out of 43.8 minutes) in producing an accurate and meaningful message.

Disclosures: D.R. Berry: None. **K.A. Colwell:** None. **E. Sellers:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

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Topic: D.18. Brain-Machine Interface

Support: NIH Bioengineering Research Partnership Grant EB00856 (NIBIB)

Title: BCI-360: Full-service support for independent home-based BCI use and for translational studies

Authors: S. WINDEN[1], C. S. CARMACK[2], D. E. CORDA[2], D. J. MCFARLAND[2], D. ZEITLIN[3], L. TENTERAMANO[3], *T. M. VAUGHAN[2], J. R. WOLPAW[2]
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Abstract: A major goal of brain-computer interface (BCI) technology is to restore communication and control capacities to people with severe motor disabilities. The BCI Project of the Program for Translational Neurological Research (Wadsworth Center/Helen Hayes Hospital) provides BCI-24/7, a portable 8-channel EEG-based BCI system, for independent home use by people with ALS and other disorders. This usage is enabled by Wadsworth BCI-360, a BCI2000-based software package that ensures reliable operation of the BCI device and provides a range of applications. Present applications use the P300-based visual speller format and include e-mail, limited Internet access, word-processing, games, and audio books.
BCI360: tracks system readiness over each 24-hour period (*Uptime*); controls real-time focus and window placement in the Windows XP environment to ensure correct sequencing of events (*WinMover* & *BCI Keyboard*); collects data to assess system operator (SO) training requirements and electrode cap maintenance requirements; provides system setup oversight for the SO (e.g.,

electrode impedances and artifacts) (*Diagnostics*); gives access to applications through a general-purpose configurable menu (*Main Menu*); transfers all data daily to the Wadsworth Center over the Internet and stores it redundantly to ensure fidelity (*RSync*); and compiles and displays daily use for each subject (including data on calibration exercises, usage time, applications used, and single selections) (*Report*). These data are monitored by Wadsworth personnel in order to ensure timely technical support as needed (Vaughan et al., adjacent poster). For example, over a total of 130 user-days, each of 5 BCI home users used *Diagnostics* an average of 1.24 times/day. The data showed that the average electrode impedance value exceeded 40 KOhm on only 4.5% of 465 calibration runs, and that there was no correlation between impedance and system performance (measured as selection accuracy), which may reflect the overall low impedance values.

We are now following 20 people who are using the BCI-24/7 system with BCI-360 in their homes. The results to date indicate that this technology enables independent BCI home use by people with severe disabilities who are supported by SOs with only modest training. We are modifying BCI-24/7 and BCI-360 to improve performance consistency, to accommodate other EEG features (e.g., sensorimotor rhythms), and to add new applications.

Disclosures: S. Winden: None. **C.S. Carmack:** None. **D.E. Corda:** None. **D.J. McFarland:** None. **D. Zeitlin:** None. **L. Tenteramano:** None. **T.M. Vaughan:** None. **J.R. Wolpaw:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

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Program#/Poster#:892.03/NN12

Topic: D.18. Brain-Machine Interface

Support: NIH Bioengineering Research Partnership Grant EB00856 (NIBIB)

Title: Providing reliable independent BCI home use for individuals with ALS

Authors: T. M. VAUGHAN[1], *L. MCCANE[1], S. M. HECKMAN[1], D. J. MCFARLAND[1], L. TENTERAMANO[2], D. ZEITLIN[2], B. ZOLTAN[2], C. S. CARMACK[1], S. WINDEN[1], D. E. CORDA[1], J. R. WOLPAW[1]
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Abstract: Brain-computer interfaces (BCIs) can restore communication and control capacities to people with severe motor disabilities. The BCI Project of the Wadsworth Center/Helen Hayes Program for Translational Neurological Research has translated laboratory-proven EEG-based

BCI technology into a system (BCI-24/7) that can be used by severely disabled people independently in their homes with minimal ongoing technical oversight (Sellers et al. ALS 11:449-455, 2010). This system is coupled with BCI-360, a BCI2000-based software package that at present provides a variety of applications in a P300 visual speller format.
Reliability (i.e., dependable performance) is the key to useful sustained independent BCI use. We are currently developing procedures for evaluating prospective users and then optimizing their daily use of the BCI system. After an initial confirmation that the person does not have a severe visual impairment, we conduct two one-hour evaluations to determine whether the system is effective for the person and whether the home environment and the caregiver(s) can support system use. If the results are positive, we install the BCI system in the home and over a series of 3-6 visits train the caregiver(s) as system operator(s) (SOs). This training includes placing the electrode cap, insuring satisfactory EEG signals, and initiating and overseeing device operation. At the same time, we introduce the user to the applications available through BCI-360 (Winden et al., adjacent poster). Currently these applications include: simple word processing using word prediction with both print and speech output; e-mail; limited Internet access; several games; and audio and video content (e.g., audio books, picture albums). We base initial system parameters (e.g., stimulus rate and number) for each BCI user on the EEG data recorded during the evaluations. Using the remote oversight capabilities of BCI360 (including data transfer via the Rsync transfer protocol), we monitor daily use in order to detect and address problems that may arise concerning hardware, software, SO training, and other issues. We can also interact remotely with the user and SO via *GoToMyPC* (Citrix). In addition, we conduct offline data analyses to optimize system operation. For example, recent analysis of chronic data from home users indicates that system accuracy could be improved by increasing the EEG decimation factor from its current value of 20 to 25 or higher. This change is now being implemented for an initial set of home users. If it proves effective online, we will incorporate it for our entire current home user population of 20 people and monitor its impact on each user.

Disclosures: T.M. Vaughan: None. **L. McCane:** None. **S.M. Heckman:** None. **D.J. McFarland:** None. **L. Tenteramano:** None. **D. Zeitlin:** None. **B. Zoltan:** None. **C.S. Carmack:** None. **S. Winden:** None. **D.E. Corda:** None. **J.R. Wolpaw:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

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Topic: D.18. Brain-Machine Interface

Support: NIH Bioengineering Research Partnership EB00856 (NIBIB)

Title: Spectral bandwidth of movement-related oscillations in scalp-recorded EEG activity

Authors: *D. J. MCFARLAND, W. A. SARNACKI, J. R. WOLPAW
Lab. Neural Injury and Repair, Wadsworth Ctr., ALBANY, NY

Abstract: Brain-computer interface (BCI) technology can restore communication and control to people who are severely paralyzed. BCIs use brain signals recorded from the scalp (EEG), the cortical surface (ECoG), or within the cortex. Activity in the gamma band (i.e., ≥30 Hz) has proven to be a very useful feature for ECoG-based BCI studies and is being considered for use with scalp EEG-based BCIs. The present study examined the utility of low frequency (i.e., 9-28 Hz) mu and beta activity and high frequency (i.e., 29-130 Hz) gamma activity recorded over sensorimotor cortices for classifying EEG associated with movement and movement imagery. Six subjects performed interleaved 3-min sessions of either movement or movement imagery. For each 4-sec trial, a target at either the left edge or right edge of a video screen cued left- or right-hand movement or movement imagery. The EEG was recorded from 64 electrodes referenced to the right mastoid and digitized at 1200 Hz. The amplitudes in 5 low frequency bands (i.e., 9-12, 13-16, 17-20, 21-24, and 25-28 Hz) and 5 high frequency bands (i.e., 29-36, 37-48, 69-84, 85-105, and 106-130 Hz) at C3, C4, and Cz were used as features in linear discriminant analyses.
Visual inspection of frequency-specific topographies of r^2 for movement vs. rest, imagery vs. rest, left vs. right hand movement, and left vs. right hand movement imagery showed that the differences were restricted mainly to activity below 30 Hz. The discriminant analyses confirmed these results. Overall, the data indicated that inclusion of activity above 30 Hz contributed little to the classification of the EEG associated with movement or movement imagery. This remained true even when the data were re-referenced to emphasize features with high-spatial frequencies, and even when analysis focused on the first or second 500 msec of the 4-sec trial.
It is possible that more complex movement or imagery tasks would have produced more prominent changes in high frequency activity. At the same time, these largely negative results regarding the discriminative value of EEG gamma activity indicate the need for careful evaluation of possible contamination by EMG or other artifacts in BCI studies involving high-frequency EEG activity.

Disclosures: D.J. McFarland: None. **W.A. Sarnacki:** None. **J.R. Wolpaw:** None.

Poster

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NIH/NIDCD 4R33 DC010470-03

NIH/NIDCD 1R15DC011002-01

Title: Enhancing brain-computer interface Performance in an ALS population: Checkerboard and color paradigms

Authors: *D. RYAN[1], S. THROCKMORTON[2], L. M. COLLINS[2], K. M. CAVES[2], E. W. SELLERS[1]
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Abstract: Noninvasive brain-computer interface (BCI) provides non-muscular communication via detection of EEG features. Compared to traditional means of communication, BCI communication is slow; however, improved techniques (e.g., paradigm manipulation and/or target classification algorithms) have been used to optimize speed and accuracy. In a non-disabled population, and in pilot data from three subjects with amyotrophic lateral sclerosis (ALS), the “checkerboard” presentation paradigm provided better performance than the standard “row/column” presentation paradigm. The checkerboard controls for adjacent flashes of items and ensures that once an item has been presented it will be excluded from at least six subsequent flash groups, which improves identification of target stimuli. In non disabled subjects, a condition that changes groups of gray matrix items to one of nine unique colors improved performance over that observed when groups of gray matrix items change to white. The color stimuli decrease non-target distraction and improve target classification as compared to the standard white stimuli. To determine if these improvements generalize to an ALS population, a within participant design tests all three paradigms. Current data suggest that the checkerboard improves performance as compared to the row/column. In addition, color stimuli enhanced performance and participants prefer the color stimuli over the white stimuli. The results suggest matrices including checkerboard and color stimuli provide more efficient communication and higher user satisfaction in an ALS population.

Disclosures: D. Ryan: None. **S. Throckmorton:** None. **L.M. Collins:** None. **K.M. Caves:** None. **E.W. Sellers:** None.

Poster

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Support: NIH/NIBIB & NINDS (EB00856)

NIH/NIDCD (4R33DC010470-03)

Title: A longitudinal investigation of non-invasive P300-based brain-computer interface

Authors: *K. E. BROWN[1], D. B. RYAN[2], J. A. ARMSTRONG[2], E. W. SELLERS[2]
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Abstract: Brain-Computer Interface (BCI) provides a method of communication for severely disabled individuals who are unable to communicate due to significant loss of muscle control. People diagnosed with amyotrophic lateral sclerosis (ALS) can typically derive the most benefit from BCI use, as ALS causes inexorable loss of muscle function that will eventually result in locked-in syndrome. The BCI presented here operates independently of muscle control. Event-related potentials (ERPs) are extracted from the EEG and analyzed. Items (i.e., alphanumeric characters and keyboard commands) attended to by the subject should produce a P300 ERP, and the unattended items should not. The computer uses a stepwise linear discriminant analysis to classify the items as attended or unattended. Many studies have shown that people disabled by ALS can communicate using the P300 BCI; however, no study has systematically examined BCI performance longitudinally. The current study examines the relationship between disease progression, BCI performance, and several other factors that may contribute to BCI performance. Presently, five patients are enrolled in the study and data are collected in intervals of approximately eight weeks. Each session involves administering the ALSFRS-r as a means of tracking disease progression followed by four EEG tasks including: resting EEG, cognitive task (counting backward by 7), an oddball task, and copy spelling task. Preliminary data suggest that BCI performance remains stable despite progressive loss of movement. Alpha power spectra is greater at rest than during the cognitive task, suggesting an appropriate response to cognitive load. The oddball task generated a P300 for attended stimuli, and no P300 for unattended stimuli. ALSFRS-r scores decreased by 1.8 points on average, measured over a 10.2 week period. Across the five subjects, mean BCI copy spelling accuracy was 88% with an average subject variability of 12%. Overall, the results suggest that BCI performance remains stable as disease progression continues. We will continue to monitor this relationship over time and compare it to data collected from healthy individuals.

Disclosures: K.E. Brown: None. **D.B. Ryan:** None. **J.A. Armstrong:** None. **E.W. Sellers:** None.

Poster

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Topic: D.18. Brain-Machine Interface

Support: NIH/NIDCD (R33 DC010470-03)

Title: Utilizing a language model to improve dynamic data collection in P300 spellers

Authors: B. MAINSAH, L. M. COLLINS, K. COLWELL, *S. THROCKMORTON
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Abstract: P300 spellers rely on event-related potentials (ERPs) that occur in scalp-measured electroencephalography (EEG) to determine the character that the BCI user intends to spell. The P300 speller provides a noninvasive method of communication that is not reliant on physical movement thus providing a method of communication for those suffering from severe neuromuscular disabilities that might preclude the use of other assistive devices. The speller operates by monitoring the EEG response of the user as subsets of characters are flashed on a monitor; when the flashed subset contains the character the user desires to spell, an ERP occurs. However, single ERPs tend to have low signal-to-noise ratios (SNR). In order to increase SNR and thereby increase classification performance, each character is flashed multiple times, and the responses are averaged. Several studies have demonstrated that spelling speed and accuracy is improved by adaptively selecting the number of flashes per character. However, these methods tend to rely in some form on past performance of participants to control the data collection rather than on a subject-independent, probabilistic method. A probabilistic method has the advantage of both assessing the quality of the data currently under consideration as well as allowing for the incorporation of *a priori* knowledge of the language of the user to increase spelling speed. The Dynamic Stopping algorithm used in this study maintains a probability distribution over characters; each classified flash response is integrated into the model via a Bayesian update. This algorithm has been demonstrated to significantly increase spelling speed and accuracy for subjects with a wide range of abilities to use the system [Colwell et al., 2011]. The Dynamic Stopping algorithm is expanded here to include a language model which is implemented by initializing each character prior to data collection based on the previously spelled character. This potentially reduces the time required for the probability of the intended character to reach the threshold used to stop data collection. We present the results of an experiment testing subjects' spelling accuracy and speed under the Dynamic Stopping and Dynamic Stopping plus Language Model algorithms.

Disclosures: B. Mainsah: None. **L.M. Collins:** None. **K. Colwell:** None. **S. Throckmorton:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:892.08/NN17

Topic: D.18. Brain-Machine Interface

Support: NIH/NIDCD Grant R33 DC010470-03)

Title: Sparse structured probit regression for online channel selection and classification for the P300 speller

Authors: *K. A. COLWELL, L. M. COLLINS, K. D. MORTON
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Abstract: The P300 Speller is a device that allows users to control a computer via measured multi-channel electroencephalogram (EEG) data without requiring neuromuscular control. As such, it provides an option for communication to populations with severe physical limitations. Home and clinical use of the P300 Speller may be better facilitated by improving the ease-of-use of the physical interface, increasing the device's accuracy, and improving system robustness to installation. We address each of these aims by introducing Sparse Structured Probit Regression (SSPR), a method for training a linear classifier that generalizes the Relevance Vector Machine classifier [Bishop 2000] to perform channel selection and classification concurrently. The generalization allows the model to search for solutions that are not only sparse in features, but sparse in channels as well; this equates to a de facto channel selection whose number of informative channels is determined from the data, rather than a priori. The resulting sparsity among channels may demonstrate that certain channels of the electrode cap are not informative and thus may be removed, easing installation, while the classifier determined by SSPR also retains or increases the accuracy obtained using a standard set of 8 channels. The ability to select channels also provides protection against installation errors and longitudinal spatial changes in brain response. We present accuracy and channel selection results obtained by simulating the application of SSPR to real BCI data. The model is then modified further to perform online training, following the methodology of [Sato 2001], such that the training process of the classifier is performed as training data is collected. Using Online SSPR, users who would benefit from collecting additional training data may do so, increasing system accuracy, and users who do not need to collect the standard amount of training data can stop training early, saving training

time. We present accuracy and channel selection results obtained by applying Online SSPR to real data, and compare these results to both results obtained with standard methods, and Batch (standard) SSPR.

Disclosures: K.A. Colwell: None. **L.M. Collins:** None. **K.D. Morton:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

Location: Hall F-J

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Program#/Poster#:892.09/NN18

Topic: D.18. Brain-Machine Interface

Support: The Strategic Research Program for Brain Sciences (SRPBS) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan.

Title: Functional recovery and cortical reorganization after Brain-Computer Interface-based neuromuscular electrical stimulation training in chronic stroke: A case study

Authors: *T. ONO[1], M. MUKAINO[2], J. USHIBA[1]
[1]Keio Univ., Kanagawa, Japan; [2]Asahikawa Med. Univ., Asahikawa, Japan

Abstract: * Ono T. and Mukaino M. contributed equally to this work.
Previous studies suggest that brain-computer interface (BCI)-based rehabilitation is useful for promoting functional recovery of paretic hand movement in chronic stroke patients. Although these studies show the effectiveness of training with BCI-driven devices, e.g. orthosis, robot, or electrical stimulation, the extent of the contribution of the BCI system per se is not clear. In this study, we compared neuromuscular electrical stimulation (NMES) with BCI-driven NMES, employing an ABAB design.
One subject with stroke participated in this study. In epoch A, the subject received NMES on the paretic extensor digitorum communis (EDC) for 60 min per day, 5 days per week for two weeks. The subject was instructed to imagine finger extension during the stimulation. In epoch B, BCI-driven NMES training was performed. During each trial, the subject imagined finger extension for 4 s following an imperative cue. The EDC was stimulated when sustained (>1 s) motor imagery was detected in the task period as event-related desynchronization of the electroencephalogram (EEG). Epoch B was carried out for 60 min per day, 5 days per week for two weeks except the last epoch B that was performed for three weeks. Surface electromyogram (EMG) of the paretic EDC, EEG and functional magnetic resonance imaging (fMRI) were monitored at the end of each epoch as evaluations of motor functions and cortical activities. In addition, Fugl-Meyer Assessment (FMA) and modified Ashworth scale were measured as

evaluations of clinical scores.
Before starting the training, attempted finger extension was associated with weak EMG activity and bilateral cortical activation. Although several previous studies have shown that NMES could be effective for rehabilitation of chronic motor impairments after stroke, epoch A was associated with no improvement except for a slight increase in FMA score. In contrast, epoch B, the BCI-training epoch, was associated with marked augmentation in EMG activity, localization of cortical activity and improvement in clinical scores. Improvement in the clinical scores continued beyond the first epoch B, however, the localization of cortical activity on fMRI and the increase in EMG activity were negated during the second epoch A, indicating the significant contribution of the BCI system to these positive results. These results suggest the superiority of closed-loop training with BCI-driven NMES to open-loop NMES for chronic stroke patients.

Disclosures: T. Ono: None. **M. Mukaino:** None. **J. Ushiba:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:892.10/NN19

Topic: D.18. Brain-Machine Interface

Support: MEXT (Japan Funding Agency) Grant : SRPBS Type A

Title: Development of brain-computer interface system for upper extremity-rehabilitation with task-oriented design

Authors: *J. USHIBA[1], A. MORISHITA[2], T. FUJIWARA[3], K. ABE[4], A. NISHIMOTO[3], M. KAWAKAMI[3], D. KAMATANI[3], M. LIU[3]
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Abstract: Motor imagery based brain-computer interface to drive motor-driven orthosis or neuromuscular electrical stimulation has been expected as one of novel rehabilitative measures for severe motor disorder due to stroke. Previous pilot studies with single-case or case-series clinical experiments showed the increase of motor-related desycnrhonization of the sensorimotor rhythm in EEG, improvement of voluntary EMG in the paretic hand, and increase of actual clinical score assessing upper extremity motor function. Though these results have generated a great deal of interest in clinical application, a system development for further easy-to-use must be needed. Time spent for both placement of EEG electrodes and calibration of parameters in an EEG feature classifier are not negligible when the system is introduced to clinical scene so that

simplification of BCI setting must be needed. Moreover, arrangement of BCI paradigm with task-oriented design is needed to stimulate patients' motivation in daily training. Therefore in this study, we developed a prototype of brain-computer interface system for upper extremity-rehabilitation with task-oriented design. The system is consisted of a headphone-style EEG holder with a pair of dry electrode, a touch panel laptop, and a motor-driven prehension orthosis combined with neuromuscular electrical stimulator, formed based on reflex inhibitory splints. A LED was on the distal end of the orthosis to display the cue. We first tested the feasibility of this developed device in views of engineering and human ergonomics. Skin-electrode impedance assessment evidenced that the headset sufficiently held the EEG electrode on the scalp, and a questionnaire with Quebec User Evaluation of Satisfaction with Assistive Technology revealed that the device is ready for clinical use. The orthosis was certificated by both occupational therapists and orthotists to prevent increase of spasticity. We then tested the developed device in repetitive reach-to-grasp training in stroke participants. Participants were asked to reach their hand to a target peg using balanced forearm orthosis, and grasp it by motor driven action. Participants then moved their hand outside the pegboard, and BCI sequence was started. After a 5 s rest, a visual cue to attempt finger-opening movement was given. The motor-driven orhosis opened their finger if more than 5% of ERD by attempting finger movement was observed over the contralateral parieto-temporal region. All the participants successfully completed 10 days of 40-min training without any adverse effect. From these results, we concluded that the developed BCI system is practically useful for clinical scene.

Disclosures: J. Ushiba: None. **A. Morishita:** None. **T. Fujiwara:** None. **K. Abe:** None. **A. Nishimoto:** None. **M. Kawakami:** None. **D. Kamatani:** None. **M. Liu:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

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Program#/Poster#:892.11/NN20

Topic: D.18. Brain-Machine Interface

Support: The Strategic Research Program for Brain Sciences (SRPBS) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan.

Title: Modulation of event-related desynchronization during hand motor imagery with transcraial direct current stimulation

Authors: *M. OTO[1], Y. SHINDO[2], J. USHIYAMA[3], A. KIMURA[2], M. LIU[2], T. FUJIWARA[2], J. USHIBA[3]

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Abstract: The event-related desynchronization (ERD) of Electroencephalogram (EEG) is supposed to reflect motor preparation and appear during motor execution and motor imagery. Since ERD is a considerable marker representing temporal excitability of the sensorimotor neural circuit, extrinsic feedback of ERD caused by attempting paretic hand movement is expected to promote motor learning and to improve motor function. Stroke patients, however, show little ERD resulting in a limited usage of BMI intervention. Possible combined technique to enhance ERD is therefore awaited.

Transcranial direct current stimulation (tDCS) is one candidate to modulate ERD. Anodal tDCS can increase excitability of the motor cortex so that ERD is expected to be increased during and after stimulation. This study tested its hypothesis by comparing the amplitude of ERD during hand motor imagery before and after applying tDCS over primary motor cortex. ERD during stimulation was also measured to assess the extent of the tDCS effect.

Six healthy subjects were asked to imagine their right fingers extending. They started right hand motor imagery after receiving a visual cue. EEG was recorded over the left motor cortex, and was used to calculate ERD. Then anodal tDCS (10 min, 1 mA) was used to increase the cortical excitability of left motor cortex. Two different types of conditions were tested in this study. The one condition applied tDCS prior to 20 times of 5-s motor imagery task ("Preceding" condition). Another condition applied tDCS during the same motor task ("Simultaneous" condition). A control condition with sham stimulus during the motor task was also tested. All the condition was conducted with a randomized sequence on different days in each subject. ERD was calculated at the periods before tDCS, during tDCS, and after tDCS (three times), and their amplitudes were compared.

The ERD significantly increased in both "Preceding" and "Simultaneous" tDCS conditions, and larger ERD was seen in the latter condition (increased 13.7 ± 21.1 %). Enhancement of ERD lasted by 30 minutes in "Simultaneous" condition, which was 15 minutes longer than "Preceding" condition. ERD value did not increase in sham condition. These findings suggest that ERD is increased by anodal tDCS, and the enhancement of ERD and its carry-over effect are larger when stimulation was applied during the motor task, rather than after motor task. Though this preliminary study was achieved in healthy individuals, the result gives us a possibility to use tDCS during ERD-based BMI training for further efficacy in rehabilitation.

Disclosures: M. Oto: None. **Y. Shindo:** None. **J. Ushiyama:** None. **A. Kimura:** None. **M. Liu:** None. **T. Fujiwara:** None. **J. Ushiba:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

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Topic: D.18. Brain-Machine Interface

Support: Strategic Research Program for Brain Sciences (SRPBS) by Ministry of Education, Culture, Sports, Science and Technology, Japan.

Title: Event-related desynchronization during hand motor imagery is correlated with excitability of both primary motor cortex and spinal motoneuron

Authors: *M. TAKEMI[1], A. KIMURA[2], M. LIU[2], Y. MASAKADO[3], J. USHIBA[4]
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Abstract: There is increasing interest in electroencephalogram (EEG)-based brain-computer interface (BCI) as a tool for rehabilitation of upper limb motor functions in hemiplegic stroke patients. This type of BCI often exploits the oscillations in the EEG occurring in the mu and beta bands recorded over the sensorimotor areas, and their event-related desynchronization (ERD) following motor imagery (MI) is believed to represent increased excitability of the sensorimotor cortex. However, it is not well understood if the temporal excitabilities at the cortical and spinal level in the motor system are correlated with ERD. The purpose of this study was to assess the association of ERD with primary motor cortex (M1) and spinal motoneuron excitability during hand MI using electrophysiological techniques.
This study comprises two experiments. Ten healthy subjects were recruited for each experiment. The subjects received real-time visual feedback of the ERD magnitude of EEG signals recorded over the contralateral hand sensorimotor area while they performed a MI task.
In Experiment 1, each subject performed 30 trials comprising 7 s of rest followed by 5 s of MI of right wrist extension. M1 excitability was tested by motor evoked potentials (MEPs), intracortical inhibition (ICI) and intracortical facilitation (ICF), using transcranial magmetic stimulation (TMS) of the contralateral hand area of M1. TMS was applied when ERD exceeded 5% or 15% thresholds during MI. EMG was recorded from the extensor carpi radialis.

In Experiment 2, each subject performed 50 trials comprising 7 s of rest followed by 5 s of MI of right thumb abduction. Spinal motoneuron excitability was assessed by F wave persistence recorded from the abductor pollicis brevis following right median nerve stimulation at the wrist level. Electrical stimulation was applied when ERD exceeded 5% or 15% thresholds during MI. One-factor repeated measures ANOVA showed there was a main effect of ERD for MEP amplitudes (p = .005), ICI (p = .0002) and F wave persistence (p = .010), but not ICF (p = .34). Post-hoc analysis revealed that MEP amplitudes were significantly larger at ERD 5% (p = .026) and ERD 15% (p = .016) compared to rest. ICI was significantly reduced at ERD 15% compared to rest (p = .004) and ERD 5% (p = .023). F wave persistence was significantly increased at ERD 15% compared to rest (p = .004), and tended to increase at ERD 15% in comparison with the ERD 5% (p = .084).
Our findings suggest that ERD magnitude during hand MI is an index of the temporal excitability of M1 and spinal motoneurons. Using BCI to associate ERD with upper limb training may be beneficial in stroke rehabilitation because it enhanced corticospinal tract excitability.

Disclosures: M. Takemi: None. **A. Kimura:** None. **M. Liu:** None. **Y. Masakado:** None. **J. Ushiba:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

Location: Hall F-J

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Program#/Poster#:892.13/OO2

Topic: D.18. Brain-Machine Interface

Support: Grant in aid for Strategic Research Program for Brain Sciences from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Clinical application of a newly developed Brain Computer Interface training system to patients with severe hemiparesis.

Authors: *T. FUJIWARA[1], A. NISHIMOTO[1], M. KAWAKAMI[1], J. USHIBA[2], A. MORISHITA[2], D. KAMATANI[1], M. LIU[1]
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Abstract: Brain
computer interface (BCI) training has been developed for upper extremity (UE) rehabilitation for patients with severe hemiparesis. It has been reported that BCI training improves UE motor function in patients with stroke. BCI can directly translate brain signals for the intension of moving fingers into commands for

control of a motor-driven hand orthosis. BCI systems estimate a patient's motor intention from the amplitude modulation of the mu rhythm (event related desynchronization; ERD).
Clinical application of BCI to rehabilitation,
however, has beenlimited because of its feasibility. We developed a new compact BCI system and applied it to clinical rehabilitation setting. In this new BCI system, we detect ERD using a pair of dry electrodes, which are attached to a headphone. It takes less than 5 minutes to put on the electrodes by using this headphone systems. Patients wear the motor-driven orthosis, which extend their paretic fingers triggered with ERD. Using this orthosis, patients can pick up and release pegs.
We applied this new BCI system to 6 patients
with severe hemiparetic stroke. BCI training consisted of a 40-min session per day and was performed for 10 days. Their mean time from stroke onset was 1712 days and mean age was 51.5 y.o. Before and after the BCI training, we assessed Fugl-Meyer upper extremity motor function (FM-U) and motor activity log (MAL) scores. We also measured short intracortical inhibition (SICI) of the motor cortex with paired pulse transcranial magnetic stimulation and reciprocal inhibition (RI) of flexor carpi radialis (FCR) H reflex in the affected forearm.
After the BCI training we found significant improvements of FM-U and MAL scores. We found disinhibition of SICI of the unaffected hemisphere in some patients before the BMI training. This disinhibition became less after the BCI training. The BCI training also increased the magnitude of RI.
Clinical
use of our new BCI system improved UE motor function and daily use of the paretic hand. The pinch and release are important functions to use the hand in activities of daily living. Our BCI system can improve these functions and improve the amount of use of the paretic hand. It is supposed that our BCI training may induce some spinal and cortical plastic changes.

Disclosures: T. Fujiwara: None. **A. Nishimoto:** None. **M. Kawakami:** None. **J. Ushiba:** None. **A. Morishita:** None. **D. Kamatani:** None. **M. Liu:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:892.14/OO3

Topic: D.18. Brain-Machine Interface

Title: Categorizing independent components based on spatial, temporal, and spectral features.

Authors: *R. J. FERNANDEZ[1], S. M. GORDON[1], J. M. VETTEL[2], S. KERICK[2], J. C. BRADFORD[3], V. LAWHERN[4], A. URBAN[2], K. WHITAKER[2], K. MCDOWELL[2]
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Abstract: Independent component analysis (ICA) is commonly employed in neuroimaging research to separate signals into maximally independent components, including EEG recordings believed to represent a linear mixing of brain and non-brain sources. Previous research has shown that ICA is an effective tool for isolating brain sources of interest in a variety of experimental tasks.
Typically, to identify brain-dominant independent components (ICs), an experimenter must manually inspect each IC after decomposing the EEG data. Each IC is first fit to a dipole model within a brain volume using either a standard MNI/Talairach template or a subject-specific MRI scan. The experimenter next cross-references the dipole fit with the IC activation plot, the dipole projection onto the scalp, and spectral measures such as log power in specific frequencies. Based on these features, the IC is then categorized as a brain, non-brain, or mixed source IC. Our group is exploring the analytic utility of seven IC categories, and to facilitate this project, we developed a categorization rubric and confidence assessment for IC features identified by multiple experimenters.Using this rubric, experimenters categorized ICs for EEG data obtained from many subjects. An expert system then learned a set of Bayesian rules for IC categorization given an input feature space and the experimenter-labeled ICs. The system returned both the most likely category as well as a confidence measure based on the marginal distribution returned by each rule. Initial results indicate that such an approach can adapt to specific experimental goals, i.e. the categorization is rubric dependent. In addition, the system can provide a reliable label and descriptive feature list for each IC to minimize burden on the experimenter during manual inspection as well as maximize the objectivity of the categorization process.

Disclosures: R.J. Fernandez: Employment; DCS Corp. Other Research Support; Army Resarch Laboratories. **S.M. Gordon:** None. **J.M. Vettel:** None. **S. Kerick:** None. **J.C. Bradford:** None. **V. Lawhern:** None. **A. Urban:** None. **K. Whitaker:** None. **K. McDowell:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

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Program#/Poster#:892.15/OO4

Topic: D.18. Brain-Machine Interface

Support: Army Contract: W911NF-10-2-0022

Title: Utility of brain and non-brain signals measured with EEG for development of Brain-Computer Interfaces

Authors: *S. M. GORDON[1], B. J. LANCE[2], J. M. VETTEL[2], M. CANNON[1], C. MANTUEFFEL[1], M. JASWA[1], T. JOHNSON[1], V. PAUL[3], J. AMMORI[3], K. S. OIE[2]
[1]DCS Corp., Alexandria, VA; [2]Army Res. Lab., Aberdeen, MD; [3]U.S. Army Tank Automotive Research, Develop. and Engin. Ctr., Warren, MI

Abstract: Brain-Computer Interface (BCI) experiments often utilize simplified environments and tasks to reliably extract brain signals that are diagnostic for either subject behavior or intent. Recently, BCI research has expanded to a broader range of applications, including targeted performance enhancement for healthy individuals in complex, multi-tasking environments. In such contexts, limiting BCI analysis to brain signals may produce suboptimal results.
Fourteen Army Sergeants completed six experimental missions that simulated vehicle operations on a low-threat security patrol through a desert urban environment. While scalp EEG was recorded, the Vehicle Commander (VC) sat in a 6-DOF ride motion simulator and performed mission tasks, including route planning and navigation, target detection, and radio communications. The number and frequency of simultaneous tasks varied during the mission, with higher taskload (more concurrent tasks) during travel in the city sector and lower taskload (sparse tasking) during travel from the sector to the city outskirts.
This analysis examined the predictive value of specific classes of independent components (ICs) for identifying VC taskload on a moment-by-moment basis. EEG data was transformed into maximally independent sources and separated into specific classes using spatial, temporal, and spectral features: (1) brain dominant, (2) eye dominant, (3) brain plus non-brain, and (4) non-brain dominant ICs. We built classifiers using various IC class combinations and projected only selected sources to the scalp. A two-stage classification approach used a support vector machine (SVM) to classify average spectral power features, and a Dynamic Bayesian Network (DBN) filtered and classified the raw SVM values.
The results indicate that no single source type was point-wise dominant across all six missions for a participant; instead, each IC class captured task-relevant processes, and their combination enabled optimal classification. These results demonstrate the feasibility of BCI applications in more complex domains and the value of both brain and non-brain, task-related sources.

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Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

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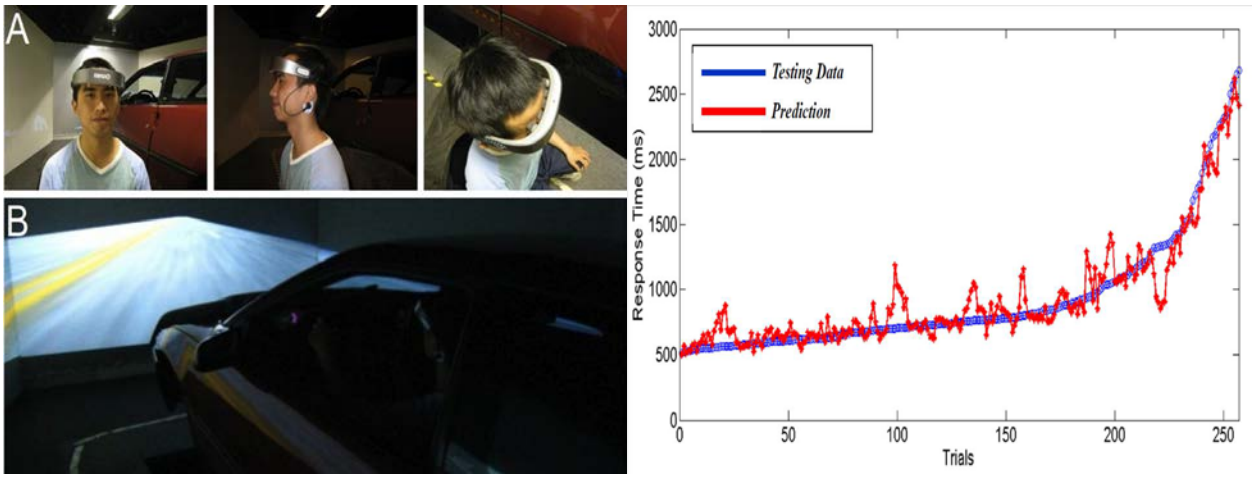
Title: Four-channel wireless EEG system for driver's fatigue estimation

Authors: L.-W. KO[1,2], C.-H. CHUANG[1], Y.-H. CHEN[1], S.-A. CHEN[1], W.-T. CHANG[1], *C.-T. LIN[3,1]
[1]Brain Res. Ctr., [2]Dept. of Biol. Sci. and Technol., Natl. Chiao Tung Univ., Hsinchu, Taiwan; [3]Natl. Chiao-Tung Univ., Hsinchu, Taiwan

Abstract: The goal of this research is to implement the value of developed mobile and wireless EEG system for monitoring driver's fatigue. Four-channel mobile and wireless EEG system, called Mindo-4, acquires EEG signals from the forehead area. Results showed the average correlation between dry and wet EEG signals were 98.14%. We then implement the dry sensor device to conduct the sustained-attention experiment in a realistic dynamic driving simulator. Support vector regression was used for modeling the spectral pattern to predict driver's fatigue state.

Eight voluntary adults participated in a sustained-attention driving task for 1.5 hours after lunch which would be easy to let the subject fall asleep and induce their fatigue level in the experiment. Each subject wearing the Mindo-4 EEG device (figure 1), sat inside the vehicle to perform the experiments. The duration time from the response to the deviation event, denoted as the response time (RT), was used as an indicator to determine subject's fatigue level. To examine the feasibility of the estimation system, experiment results in Figure 2 demonstrate the prediction performance of using Mindo-4 with dry EEG sensors in the driving environment. Furthermore, experiment evidences including power spectral analysis and prediction performance had showed the feasibility of using the mobile and wireless

EEG system can obtain good performance and be applied in the real world.



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Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

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Program#/Poster#:892.17/OO6

Topic: D.18. Brain-Machine Interface

Support: 0903622

Title: Improving EMG-based gesture control by using symmetric bimanual gestures

Authors: A. AKHTAR[1], J. NORTON[1], *S. N. BESHERS[2], T. BRETL[1]
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Abstract: The use of gestures as an input device for controlling devices and other multimedia have seen great increases in recent times. Several studies have investigated using electromyography (EMG) as the signal source for characterizing these gestures. A recent study by Kim et al. in 2008 [1] explored the use of a single-channel EMG placed on the wrist to control an RC car with four gestures. We extend this work by performing corresponding bimanual gestures for better accuracy, as well as

improving on the timescale of classification. Our results suggest that bimanual gestures improved classification accuracy, Linear Discriminant Analysis (LDA) performs better than the methods used in [1], and that the windowing method used in this paper allows for classification at a faster rate.

Disclosures: A. Akhtar: None. **J. Norton:** None. **S.N. Beshers:** None. **T. Bretl:** None.

Poster

892. Brain Machine Interfaces: Neurophysiology and Non-Invasive Approaches

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Topic: D.18. Brain-Machine Interface

Support: NSERC Alexander Graham Bell Canada Graduate Scholarship

CERC program

Title: A p300 based cognitive assessment battery for severely motor impaired and overtly non-responsive patients

Authors: A. KIRSCHNER, *A. M. OWEN, A. HAMPSHIRE
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Abstract: Diagnosing disorders of consciousness is notoriously difficult, with estimates of misdiagnosis rates as high as 40%. Moreover, recent studies have demonstrated that patients who do not show signs of volitional motor responses can exhibit preserved command following as detected by functional magnetic resonance imaging (fMRI) and electroencephalography (EEG). However, although these patients clearly retain some cognitive abilities, administration of standard neuropsychological tests is impossible with such severally motor impaired patients. Consequently, the extent of their cognitive function is unknown.
In the current study, we sought to develop a p300 event related potential (ERP) paradigm in healthy subjects to assess three distinct components of higher cognition in the absence of motor output.
First, to assess covert command following subjects were instructed to attend to a target auditory stimulus, counting each time it occurred, while ignoring distractor auditory stimuli. At the single subject level, 10/10 individuals displayed a significantly larger p300 response to attended vs. unattended words, demonstrating that selective attention can be measured using only electrophysiological markers and used to assess command following.

In order to assess working memory capacity we adapted a standard neuropsychological test (digit span) to use a similar p300 response mechanism. Subjects were instructed to remember 4-8 words in order over a 10 second delay period and asked to retrieve the letter in a given position when the delay was over. Subjects indicated their answer in each trial by attending to the word corresponding to their response. In 8/10 subjects, the p300 responses were significantly larger to correct vs. incorrect words, indicating that they had correctly solved the majority of the problems and could signal their answer using selective attention.
Finally, to assess reasoning ability, we adapted a verbal reasoning task in the same manner. Subjects were given sentences describing the relative position of two items (i.e. “boss does not precede seal”) and asked to report which of the two items logically came first. P300 responses were significantly greater to stimuli corresponding to correct vs. incorrect answers in 8/10 subjects, again demonstrating that they had successfully solved the majority of the problems. These results demonstrate that covert control of attention, as measured by the p300 ERP, can be used to assess attention, working memory and reasoning abilities with a high degree of reliability. We propose that this approach could provide a viable cognitive assessment battery for patients with disorders of consciousness.

Disclosures: A. Kirschner: None. **A.M. Owen:** None. **A. Hampshire:** None.

Poster

893. Brain-Machine Interface: Tissue Response and Analytic Methods

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NIH K-Grant (1K01NS066131-01A1)

Title: *In vivo* two photon microscopy reveals immediate microglial reaction to implantation of microelectrode through extension of processes

Authors: *T. D. KOZAI[1], A. L. VAZQUEZ[2], C. L. WEAVER[1], S.-G. KIM[2], X. CUI[1]
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Abstract: Penetrating cortical neural probe technologies allow investigators to record electrical signals in the brain. Implantation of probes result in acute tissue damage and microglia density increases around these devices over weeks. However, the mechanism(s) underlying this encapsulation is not well understood in the acute temporal domain. Using two-photon in vivo

microscopy, microglia at a depth of ~200 µm below the surface of the cortex were imaged every minute in mice with green fluorescent protein-expressing microglia. Following probe insertion, nearby microglia immediately extended processes toward the probe at (1.6 ± 1.3) µm/min during the first 30-45 min, but showed negligible cell body movement for the first six hours. Six hours after probe insertion, microglia at distances <130.0 µm ($p=0.5$) from the probe surface exhibit morphological characteristics of transitional stage (T-stage) activation, similar to the microglial response observed with laser-induced Blood Brain Barrier (BBB) damage. T-stage morphology and microglia directionality indexes were developed to quantify the microglial response to implanted probes. Evidence suggesting vascular reorganization after probe insertion and distant vessel damage was also observed hours after probe insertion. A precise temporal understanding of the cellular response to microelectrode implantation will facilitate the search for molecular cues initiating and attenuating the reactive tissue response.

Disclosures: T.D. Kozai: None. **A.L. Vazquez:** None. **C.L. Weaver:** None. **S. Kim:** None. **X. Cui:** None.

Poster

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Support: NIH R01 EB005157

DARPA N66001-11-1-4015

Title: Changes in astrocyte, microglia and neuron morphologies and distributions around implanted microfabricated neural prosthetic devices of different designs

Authors: *W. SHAIN[1], C. HARRIS[1], P. CHONG[1], K. TRETT[1], B. ROYSAM[2], B. BUSSE[2], R. PADMANABHAN[2], Y. XU[2], Y. LU[2], J. LUISI[2], M. SAVELONAS[2], V. SOMASUNDAR[2], P. KULKARNI[2], H. CHEUNG[2], A. CHEONG[2], A. CHEONG[2], L. CARIN[3], D. KIPKE[4], C. STOETZNER[4], R. VETTER[5]
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Abstract: Our overall goal is to determine particular device design features that are associated with long-term recording performance of implanted neuroprosthetic devices. In these

experiments we have examined a design space including 12 different devices with different shank widths, open architectures, electrode placements, and electrode designs. Animals were implanted and recordings performed three-times a week for 30 days while animals were performing a trained task. Following the last recording session animals were perfusion fixed with 4% paraformaldehyde and brains prepared for high resolution, high throughput, multichannel, 3D imaging using an Olympus spinning disc confocal system. Tissue slices were prepared to include the entire device within a single section. Nuclei labeled with Hoechst 3453; neurons labeled with NeuroTrace; and astocytes and microglia labeled using immunohistochemistry to GFAP and Iba-1, respectively. All images were collected as a series of optical sections through the entire tissue slice. A series of overlapping images were collected around each site resulting in data collection of as much as a 2x2x0.1mm tissue volume. FARSIGHT image analysis was performed on each data set providing detailed tracing of microglia and astrocytes and mapping of all neurons, microglia, and astrocytes relative to both the device shank and individual electrodes. In addition, analyses have been completed describing different classes of microglia, changes in astrocyte morphologies, neuron distributions, and changes in their distribution relative to control brain samples. These data are being correlated with electrophysiological recording data to identify critical device features and electrode performance.

Disclosures: W. Shain: None. **C. Harris:** None. **P. Chong:** None. **K. Trett:** None. **B. Roysam:** None. **B. Busse:** None. **R. Padmanabhan:** None. **Y. Xu:** None. **Y. Lu:** None. **J. Luisi:** None. **M. Savelonas:** None. **V. Somasundar:** None. **P. Kulkarni:** None. **H. Cheung:** None. **A. Cheong:** None. **A. Cheong:** None. **L. Carin:** None. **D. Kipke:** Ownership Interest; NeuroNexus. **C. Stoetzner:** None. **R. Vetter:** Employment; NeuroNexus.

Poster

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Topic: D.18. Brain-Machine Interface

Support: DARPA N66001-11-1-4015

R01 EB005157

Title: A machine-learning method for identification and tracing of astrocyte cells in 3D confocal microscopy images

Authors: P. KULKARNI[1], *M. SAVELONAS[1], J. LUISI[1], B. BUSSE[1], R. K. PADMANABHAN[1], V. SOMASUNDAR[1], K. TRETT[2], C. HARRIS[2], P. CHONG[2], W.

SHAIN[2], B. ROYSAM[1]
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Abstract: Astrocytes are glial cells of particular importance for studying several disease mechanisms, as well as brain reaction to the presence of implanted devices. Astrocyte nuclei detection is a complicated task due to the presence of microglial cells, neurons and endothelial nuclei. In addition, astrocytes are characterized by a complex octopus-like morphology, and complex cell-cell and cell-vessel contact patterns. Given that astrocyte nuclei and processes are stained in different channels, there is a substantial challenge for automated quantification methods. This work introduces a novel machine-learning method for the quantification of astrocytes, based on a combination of morphological and associative features derived from 3D multi-channel confocal microscopy images. Astrocyte roots, i.e. regions of basal convergence of astrocyte processes, are strong indicators of the presence of astrocyte nuclei within their proximity. In the proposed method, nuclei classification and root detection are effectively coupled in a 2-stage classification scheme, employing active learning so as to allow the expert biologist to provide the most informative training examples. Starting from the identified roots, a tree-based tracing algorithm is applied to enable quantification of astrocyte morphology. Experimental results demonstrate the usefulness of the proposed method for quantifying tissue perturbations caused by implanted neuroprosthetic devices.

Disclosures: P. Kulkarni: Research Grant; DARPA N66001-11-1-4015. **M. Savelonas:** Research Grant; DARPA N66001-11-1-4015. **J. Luisi:** Research Grant; DARPA N66001-11-1-4015. **B. Busse:** Research Grant; DARPA N66001-11-1-4015. **R.K. Padmanabhan:** Research Grant; DARPA N66001-11-1-4015. **V. Somasundar:** Research Grant; DARPA N66001-11-1-4015. **K. Trett:** Research Grant; DARPA N66001-11-1-4015. **C. Harris:** Research Grant; DARPA N66001-11-1-4015. **P. Chong:** Research Grant; DARPA N66001-11-1-4015. **W. Shain:** Research Grant; DARPA N66001-11-1-4015. **B. Roysam:** Research Grant; DARPA N66001-11-1-4015.

Poster

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NIDCR Grant R01-DE011451

Title: Recording quality, distribution and morphometric analysis of interneurons following chronic multielectrode implants

Authors: *M. A. FREIRE[1], E. MORYA[1], J. FABER[1,2], J. SANTOS[1,3], N. A. M. LEMOS[1,3], K. SAMESHIMA[4], M. A. L. NICOLELIS[1,5]
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Abstract: One of the main medical challenges involves the development of therapies to improve the quality of life of people suffering from different types of body paralysis, through the reestablishment of its sensory and motor functions. Brain-machine interface (BMI) emerges as a potential candidate, allowing the control of paralyzed body parts directly by brain activity. Chronically implanted multielectrodes are one of the fundamental components of an invasive BMI, recording neural activity directly from the brain parenchyma. However, before this technique can be effectively available to human clinical trials, it is necessary to comprehensively characterize its impact on the nervous tissue. Here we evaluated the impact of chronic implanted tungsten multielectrode arrays on the distribution and morphology of some classes of inhibitory neurons across rat's motor cortex. In addition, we also correlated the results of cell distribution with electrophysiological signal, in order to estimate the impact of post-implant elapsed time on neuronal recordings. Chronic electrode arrays were well tolerated by the nervous tissue, with recordings remaining viable for up to 6 months after implantation. A restricted neuronal loss and glial activation were observed on the implanted sites. In addition, an interesting result supports future application, since both morphology and distribution of inhibitory neurons were not broadly affected. Altogether, our results suggest that tungsten electrodes can be defined as a feasible candidate to future human clinical interventions involving BMI.

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Poster

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Support: Defense Advanced Research Projects Agency (DARPA) as a part of the Reliable Neural Technology Program; grant No. N66001-11-1-4008.

Title: Normal ATF-3 and cJUN expression in sensory neurons of peripheral nerve interfaced animals despite nerve stretching

Authors: *V. H. DESAI[1], S. BHETAWAL[1], N. KHOBRAGADE[2], C. WATSON[2], A. NGUYEN[2], J. SEIFERT[2], M. ROMERO-ORTEGA[2]
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Abstract: Peripheral nerve interfaces (PNI) have been shown to record motor intent in amputee volunteers and to elicit specific sensation modalities after electrical stimulation. While several types of PNI exist, those implanted directly into the nerve provide higher sensitivity in the recording of single unit neural spikes. However, peripheral nerves are intrinsically elastic and movement of interfaced nerves likely exerts mechanical stress at the electrode-nerve interface. Whether such movement causes tissue micro-injuries and possibly contributes to signal decay over time, is currently unknown. Transcription factors such Activating Transcription Factor ATF-3 and cJUN are established markers of neuronal cell damage and nerve regeneration, respectively. In PNIs such as the Regenerative Multielectrode Interface (REMI), in which the nerve is transected to implant the electrode array, one can anticipate an initial and temporal increase in both ATF-3 and cJUN. In contrast, a persistent expression of both transcription factors, would indicate continued micro-injuries caused by the mechanical mismatch at the electrode/nerve interface. Here we evaluated the expression of ATF-3 and cJUN in the L4/L5 Dorsal Root Ganglions (DRG) of 18 adult Lewis rats, implanted with REMI in the sciatic nerve. REMI consists of an 18 electrode array fixed in the lumen of a 7 mm polyurethane conduit. Controls were implanted with an empty conduit. Histological evaluation of the transcription factors was achieved at 15, 30 and 60 dpi (n=6 for each time point). In REMI implanted animals, quantitative analysis revealed that the percentage of ATF-3 expressing DRG neurons decreased from 15 dpi (18%) to 60 dpi (7%). Similarly, cJun positive cells significantly decreased from 30 days (47.8%) to 60 days (13.4%; p< 0.005). Importantly, these results were comparable to those observed in the tube-only control subjects, suggesting the lack of exacerbation injury by micro-motion in the interfaced animals. To test further the effect of nerve stretching in REMI induced microinjuries, a separate cohort of 18 animals undergo cyclic limb stretching at full extension weekly after REMI implantation for 15, 30 and 60 days. Routine limb extension did not affect the levels of ATF-3 or cJUN observed in the DRG neurons. Together, these results indicate normal progression of nerve repair and regeneration over time despite limb movement or nerve stretching, and support the notion that the REMI forms a monolithic structure with the

nerve. These findings provide evidence of limited microinjuries by regenerative PNIs and suggest that such interfaces will be likely more stable over time.

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Poster

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Topic: D.18. Brain-Machine Interface

Support: IWT SBO Project SBO090054

Title: Histological comparison of acute and chronic tissue response to flexible polyimide and rigid silicon neural implants

Authors: *M. WELKENHUYSEN, A. ANDREI, O. KRYLYCHKINA, S. MUSA
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Abstract: The sustained tissue response during chronic implantation of neural implants is considered one of the main factors preventing long-term recording of neural activity. The micromotion that induces strain between the brain tissue and the implant has been identified to play a key role in this response. Theoretical models have suggested that the induced tissue strain and hence micromotion may be significantly reduced by using flexible probes. The reduced strain would alleviate tissue encapsulation of the implant caused by the inflammatory response and increase the possibility to record chronically from nearby neurons. One way to achieve more flexible probes is to replace part of the silicon (Si) substrate used for most probes by a softer polymer such as polyimide.
We have designed and fabricated a novel hybrid neural probe consisting of a flexible polyimide shank and a rigid Si tip. The Si tip provides stability during insertion and allows integration of large electrode arrays with possibly local CMOS circuitry later on. We compared the short and long term tissue response to the hybrid probe with the response to a full Si probe with the same shank dimensions. In this randomized blinded study, 40 rats were implanted in a deep brain structure with either a 10 mm parylene-coated hybrid or Si probe. The tissue response was immunohistologically assessed after 1 and 4 weeks by the amount of inflammation, glial scarring and neuronal cell loss around the implant site at three depths; at the base, middle and tip of the shank.
During stereotactic implantation, we did not observe any buckling. When comparing the

different time points, we observed a decrease in inflammation over time at the middle of the shank of the Si probes. A decrease over time of the gliosis was also observed at the middle and tip of the Si probes. The neuronal cell loss also decreases over time at the tip of the Si probes. These results were expected and are in agreement with other studies reporting the effect of Si probes over time. For the hybrid probes, we observed an unexpected increase of neuronal cell loss over time at the base and at the tip. Most interestingly, less gliosis was observed at the middle and tip of the shank, and less neuronal cell loss occurred at the tip of the hybrid probes compared to the Si probes, but these significant differences only appeared at short term.
In conclusion, we successfully implanted a novel flexible, hybrid polyimide-Si neural probe in rats and proved that neuronal cell loss at the tip is significantly reduced compared to a full Si probe. This result suggests that the hybrid probes may be more likely to record neuronal activity at the probe tip than Si probes. However, this effect may only be short term.

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Poster

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Support: Indiana SCBI grant (Fund # 00015115).

Title: Quantitative evaluation of polyethylene glycol (PEG) as a therapeutic for the reactive response of brain cells in primary mixed cortical cultures

Authors: *S. SOMMAKIA[1], J. L. RICKUS[1,2,3], K. J. OTTO[1,4]
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Abstract: Implantable intracortical microelectrodes have the potential to treat many neurological disorders, but are limited by a reactive response of brain tissue. This reactive response manifests in a glial scar, composed of a core of activated microglia and a sheath of reactive astrocytes, and a loss of neural density in the vicinity of the implanted electrode. The scar is accompanied by a loss of functionality, with a decrease in recordable signal quality and an increase in electrode impedance to unacceptable levels. It is thought that the initial phases of the injury response play a critical role in defining the extent of the chronic response. Polyethylene glycol (PEG) possesses antifouling and membrane sealing properties, making it an attractive therapeutic capable of

modulating protein and cell adhesion. We use an *in vitro* primary mixed cortical cell culture system to provide a controlled environment for testing PEG as a therapeutic. To simulate vascular injury and accumulation of blood borne components, we use lipopolylysaccharide (LPS), a bacterial endotoxin that activates some of the same receptors as do other proinflammatory blood proteins such as fibrinogen and thrombin. Forebrains from Day 17 embryonic rat pups were dissociated and plated in 96 well plates for 1 week, at which point segments of 50um-diameter tungsten microwire were placed atop the cultures, one microwire per well, and left to incubate with the cells for an additional 1 week. The microwires were coated with either LPS only, PEG only, a 1:1 mixture of LPS and PEG, or uncoated. The cells were then fixed with 4% paraformaldehyde, labeled with primary antibodies for Iba-1 (microglia), GFAP (astrocytes), and beta-3-tubulin (neurons), followed by the appropriate secondary antibodies and imaged. The resulting images were analyzed to obtain a cellular response index for each cell type in interface and distant regions. Our results show that microglia respond to LPS-coated microwire with an elevated response index in both the interface and distant regions, and that the addition of PEG to LPS prevents any statistically significant increase in the microglial response index compared to uncoated and PEG coated response index. In contrast, the astrocyte response index to LPS-coated and uncoated microwire in the interfacial region was not statistically different, whereas PEG-treated microwire (with and without LPS) resulted in a lower response index, indicating that astrocytes were responding to the presence of the microwire rather than the LPS treatment. Neurons exhibited a similar response index to astrocytes, indicating a preferential attachment and growth to an underlying astrocyte layer.

Disclosures: S. Sommakia: None. **J.L. Rickus:** None. **K.J. Otto:** None.

Poster

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Support: RO1 NIH 0467700

Title: Towards informed device design: Modeling the foreign body response to neural recording devices

Authors: *N. NOLTA, J. L. SKOUSEN, K. BOLICK, P. A. TRESCO
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Abstract: Recent studies have shown the potential for using implantable microelectrode arrays to provide volitional control of external devices in patients suffering from spinal cord injury. However, despite the promising potential of this technology, achieving widespread clinical application requires improving long-term recording consistency over a clinically relevant time frame. There is near consensus in the field that the foreign body response (FBR) mounted against implanted devices contributes to the observed recording instability over chronic indwelling times. While a number of commercial devices are available, none were designed using criteria derived from a study of the foreign body response that the body mounts against such devices. Our studies suggest that device surface bound macrophages and more specifically their released factors are responsible for the evolution and maintenance of the FBR. It follows then, that device surface area and geometry are major contributing factors to the overall neuro-inflammatory burden of the device and its effects on surrounding neural tissue and beyond. To validate this idea and to create a predictive tool to investigate future device designs, we have created a finite element model to predict the distribution of macrophage-released factors surrounding a number of commercially available recording devices and used histological tools to validate our predictions. Based on findings from these studies we present a number of simple design changes to traditional device architectures and geometries that our model suggests would be effective in reducing the FBR to these devices and should improve recording consistency and longevity.

Disclosures: N. Nolta: None. **J.L. Skousen:** None. **K. Bolick:** None. **P.A. Tresco:** None.

Poster

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Topic: D.18. Brain-Machine Interface

Support: DARPA

Title: Cellular and molecular tissue response to indewlling and regenerative peripheral neural interfaces

Authors: *A. KANNEGANTI[1], N. KOBRAGADE[1], V. DESAI[1], T. MUSA[2], E. KEEFER[2], Y. KIM[1], M. ROMERO-ORTEGA[1]
[1]Bioengineering, Univ. of Texas At Arlington, Arlington, TX; [2]Plexon Inc.,, Dallas, TX

Abstract: Natural control of advanced prosthetic devices demand sensitive recordings of neural activity, and while different types of neural electrode arrays have been designed, placement of multi-electrode interfaces in the residual nerve of amputees offer a natural portal to recording of

individual action potentials activated during intended limb movement. However, achieving long-term reliability of intraneural peripheral interfaces remains a formidable challenge. We developed a non-obstructive regenerative multi-electrode neural interface (REMI) that record single spike activity from amputated peripheral nerves in freely moving animal in as early as 7 days. Here we report on the histological evaluation of foreign body response to chronic implantation of the REMI electrode at 15, 30 and 60 days post-implantation, and compared it to that observed with the Utah slanted electrode array (USEA). Co-visualization of activated macrophages (ED-1) and inflammatory cytokines (TNF alpha), with myelinating Schwann cells (P0) and axons (NF200), revealed progressive nerve repair and reduction in the size of the macrophage coating of the electrodes. For both the REMI and the USEA, the electrode-nerve interface stabilized overtime with reduction in cytokine expression and cellular proliferation (Ki-67) close to the electrode-tissue interface. Using RT-PCR for 84 different genes associated with nerve function and inflammation, we confirmed that the peripheral nerve sustains significant damage during implantation of both REMI and USEA electrode arrays. However, the peripheral nerve undergoes spontaneous axonal repair, and showed progressive reduction in markers of inflammation. This cellular and molecular tissue response is contrary to increase scar formation observed in the brain after electrode insertion, and underlies the different tissue response to peripheral intraneural interfaces. No correlation was found between the inflammatory response and the signal decay observed over time in both types of electrodes. The data support the notion that cellular responses other than foreign-body reaction to the electrodes might be responsible for signal decay observed over time in intraneural peripheral nerve interfaces.

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Poster

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Topic: D.18. Brain-Machine Interface

Support: NIH R01 (5R01NS062019-03)

Title: Neuroprotection and antioxidant effects of a SOD mimic on neural implants

Authors: *N. R. SNYDER[1], X. T. CUI[2]
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Abstract: Within the last decade, the development of neural prosthetics through a brain tissue-electrode interface has become increasingly popular as a potentially viable method to restore lost sensory and motor function. In order for proper function of neural prosthetics, high-quality signals must be recorded. Furthermore, for human use, the quality of these signals must be reliable on the order of years to ensure proper function without the need to cause unnecessary damage to the cortex by surgically removing and inserting a new implant. Thus far, the brain tissue response to implanted electrodes has prevented reliable chronic recordings for human clinical applications. After electrode implantation, neuronal density around the implant site decreases due to both acute (initial cell death, inflammatory response) and chronic (glial scar formation and neurodegeneration) responses. To reduce local neurodegeneration at the electrode site, a synthetic manganese chelator complex was utilized that has demonstrated in vivo neuroprotective effects in the presence of oxidative stress. This complex mimics the endogenous metalloprotein superoxide dismutase (SOD) which functions as a scavenger of reactive oxygen species (ROS). To test the performance of the SOD mimic (SODm), a synthetic derivative of the compound is covalently attached to a silicon surface and tested for neuroprotective effects. To determine the radical scavenging capabilities of the immobilized SODm, a lipopolysacharide (LPS) stimulated microglial cell line was used to model inflammatory-triggered ROS production. After the cells were cultured on the SODm surface and activated with LPS, the levels of various ROS were determined using the Griess reagent assay as well as a series of synthesized fluorescent sensors specific for several oxidative species. Further evidence for the activity of the SODm is examined via a co-culture of activated microglia and primary rat neurons and the subsequent assessment of cell viability. By mitigating the effects of reactive oxygen species (ROS), we target specifically the processes that mediate the onset of neurodegeneration around implant sites. To verify the potential of the SODm for further in vivo use on neural electrodes, both in vitro ROS production, and the cell viability of neurons cultured together with activated microglia in the presence of the SODm are evaluated.

Disclosures: N.R. Snyder: None. **X.T. Cui:** None.

Poster

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Support: KAW 2004-0119

Linné Grant 600012701

Title: Is demyelination one factor for failure of Brain Machine Interfaces?

Authors: A. TROBÄCK[1], P. KÖHLER[2], F. EJSERHOLM[1], J. SCHOUENBORG[2], M. BENGTSSON[1], *N. P. DANIELSEN[3], L. WALLMAN[1], C. ERIKSSON LINSMEIER[2]
[1]Dept. of Measurement Technol. and Industrial Electrical Engin., Lund, Sweden; [2]Neuronano Res. Ctr., Lund, Sweden; [3]Lund Univ., Lund, Sweden

Abstract: Brain Machine Interfaces are tools for recording neural signals but they can also be used as devices to prevent symptoms from specific neurological disorders. The long-term function of these devices depends greatly on their biocompatibility and long-term stability. Studies have shown that the evoked brain tissue reaction after implantation is of importance for the gradual loss of electrode recording capacity over time. This loss of function may be due to a loss of neurons and/or an excessive astroglial sheath surrounding the implant, effectively insulating the neurons from the electrode.
In a previous study, we found that even though there was an overall decrease in neuronal density in the 0-50µm area surrounding the implant as compared to naïve rat brain tissue there were still NeuN-positive cells close to the implant. This indicates that neuron loss may not be the major factor for signal loss. The difference in the numbers of NeuN positive cells between the two time-points (6 and 12 weeks) studied was not evident in this previous study. One striking finding was that with a greater GFAP response there were significantly more NeuN-positive cells in the vicinity of the implant compared to a smaller GFAP response.
Even though our previous study clearly demonstrated that there are viable neurons within the recording distance, the question of what causes the signal degradation remains. By staining the tissue with RIP for oligodendrocytes and myelin we observed a loss of myelin in the tissue surrounding the implant. We can clearly demonstrate neurons in the area close to the implant and that should in theory allow for signal recording. The loss of electrode function, despite the close vicinity of neurons and a small GFAP response, may thus be explained by axonal damage or loss of myelin and/or comprised oligodendrocytes.
Our current working hypothesis is that the number of neurons does not vary that much over time. The gradual loss of electrode function over time may therefore be explained by demyelination and affected oligodendroglia function.

Disclosures: A. Trobäck: None. **P. Köhler:** None. **F. Ejserholm:** None. **J. Schouenborg:** None. **M. Bengtsson:** None. **N.P. Danielsen:** None. **L. Wallman:** None. **C. Eriksson Linsmeier:** None.

Poster

893. Brain-Machine Interface: Tissue Response and Analytic Methods

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:893.12/OO19

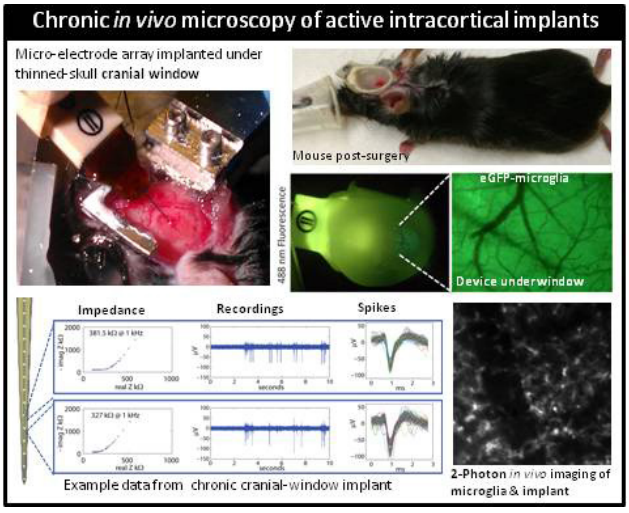
Topic: D.18. Brain-Machine Interface

Support: DARPA BAA-10-32, Grant No. N66001-11-1-4013

Title: *In vivo* microscopy of neural tissue dynamics surrounding intracortical microelectrode arrays

Authors: *A. J. WOOLLEY[1], H. A. DESAI[1], T. J. RICHNER[3], S. K. BRODNICK[3], K. W. ELICEIRI[3,4], J. C. WILLIAMS[3,5], K. J. OTTO[1,2]
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Abstract: Penetrating brain-implants, such as microelectrode arrays, alter their surrounding tissue, which in turn affects the long term functionality of these devices to record and stimulate nearby neurons.
To better understand how biological changes influence physiological recordings, we implanted microelectrode arrays under thinned-skull cranial windows in mice. Multiphoton based imaging of transgenic, fluorescent markers over time in these mice has revealed significant restructuring of tissue components local to implanted devices. Active electrode arrays were utilized, and changes in physiological recordings and electrical impedances were captured. The movement dynamics of immune cells near the implant were assessed, providing insight into the changing nature of the device/tissue interface. This research intends to characterize the impact of intracortical implants, and inform the development of strategies to maintain long-term physiological recordings from implanted microelectrodes. The ultimate goal is to help extend the reliability of such devices, and see them utilized clinically for the treatment of nervous system impairments and disorders.



Disclosures: A.J. Woolley: None. **H.A. Desai:** None. **T.J. Richner:** None. **S.K. Brodnick:** None. **K.W. Eliceiri:** None. **J.C. Williams:** None. **K.J. Otto:** None.

Poster

893. Brain-Machine Interface: Tissue Response and Analytic Methods

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Program#/Poster#:893.13/OO20

Topic: D.18. Brain-Machine Interface

Support: NIH RO1 NS0467700

Title: Permeable cytokine sinks: A new approach to passively reduce inflammation surrouding biomedical devices implanted chronically in the cns.

Authors: *J. L. SKOUSEN, P. A. TRESCO
Dept. of Bioengineering, Univ. of Utah, Salt Lake City, UT

Abstract: All chronic indwelling, biomedical devices would benefit from approaches that reduce chronic neuroinflammation at the brain tissue-device interface including hydrocephalic shunts, drug delivery systems, nerve regeneration substrates, DBS electrodes and chronic recording devices. Not only does persistent inflammation affect the funciton and viability of nearby neural cells but recent evidence suggests that it may reduce neurogenesis at more distant sites in the brain. Available evidence indicates that macrophage-secreted factors at the biotic-abiotic interface are likely responsible for the negative inflammatory sequelae. Based on this assumption and the fact that such molecules have a short biological half-life implant designs that reduce the concentration and impact of inflammatory soluble factors should reduce gliosis, BBB dysfunction, loss of neural cells and affects on neurogenesis. To explore this hypothesis, we studied the chronic brain tissue response to planar solid silicon microelectrode arrays with thick hydrogel coatings (cytokine permeability sinks) designed to passively absorb pro-inflammatory factors that are released by reactive immune cells into the device and away from adjacent brain tissue. Using quantitative immunohistochemical approaches these devices were compared to uncoated cohorts as well as arrays with a thin hydrogel coating, serving as a surface chemistry and functional sink control. After a16-week implantation period in adult Sprague-Dawley rat cortex, we observed that the incorporation of the thick permeable hydrogel was accompanied by a significant reduction in all of the classic hallmarks of the FBR including a drastic reduction in macrophage recruitment and activation, reduced neuronal cell and fiber loss, as well as reduced astrocyte hypertrophy when compared to the reaction observed surrounding either control group. The appearance of the interface was pristine and looked like the implantation tract was cut from

normal brain tissue with a hole punch with the only noticeable change being the bending of cell processes at the coating brain tissue interface. Modeling studies suggest that the thickness of the coating needs to be at least 400 microns for maximal impact. Together, the results suggest a simple, passive approach to improving the biocompatibiltiy of all types of CNS implants that are used in chronic applications.

Disclosures: J.L. Skousen: None. **P.A. Tresco:** None.

Poster

893. Brain-Machine Interface: Tissue Response and Analytic Methods

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:893.14/PP1

Topic: D.18. Brain-Machine Interface

Title: Hippocampal neurogenesis is reduced in animals with chronic peripheral nerve cuffs

Authors: *M. B. CHRISTENSEN, B. D. WINSLOW, A. E. HIGGINS, P. A. TRESCO
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Abstract: Recent studies have demonstrated that the administration of pro-inflammatory agents into both the central nervous system (CNS) and peripherally are sufficient to reduce neurogenesis in the dentate gyrus. Previous research from our lab has shown that persistent inflammation surrounding chronically implanted single-shank silicon microelectrode arrays, implanted in cortex above and a few millimeters anterior to the hippocampus, was sufficient to reduce neurogenesis in the dentate gyrus. Here we investigated whether persistent inflammation associated with the surface of peripheral nerve cuffs was sufficient to reduce hippocampal neurogenesis. Gold-mesh peripheral nerve cuffs were implanted around the right sciatic nerve of Fischer 344 rats for eight weeks. Non-implanted age-matched animals were used as controls. Following the implantation period, animals were transcardially perfused and the brain tissue post-fixed for 24 hours. Horizontal sections 40µm in thickness were cut using a vibratome. Every 10th section was placed in a blocking solution, consisting of 4% (v/v) donkey serum and 1.0% (v/v) Triton X-100 with 0.1% (w/v) sodium azide, overnight, incubated with antisera against DcX for immature neurons for 48 hours, rinsed, incubated in appropriate secondary antibodies for 72 hours, rinsed, then mounted on glass slides and coverslipped. Z-stack montages of the entire dentate gyrus of each brain section were obtained at 200x magnification on an Olympus FV1000 confocal microscope through the use of an automated stage. DcX+ cells were manually identified through co-localization with DAPI and the number of DcX+ neurons in the dentate gyrus was extracted from these counts. The data indicates that the number of DcX+ cells

within the dentate gyrus of animals implanted with peripheral nerve cuffs is reduced, despite their distance from the CNS and the low-level of persistent inflammation present around these devices. The findings suggest that low-levels of persistent inflammation surrounding devices away from the CNS, such as is found associated with conventional medical devices, may be sufficient to decrease the production of new neurons in the dentate gyrus.

Disclosures: M.B. Christensen: None. **B.D. Winslow:** None. **A.E. Higgins:** None. **P.A. Tresco:** None.

Poster

894. Evolution

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:894.01/PP2

Topic: D.19. Comparative Anatomy and Evolution

Title: A computed tomography study of brain development in the coyote (Canis latrans).

Authors: B. A. WHITT[1], B. M. ARSZNOV[1], B. L. LUNDRIGAN[2], *S. T. SAKAI[3]
[1]Dept. of Psychology, [2]Dept. of Zoology and Michigan State Univ. Museum, [3]Dept. of Psychology and Neurosci. Program, Michigan State Univ., EAST LANSING, MI

Abstract: Previous work in the highly gregarious spotted hyena (Crocuta crocuta) revealed that both brain and skull growth occurs over a lengthy developmental period, not reaching completion until well after sexual maturity. In addition, a significant sex difference exists where adult male spotted hyenas possess more frontal cortical volume than females, reflecting differing social life histories between the sexes. It is hypothesized that social species possess larger brains and require a prolonged postnatal brain development relative to that required of solitary species. The central aim of this study was to examine total and regional brain development in one species of the highly social canid family, coyotes (Canis latrans). In order to analyze brain development in coyotes, three-dimensional virtual endocasts were created from computed tomography scans of 42 coyote skulls (23 female, 19 male) ranging in age from 1 day to 9 years. Age was either known or estimated from dentition patterns. A logistic growth curve was fit and used to determine the age for 95% asymptotic (adult) growth for total endocranial volume, skull basal length, and three brain regions (anterior cerebrum (AC), posterior cerebrum (PC), cerebellum + brainstem (CB+BS)) in 39 specimens (21 female, 18 male). The 95% of asymptotic growth for total endocranial volume and every brain region measured occurred within the juvenile life stage (12-26 weeks). An independent t-test of the residuals from linear regressions of regional brain volumes and total endocranial volume showed no significant sex differences. Similarly, no

differences were found between adults (≥93 weeks) and subadults (< 93 weeks) based on the same measures. However, a t-test of the residuals from a linear regression of total endocranial volume and skull basal length, a proxy for body size, yielded a significant difference between adults and subadults. Relative brain volume was larger in subadults than adult coyotes. This finding suggests that brain development concludes well before adult body size has been attained and contrasts with the relatively prolonged brain developmental period in the spotted hyena. The early brain development noted here parallel behavioral observations of early social development observed in coyotes in comparison to other canids. Finally, similar social life histories experienced by male and female coyotes may be reflected in the similarities in total and regional brain volumes found here.

Disclosures: B.A. Whitt: None. **B.M. Arsznov:** None. **B.L. Lundrigan:** None. **S.T. Sakai:** None.

Poster

894. Evolution

Location: Hall F-J

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Program#/Poster#:894.02/PP3

Topic: D.19. Comparative Anatomy and Evolution

Title: The life aquatic: Relative neocortical surface area and brain volume is greater in aquatic than terrestrial mustelid species.

Authors: *B. M. ARSZNOV[1], J. M. KOTT[1], B. L. LUNDRIGAN[2], S. T. SAKAI[3]
[1]Psychology Dept., [2]Dept. of Zoology and Michigan State Univ. Museum, [3]Dept. of Psychology and Neurosci. Program, Michigan State Univ., East Lansing, MI

Abstract: Variations in brain size are hypothesized to be related to multiple factors including: sociality, diet, habitat, body size, and phylogeny. Here, we sought to determine if these factors influence both total and regional brain volumes in the highly diverse carnivore family Mustelidae since variations within taxa may reveal functional features that influence encephalization separate from the broad constraints associated with comparisons made across taxa. This study used computed tomography to create three-dimensional virtual endocasts based on serial analysis of coronal sections through the adult endocranium in 11 species. Eight genera of the mustelid family were analyzed including: badgers (Taxidea taxus), grisons (Galictis vittata and Galictis cuja), fishers (Martes pennanti), american marten (Martes americana), tayras (Eira barbara), wolverines (Gulo gulo), mink (Neovison vison), honey badger (Mellivora capensis) and two river otter species (Lontra longicaudis and Lontra canadensis). A strong allometric correlation

between total endocranial volume and skull basal length, a proxy for body, size was found among mustelids. Analysis of mean residuals from linear regressions revealed that relative endocranial volume was significantly greater in aquatic compared to terrestrial mustelids. Additionally, the natatorial species possessed more neocortical surface area relative to endocranial volume than terrestrial species. These findings may be related to habitat complexity, where an aquatic habitat presents greater cognitive, spatial memory and sensorimotor challenges, such as having to navigate a complex three-dimensional environment, compared to a terrestrial habitat. These differences in the relative brain size and the relative amount of neocortex in mustelids support the notion that intrafamily comparisons may reveal family-level variation in morphological and ecological traits not detected by broader taxonomic comparative studies.

Disclosures: B.M. Arsznov: None. **J.M. Kott:** None. **B.L. Lundrigan:** None. **S.T. Sakai:** None.

Poster

894. Evolution

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:894.03/PP4

Topic: D.19. Comparative Anatomy and Evolution

Support: FONDECYT 1120124

Title: Thylamys elegans: A lowly marsupial with a regal dorsal geniculate nucleus

Authors: D. SEVERIN[1], N. MADRID[1], L. OSSA-FUENTES[1], *J.-C. LETELIER[1], G. MARIN[1,2], T. VEGA-ZUÑIGA[1], J. MPODOZIS[1]
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Abstract: In mammals binocular vision is dependent of the visual projections to the thalamic dorsal nucleus of the lateral geniculate (GLd). Afferences from both eyes end segregately into different layers of the GLd in an interdigitated fashion. Among eutherians, the laminar composition of the GLd is variable, and appears to be subject to phylogenetic restraints. A high degree of laminar complexity is a characteristic of the primate order only. In contrast, among metatherians this trait does not correlate simply with phylogeny. Close related species can have differences in the degree of lamination of this nucleus. Several documented cases indicate that close marsupial species that differ in visual usage also differ in GLd complexity. Members of the family Didelphidae, a clade of nocturnal American marsupials constitute a representative example of this: Didelphis virginiana, a cursorial omnivorous, exhibit a simple oligolaminar GLd structure, while Didelphis marsupialia, a cursorial predator, and Marmosa robinsoni, an arboreal

predator, both exhibit a complex multilaminar GLd. In an attempt to relate visual habits with GLd lamination we studied the visual projections in another member of the Didelphidae clade: *Thylamys elegans*, a visually active arboreal insectivorous. To this end, we performed monocular intravitreal injections of the anterograde tracer CTb, and prepared series of CTb reacted, nissl counterstained brain sections. We found that *T. elegans* features a well-developed system of visual projections, in which all major retinal targets described in mammals were easily identifiable. The suprachiasmatic nucleus, the accessory optic nuclei and the pretectal nuclei received only contralateral retinal afferences. The ventral lateral geniculate complex, the superior colliculus, and the GLd received ipsi and contralateral retinal projections. The GLd showed in its dorsal pole a striking pattern of lamination. Seven layers, latero-medially disposed were readily distinguishable. These layers displayed an alternate pattern of retinal terminals, which was complementary between the ipsi and contralateral sides. The ventral pole of the GLd does not appear to be laminated, and only at the contralateral side displayed a homogeneous plexus of labeled retinal terminals. In general, these results sustain the notion that in marsupials GLd lamination is closely related to visual usage. In particular, the results indicate that in visually active animals arboreal/predatory habits are associated with an enhanced GLd complexity, at least in the case of Didelphidae. More extensive comparative studies will be required to generalize this conclusion.

Disclosures: D. Severin: None. **N. Madrid:** None. **L. Ossa-Fuentes:** None. **J. Letelier:** None. **G. Marin:** None. **T. Vega-Zuñiga:** None. **J. Mpodozis:** None.

Poster

894. Evolution

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Program#/Poster#:894.04/PP5

Topic: D.19. Comparative Anatomy and Evolution

Support: NIH R21NS071225

Title: Differential distribution of neurons within the neocortex of short-tailed opossums (Monodelphis domestica)

Authors: *A. M. SEELKE[1], J. C. DOOLEY[1], L. A. KRUBITZER[2]
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Abstract: The mammalian neocortex varies along a number of dimensions including relative size, number of cortical fields, connections, and neuron number and density. These differences give rise to the variety of cognitive and behavioral specializations that different species exhibit.

Neurons are the primary unit of neural processing, and it is thought that the number, distribution and organization of neurons and synapses is related to processing capacity and cognitive abilities. Further, it is also hypothesized that the amount of area within the cortex given to processing a certain modality reflects the relative importance of that modality for species specific behaviors. For example, humans are highly visual animals, and as such a large proportion of their cortex is devoted to processing visual information.
Using isotropic fractionation, we examined the cellular composition, including neural number and density, of different areas within the neocortex of short-tailed opossums (Monodelphis domestica), a highly visual marsupial. The brains of adult opossums were dissected, isolating the neocortex. The neocortex was then further subdivided into the primary visual (V1), auditory (A1) and somatosensory (S1) areas. The tissue was homogenized and nuclei were dissociated. All nuclei were stained with DAPI and neuronal nuclei were also stained for NeuN. Nuclei were counted using standard stereological techniques, and a flow cytometer was used to determine the proportion of neuronal and non-neuronal nuclei. For each cortical region we calculated the total number of cells, total number of neurons, total cell density, neuronal density, and proportion of neurons.
The total number of cells in each primary cortical area was significantly different with A1 having the fewest number of cells and V1 having the largest number of cells. Likewise A1 also had the fewest number of neurons and V1 had the largest number of neurons. The total cell density (number of cells per mg of tissue) did not differ significantly between cortical areas. In contrast, neuronal density (number of neurons per mg of tissue) was significantly higher in A1 and S1 compared to V1. Finally, the percent of neurons was higher in A1 and S1 than in V1.
These data indicate that the opossum neocortex has a differential distribution of neurons. However, contrary to our expectations, the largest neocortical sensory region, V1, had a smaller proportion of neurons than A1 and S1. This suggests that the number and density of neurons alone may not be responsible for the increase in processing capacity, but some other factors, such as number and position of synaptic contacts and concentration of glial cells, likely contribute as well.

Disclosures: A.M. Seelke: None. **J.C. Dooley:** None. **L.A. Krubitzer:** None.

Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: NIH Grant EY006821

Title: Chemoarchitectural characterization of the tree shrew frontal cortex

Authors: *M. BOLTON[1], C. A. BAKER[1], D. FITZPATRICK[2]
[1]Disorders of Neural Circuit Function, [2]Functional Architecture and Develop. of Cerebral Cortex, Max Planck Florida Inst., Jupiter, FL

Abstract: The massive expansion of the frontal cortex of the brain during the evolution of the mammalian lineage is thought to be responsible for the increasing complexity of cognitive function and social behavior apparent in primates. Areas within primate frontal cortex have been implicated in various human psychiatric disorders but have no clear homolog in rodents. As one of the closest living relatives of primates, the northern tree shrew (Tupaia belangeri) has a highly developed cerebral cortex with somatosensory and visual systems that are well characterized, but studies on how its frontal cortex compares to that of primates and rodents remain incomplete. Here we utilize an array of cytochemical stains and molecular markers for particular neuronal subtypes to delineate particular areas within the shrew frontal cortex. We also demonstrate a dramatic expansion of the frontal cortical regions in the shrew relative to that of the mouse. The thalamic innervation and functional specialization of these areas, as well as their relation to subdivisions in rodent and primate frontal cortex, remain to be determined. The tree shrew represents a novel system for exploring the functional organization of frontal cortex and its contribution to behavior.

Disclosures: M. Bolton: None. **C.A. Baker:** None. **D. Fitzpatrick:** None.

Poster

894. Evolution

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Topic: D.19. Comparative Anatomy and Evolution

Support: NIH grant EY02686

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Title: Cytoarchitectonic characteristics of the neocortex of the gray mouse lemur (microcebus murinus).

Authors: *P. BALARAM[1], H. KENNEDY[2], J. H. KAAS[1]
[1]Psychology, Vanderbilt Univ., Nashville, TN; [2]INSERM, Bron, France

Abstract: The gray mouse lemur is a small lemuroid primate native to Madagascar that may resemble, in many ways, the ancestral primate that gave rise to divergent lemuroid, tarsoid, and anthropoid primate lineages (Le Gros Clark, 1931). Mouse lemurs share many ecological and behavioral traits with other members of the primate order, yet their brains appear much more primitive when compared to most other living primates. The neocortex of the gray mouse lemur is small, roughly 17mm from the rostral to caudal pole, and relatively smooth, with only two distinct sulci interrupting the surface. Previous histological studies have identified an average of 20 distinct neocortical areas based on the density of Nissl bodies in serial sections, yet the relative extent and location of these areas varies between accounts (Le Gros Clark, 1931; Zilles et al, 1979; Zilles and Schleicher, 1980). In order to establish a reliable cortical map that may be used to guide further anatomical studies in this species, we have reexamined the areal organization of the gray mouse lemur neocortex using modern immunocytochemical and analytical techniques. Four cortical hemispheres from two mouse lemurs were stained with a battery of known immunohistochemical markers that identify cortical areal boundaries, reconstructed in three dimensions, and differentiated into cortical areas based on changes in staining patterns across the neocortical surface. We find that the neocortex of the gray mouse lemur is indeed comprised of roughly 15-20 distinct cortical areas. The majority of the neocortical surface is occupied by primary sensory areas, with large amounts of cortical space devoted to V1, A1, and 3b. Frontal and motor areas occupy only a limited expanse of cortical surface, only extending a few millimeters caudal to the frontal pole. The areal organization of the neocortex of the gray mouse lemur suggests a more ancestral form of the primate cortex, with fewer anatomically distinct cortical areas and larger primary sensory areas in proportion to total brain volume. However, the relative locations of most cortical areas in the mouse lemur neocortex are similar to those seen in other primates and extant mammals, indicating that patterns of cortical organization are a conserved feature of mammalian evolution. Our results are presented in the form of areal maps of the neocortical surface of the gray mouse lemur brain. These architectonic maps of cortical areas generated by this study will inform further studies of cortical organization across primate species.

Disclosures: P. Balaram: None. **H. Kennedy:** None. **J.H. Kaas:** None.

Poster

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Support: NSF 0929572

NIH R03AI081701

Title: Brain development in an insect with extensive maternal care, the tsetse fly Glossina morsitans (Diptera: Glossinidae)

Authors: *S. M. FARRIS[1], R. V. M. RIO[2]
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Abstract: Like birds and mammals, insects vary in the degree of maturity at birth, which is correlated with the availability of parental investment. Precocial young are born relatively independent of adult care and are mobile with well-developed sensory organs and nervous systems and external structures such as feathers, fur, and exoskeletons. In contrast, altricial young are dependent upon parents or other adults that provide food and shelter, and show reduced mobility and delays in development of the brain, sensory organs and external structures. Altricial young likely have fewer sensory and behavioral demands relative to free-living precocial young, permitting the evolution of delayed development of structures required for more independent behavior due to reduced necessity. In insects, altricial young are often observed in eusocial species such as the honey bee Apis mellifera. Honey bee larvae are contained within brood cells and are provided with food by adult nestmates. Compared with free-living larvae such as those of the fruit fly Drosophila melanogaster, sensory and integrative brain centers such as the antennal lobes and mushroom bodies show delayed development in the honey bee, first appearing at the late larval or early pupal stage rather than during embryogenesis. Delayed mushroom body development is also observed in altricial larvae of non-social species such as dung beetles, in which young develop enclosed within a food source provided by the parent. Perhaps the most extreme form of parental care in insects is adenotrophic viviparity. Obligate viviparity may be observed in the hippoboscoid flies, in which the entirety of larval development takes place within a uterus-like organ within the female in a manner similar to placental mammals. Given the strong correlation between brain development and parental care of altricial young, observed in animals across phyla, we predict that hippoboscoid larvae and pupae will display profound delays in brain development associated with the paucity of sensory and behavioral needs of a larva developing in utero. Development of the brain, with emphasis on sensory neuropils and the mushroom bodies, will be investigated in a hippoboscoid species of the family Glossinidae, the tsetse fly Glossina morsitans. We predict brain development in this species will be delayed compared to that of related flies with free-living larvae such as Drosophila melanogaster; however, due to the greater degree of maternal care and the complete lack of sensory and behavioral demands due to the restricted ecology of the larva, the delay will be far more extensive than has been observed in other altricial species.

Disclosures: S.M. Farris: None. **R.V.M. Rio:** None.

Poster

894. Evolution

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Support: NSF GRFP to GHW

University of Arizona Regents Fund to NJS

University of Arizona Neuroscience GIDP

Title: Expression of DC0 in forebrains supports genealogical correspondence of learning and memory centers across Lophotrochozoa and Ecdysozoa

Authors: *G. H. WOLFF[1], X. MA[2], N. J. STRAUSFELD[1]
[1]Dept. of Neurosci., The Univ. of Arizona, Tucson, AZ; [2]Dept. of Palaeontology, Natural History Museum, London, United Kingdom

Abstract: In arthropods, primary sensory brain neuropils project to higher order centers, such as connections from olfactory glomeruli to the mushroom bodies in insects, chelicerates, and myriapods, and from the olfactory lobes to the hemiellipsoid bodies in malacostracans. These higher centers may integrate visual, olfactory and haptic inputs, and have been described as “learning and memory” neuropils. Previously, an immunohistological study of the insect mushroom body and malacostracan crustacean hemiellipsoid body has shown that both structures express the major catalytic subunit of cAMP-dependent protein kinase A (DC0), to the exclusion of the rest of the brain, with the exception of the lower division (ellipsoid body) of the central complex, which is DC0 immunoreactive in both groups. This evidence, along with corresponding morphological elements, suggests that insect mushroom bodies and crustacean hemiellipsoid bodies are genealogically homologous, but it is unknown whether this organization is part of the ground plan for all arthropods. In order to explore homology across higher order centers in arthropods, we used immunohistochemistry methods to localize and compare DC0 expression in taxa belonging to Insecta, Crustacea, Chelicerata, and Myriapoda. We also compared these results to DC0 localization in an annelid, the polychaete worm, Nereis virens to determine whether the mushroom body ground plan may have been present in the common ancestor of Ecdysozoa and Lophotrochozoa. Despite superficial differences in morphology of higher order centers between arthropod groups, we have found DC0 expression localized to these

structures in representative taxa of Insecta, Crustacea, Chelicerata, and Myriapoda as well as in the mushroom body of the annelid, N. virens. DC0 expression has also been found in insect and crustacean central complexes, the arcuate body in chelicerates, and corresponding unpaired midline neuropils in myriapods and annelids. These studies provide further evidence for an origin of the mushroom body ground plan preceding the divergence of Lophotrochozoa and Ecdysozoa from a common ancestor, as well as correspondence of the unpaired midline neuropils. These may also serve learning and memory as well as control of multijoint appendages or, in errant annelids, segmental setae. Such findings add kindling to the debate whether a common ancestor of "Articulata" and chordates was equipped with a mushroom body-like sensory association center and a mid-line center mediating choice of behavioral actions. These considerations currently drive research into seeking corresponding patterns of protein expression in arthropod and vertebrate brains.

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Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: National Science Foundation

Title: Detection of hydrodynamic stimuli by Florida manatees (Trichechus manatus latirostris)

Authors: *R. L. REEP[1], J. C. GASPARD III[2]
[1]Univ. Florida, GAINESVILLE, FL; [2]Mote Marine Lab., Sarasota, FL

Abstract: Manatees possess a unique arrangement of specialized sensory hairs (vibrissae), present on the face and across the body. Previous research conducted on the facial vibrissae has demonstrated that touch may be the manatee's primary sensory system, highlighted by acute detection of oscillatory water movement with a particle displacement threshold as low as 1 nm and sensitive active touch discrimination (Weber fraction = 0.025). These hairs appear to be represented in the somatosensory cortex by neuronal aggregations similar to the barrels in rodents. Two Florida manatees were tested in a go/no-go paradigm to determine their abilities to detect hydrodynamic stimuli ranging from 5 - 200 Hz utilizing their post-facial vibrissae. The stimuli were generated by a sinusoidally oscillating sphere which generated a dipole field. Trials

were conducted using a staircase method and counterbalanced stimulus-present and stimulus-absent trials. Detection thresholds of the post-facial hairs were shown to be elevated in comparison to the facial vibrissae but still fairly sensitive, approaching 1 μm particle displacement for most frequencies. To further elucidate the role of the vibrissae, the manatees were trained to wear a neoprene wrap which allowed a series of isolation tests. Detection thresholds were similar at lower frequencies to the unrestricted trials even when only a small number of hairs were exposed. With over 3,000 of these tactile hairs distributed across the body, manatees may utilize their vibrissae as a sensory array, analogous to the lateral line system of a fish.

Disclosures: R.L. Reep: None. **J.C. Gaspard III:** None.

Poster

894. Evolution

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:894.10/PP11

Topic: D.19. Comparative Anatomy and Evolution

Support: MICINN-FEDER Grant BFU2009-07212/BFI

Title: Expression of developmental regulatory genes help to delineate the pallial amygdala in a lacertid lizard

Authors: E. DESFILIS[1], A. ABELLAN[2], *L. MEDINA[2]
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Abstract: The amygdala is a telencephalic center essential for control of emotions and social behavior that is present in all tetrapods. Based on the expression of developmental regulatory genes and other data, pallial and subpallial amygdalar divisions have been identified. In mammals, the pallial amygdala consists of deep nuclei, highly interconnected with the cerebral cortex and hippocampal formation, and olfactory-related superficial cortical areas. Genoarchitecture and fate mapping data in the mouse have shown that part of the mammalian pallial amygdala specifically derives from the ventral pallium, and is characterized by expression of the pan-pallial gene Tbr1 and, specifically, by Lhx9. A similar combinatorial gene expression characterizes the ventral pallial amygdala in the chicken and the anuran Xenopus laevis. However, data in reptiles are scarce and only pertain to turtles, which like birds show a diminished olfactory system. To better understand the evolution of the amygdala, we have started a research project to clone and analyze the expression of a battery of developmental regulatory genes in the embryonic forebrain of the lizard Psammodromus algirus (Reptilia,

Lacertidae), which is a sauropsid with well-developed olfactory and vomerolfactory systems. Herein we present data on the expression of Dlx2, Tbr1, Lhx2 and Lhx9 in the embryonic telencephalon of P. algirus from intermediate to late embryonic stages (S36-S40). Similarly to other vertebrates, the expression of Dlx2 and Tbr1 helps to distinguish pallial and subpallial structures of the lizard telencephalon. At S36, Dlx2 is highly expressed only in the ventricular/subventricular zone of the subpallium, but not the pallium. Nevertheless, Dlx2-expressing cells tangentially migrating from the subpallium to the pallium are observed. On the contrary, Tbr1 is expressed in all pallial derivatives and these include the primordia of lizard amygdalar structures such as the lateral and dorsolateral amygdala, the posterior dorsal ventricular ridge and nucleus sphericus. Lhx2 and Lhx9 are also expressed in these pallial amygdalar structures during development. However, the anterior dorsal ventricular ridge is free of Lhx9 expression from S37 onwards. These data help to delineate the ventral pallial amygdala of lizards and are important for trying to understand the evolution of olfactory- and association-related amygdalar areas.

Disclosures: E. Desfilis: None. **A. Abellan:** None. **L. Medina:** None.

Poster

895. Comparative Anatomy

Location: Hall F-J

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Program#/Poster#:895.01/PP12

Topic: F.04. Neuroethology

Support: NIH R01 NS045193

NIH R90 DA023419

Title: Quantitative analysis of canine temperament and neuroanatomical variation

Authors: *D. DAS[1,2], S. ALI[1,2,3], J. A. SERPELL[4], S. S.-H. WANG[1,2]
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Abstract: Dogs are the most morphologically variable of mammalian species and also show considerable breed-specific temperamental variation. We analyzed 3565 responses to the Canine Behavioral Assessment and Research Questionnaire (C-BARQ). We restricted our analysis to 63 questionnaire items representing 10 traits: Attachment or Attention-Seeking Behavior, Chasing, Dog-Directed Fear or Aggression (DFA), Excitability, Non-Social Fear (NSF), Owner-Directed

Aggression (ODA), Stranger-Directed Aggression (SDA), Stranger-Directed Fear (SDF), Separation-Related Behavior, and Trainability. Animals with health conditions expected to affect behavior were removed from the analysis.
The item-item correlation matrix showed that traits were not independent but strongly covaried. Five fear- and aggression-related traits (DFA/SDA/SDF/NSF/ODA) could be combined with Separation-Related Behavior to form a single meta-trait, Fear-Aggression-Separation (FAS). Within the FAS meta-trait, DFA, SDA and SDF also formed a more restricted meta-trait, Dog- and Stranger-Directed Fear and Aggression (DSFA) in which DFA and SDA were more closely related to one another than to SDF.
Some traits varied in close association with body size, a parameter that is associated with thyroid function. Three traits were uncorrelated with body size: Trainability, Chasing, and the metatrait of DSFA. Between pairs of breeds of similar body (and brain) size, the largest trait difference observed was up to 2.1 standard deviations (Cohen's d, using SD calculated from 2352 surveys of neutered dogs aged 2-10 years), approximately 10 times larger than differences by sex, age, or neuter status. Since dog brain volumes span an approximately 3-fold range, absolute volumes are unlikely to be a major determinant of these temperamental traits. We propose internal brain proportions ("cerebrotype"; Clark DA, Mitra PP, and Wang SS-H 2001 Nature 411:189) as a target for future neuroanatomical analysis. To this purpose we are gathering veterinary MRI scans for gross shape and volume reconstruction of identified brain structures.

Disclosures: D. Das: None. **S. Ali:** None. **J.A. Serpell:** None. **S.S. Wang:** None.

Poster

895. Comparative Anatomy

Location: Hall F-J

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Program#/Poster#:895.02/PP13

Topic: D.19. Comparative Anatomy and Evolution

Support: NIH Grant MH60744

Title: Ultrastructural characterization of tyrosine hydroxylase in the nucleus accumbens core and shell of the tree shrew

Authors: *L. A. MCCOLLUM[1], R. C. ROBERTS[2]
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Abstract: Many behavioral, physiological, and anatomical studies utilize animal models to investigate human striatal pathologies. Although commonly used, rodent striatum may not

present the optimal animal model for certain preclinical studies due to a lesser morphological complexity than that of non-human primates, which are increasingly restricted in research. As an alternative, the tree shrew could provide a beneficial animal model for studies of the striatum. The gross morphology of the tree shrew striatum resembles that of the primate, with the separation of the caudate and putamen by the internal capsule. However, past research of the tree shrew has largely been limited to its well-developed visual and acoustic systems. The neurochemical anatomy of the dorsal striatum has recently been characterized, but the synaptic organization of the tree shrew ventral striatum, specifically the nucleus accumbens, has not yet been examined. The nucleus accumbens, a major region of the limbic system, plays a role in normal physiological functioning, such as drive-related behaviors and reward, as well as in addiction, and thus is an area of interest for research on human striatal pathologies. The current study uses immunohistochemistry of calbindin (to identify shell and core), and tyrosine hydroxylase to determine the ultrastructural organization of dopamine synapses in the nucleus accumbens of the tree shrew (*Tupaia glis belangeri, n*=3 animals). The distribution of these markers in the tree shrew was similar to that found in rodent and primate studies of the nucleus accumbens. The core contains dense immunoreactivity for calbindin while the shell displays weaker heterogeneous immunoreactivity for calbindin. Stereology was used to quantify the ultrastructural localization of tyrosine hydroxylase, which displays weaker immunoreactivity in the core (~15% of axon terminals forming synapses contain labeling for tyrosine hydroxylase), and denser immunoreactivity in the shell (~37% of axon terminals forming synapses contain labeling for tyrosine hydroxylase). In both shell and core, synapses with tyrosine hydroxylase-labeled axon terminals were primarily symmetric and were presynaptic most often to dendritic spines (69.8% and 54.8%, in core and shell respectively). The results from this study support that the tree shrew nucleus accumbens has similar neurochemical and structural anatomy to the primate striatum and is a potential model for studies of human striatal pathologies.

Disclosures: L.A. McCollum: None. **R.C. Roberts:** None.

Poster

895. Comparative Anatomy

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:895.03/PP14

Topic: D.19. Comparative Anatomy and Evolution

Title: Retinal ganglion cell in Japanese catshark, Scyliorhinus torazame

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Abstract: The retinal ganglion cells (GCs) represent the output channel from the eye, and their axons provide the only pathway from the eye to the brain. Therefore GC is thought to place a theoretical upper limit on the spatial resolving power (SRP) of the eye. Also, the distribution of GCs varies according to the lifestyle of the species. By examining these retinal features, we can gain insight into visual capabilities of the species and their relationships with the lifestyle. Additionally, in vertebrates, it is generally thought that the morphology of GCs varies according to their functions and their projection sites. Morphological classification of GCs has been well established in most vertebrates. However, there is only one detailed study in cartilaginous fishes (Stell and Witkovsky, 1973). The number of subtypes reported in that study is less than those of other vertebrates, hence there might be additional subtypes in cartilaginous fishes yet to be described. In the present study, the distribution of GCs and SRP in the Japanese catshark, *Scyliorhinus torazame* were investigated, and the morphology of GC was also examined by the retrograde labeling method with neural tracers (biotinylated dextran amine) in *S. torazame.* With Nissl-stained preparations, morphologically distinct populations of GCs (small-sized and large-sized GCs) were identified in the ganglion cell layer of *S. torazame*. These GC groups showed different spatial distribution patterns. A horizontal streak of high cell density was recognized in the dorsal retina for small-sized GCs, while two spots of high cell densities, or *area centrales*, were identified for the large-sized GCs, one in the nasal and the other in the temporal retina. The maximum predicted SRPs of three catsharks were 2.58-3.51 cells/deg and they are at the lower range of those among elasmobranchs. Based on the soma size and the dendritic morphology, retrogradely labeled GCs could be classified into five populations. Although the depths where the somata and dendrites are positioned are still unclear, the presence of additional subtypes in GCs was suggested in the present study.

Disclosures: K. Muguruma: None. **N. Yamamoto:** None.

Poster

895. Comparative Anatomy

Location: Hall F-J

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Program#/Poster#:895.04/PP15

Topic: D.19. Comparative Anatomy and Evolution

Support: Fondecyt 1120124

Title: Modular recurrent circuits in the avian visual collopallium.

Authors: P. AHUMADA[1], S. TAPIA[1], M. FERNANDEZ[1], J. LETELIER[1], G. MARIN[1,2], *J. MPODOZIS[1]
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Abstract: In the telencephalon of birds, the main zone receiving collothalamic afferences is a pallial region known as the nidopallium. Previous studies have defined the general pattern of thalamic-nidopallial connectivity, as well as the overall pattern of nidopallial projections. Nidopallial nuclei are known to form reciprocal, short-range projections with their overlaying mesopallial areas. Recent findings involving the Field -L (auditory nidopallium) have shown that these projections are organized as radially oriented columns, in a way that resembles the columnar intralaminar arrangement of the mammalian neocortex. We have re-examined in detail the organization of the visual nidopallium practicing small, circumscribed injections of neuronal tracers in brain slices and *in vivo*, as well as intracellular fillings in brain slices. We found that the visual nidopallium (VN) is organized in two main laminas: An internal lamina (the entopallium proper, E), in receipt of very dense, topographically organized afferences from its thalamic source, the nucleus rotundus, (Rt), and a dorsally adjacent lamina (intermediate nidopallium, NI, former entopalial "belt"), in receipt of more sparse afferences from the Rt. As in the case of the Field-L, we found that the system of reciprocal projections between the VN and the adjacent ventro lateral mesopallium (MLV) is formed by dorso-ventrally oriented, discrete columnar bundles of axons, which follow a precise topographic homotopic arrangement. VN-mesopallial projecting neurons were located mainly in the dorsal half of the E. Axonal terminals of these cells form a dense band that covers the internal-most side of the MVL. In turn, MVL cells contributing to the "columns" lie intermingled with or immediately dorsal to the field of VN terminals, and send its axons to the E and NI. Cells in the NI, but not in the E or MVL generate efferences that target several other pallial regions, such as the arcopallium and the frontal, lateral and caudal nidopallium. Based on these results we regarded the VN/MVL complex as composed of three differentiated layers: internal (thalamo-recipient, E), intermediate (efferent, NI) and external (associative, MVL). Interconnections between them may form operational modules in which cells located in homotopic positions in each layer are synaptically linked by an intralaminar local loop of recurrent axonal processes. The activity of each module would be driven by the incoming thalamic axon, and would result in a modulation of the activity of the efferent cells. Thus, if effective, the physiological operation of these proposed modules will close resembles that of the cortical columns.

Disclosures: P. Ahumada: None. **S. Tapia:** None. **M. Fernandez:** None. **J. Letelier:** None. **G. Marin:** None. **J. Mpodozis:** None.

Poster

895. Comparative Anatomy

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Program#/Poster#:895.05/PP16

Topic: D.19. Comparative Anatomy and Evolution

Support: NIH Grant R01MH066128

Title: Vesicular Glutamate Transporter 2 (vGlut2), but not vGlut1, is present in avian pallium.

Authors: *M. FEIL[1], A. L. BODOR[1], P. V. LOVELL[2], C. V. MELLO[2], D. J. PERKEL[1]
[1]Univ. of Washington, Seattle, WA; [2]Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Vesicular Glutamate Transporters (vGluts) are proteins that package glutamate into vesicles prior to synaptic transmission. Three isoforms have been identified in mammals (vGlut1-3), with 1 and 2 showing a mostly complementary pattern of expression, and 3 co-localizing in a small number of cells with both 1 and 2. vGlut1 is mainly expressed in the cortex, hippocampus, and cerebellum while vGlut2 is expressed primarily in the thalamus, hypothalamus and brainstem. Knockout mice for the vGlut1 gene die shortly after weaning, indicating a crucial role for vGlut1 in development. Intriguingly, the avian genomes that have been sequenced contain no vGlut1 orthologs, despite the fact that lizards, a common ancestor of both mammals and birds, have orthologs of both vGlut1 and 2. The fact that birds function without vGlut1 suggests there may be an increased functional role for vGlut2 in the avian brain. Indeed, vGlut2 expression in the chicken brain is similar to the combined pattern of both vGlut1 and vGlut2 in mammals. To further demonstrate the presence and potential role of vGlut2 in avian pallial circuitry, we used immunocytochemical methods to study the brain distribution of vGlut2 protein in zebra finch, a representative avian species with well-defined neural circuitry. To confirm the specificity of antibodies to vGlut2, we first performed co-localization of anterograde tracer with vGlut2 in known glutamatergic terminals in RA that originated from the pallial song nucleus HVC. Electron microscopy further confirmed that labeling was restricted to synaptic terminals containing vesicles, and in situ hybridization was used to confirm the presence of mRNA in somata. We found that a large number of vGlut2-positive cells are present throughout the zebra finch pallium, and also found evidence for vGlut2 positive synapses in three different song nuclei (nucleus HVC, Area X, and robust nucleus of the arcopallium), which were imaged and analyzed for size, and the potential identity of post-synaptic elements. Our results are consistent with the notion that avian vGlut2 has largely taken over vGlut1 functions. This could have happened through an expansion of the vGlut2-expressing neuronal populations in the avian pallium, or by turning on vGlut2 expression in pallial cells that did not express it. We believe our study contributes important facts to the long disputed evolutionary relationship between the avian pallium and the mammalian cortex.

Disclosures: M. Feil: None. **A.L. Bodor:** None. **P.V. Lovell:** None. **C.V. Mello:** None. **D.J. Perkel:** None.

Poster

895. Comparative Anatomy

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Program#/Poster#:895.06/PP17

Topic: D.19. Comparative Anatomy and Evolution

Support: FONDECYT # 1110281

Title: Retinal projections in a palaeognath bird, the chilean tinamou (Nothoprocta perdicaria)

Authors: Q. KRABICHLER[1], H. LUKSCH[1], J. MPODOZIS[2], C. MORALES[2], *G. J. MARÍN[2,3]
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Abstract: The Tinamidae is a family comprising 47 species of birds, which forms a monophyletic group with the flightless 'Ratites'. Together they establish the avian line of the Palaeognathae. They are generally considered to be a very basal group, which segregated from the Neognathae (containing all other extant birds) very early in the evolution of birds. It is thus of interest to compare the basic neuronal circuitry of these groups to gather insights into evolutionary trends in the conservation and variations of the avian visual system.
We investigated the retinal ganglion cell layer and the retinofugal pathways in the palaeognath Chilean Tinamou, *Nothoprocta perdicaria,* by means of retinal wholemounts and injection of cholera toxin B subunit into the vitreous body of the eye of deeply anesthetised animals. As is the general pattern in birds, retinal projections were confined to the contralateral side and led to known retinorecipient regions, including the layers 2 – 7 of the Optic Tectum, the nuclei of the Lateral Geniculate Complex (nucleus geniculatus lateralis pars ventralis (GLv) and the substructures of the nucleus geniculatus lateralis pars dorsalis (GLd)), the intergeniculate leaflet (IGL), a prominent lateral anterior nucleus (LA), the pretectal nuclei (tectal grey (GT) and nucleus lentiformis mesencephali (LM)) and the nucleus of the basal optic root (nBOR). In addition, retrogradely labelled neurons were found in the nucleus isthmo-opticus (ION). The Optic Tectum showed some conspicuous differences compared to typical avian neognath model organisms such as the chicken (*Gallus gallus*) and the pigeon (*Columba livia*). Most notably, the retinorecipient layers 3 and 5 were thicker throughout their entire extent, whereas layer 4 was

substantially thinner.
Our results indicate that the retinal projections in palaeognath birds reflect an evolutionarily conserved avian pattern and show only minor differences on a macroscopic level. Future studies will determine whether this also applies to the microcircuitry in identified circuits such as the tecto-isthmic loops and the tecto-rotundal pathways.

Disclosures: Q. Krabichler: None. **H. Luksch:** None. **J. Mpodozis:** None. **C. Morales:** None. **G.J. Marín:** None.

Poster

895. Comparative Anatomy

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Program#/Poster#:895.07/PP18

Topic: D.19. Comparative Anatomy and Evolution

Title: Scaling of olfactory system components across mammalian species

Authors: S. SRINIVASAN, *C. F. STEVENS
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Abstract: The architecture of the olfactory system, the chemo-sensory system responsible for processing and discriminating odors, bears striking resemblance across species. This similarity naturally raises the possibility that a single theory might explain olfaction across species. As a step towards answering this question, we provide a quantitative description of two important components of the olfactory system: the glomerular layer that carries a stereotypic representation of odors, and serves as an input to the piriform cortex where much of the odor processing is thought to occur. Such a quantitative description would serve multiple purposes. First, it provides a constraint that any theory would have to satisfy. Second, it would help answer the question of whether increased odor perception arises from a greater number/density of olfactory glomeruli and/or piriform cortex neurons. We examined two species of rodents and carnivores. In these species, we estimate that each neuron in layer II averages around one synapse with each glomerulus from the bulb. This result, though surprising, becomes more clear when we consider other trends associated with brain size changes. As brain sizes increase, the number of synapses from the bulb to the piriform cortex also increases. This increase, however, is matched by a decrease in neuronal density as well as an increase in glomeruli number. Thus, the number of synapses per neuron from a glomerulus roughly stays the same.

Disclosures: S. Srinivasan: None. **C.F. Stevens:** None.

Poster

895. Comparative Anatomy

Location: Hall F-J

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Program#/Poster#:895.08/PP19

Topic: D.19. Comparative Anatomy and Evolution

Title: Biotin detection methods revealed novel neuronal subcellular structure in the nervous system of different vertebrate species

Authors: *A. L. BODOR[1], D. J. PERKEL[2]
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Abstract: Biotin is a cofactor for several mitochondrial and other enzymes that contribute to brain energy metabolism, myelination and neurotransmitter synthesis. In the case of biotin or biotinidase deficiency, biotin holocarboxylase synthase cannot attach biotin to the enzymes that require it. Consequently mitochondrial and other carboxylase function is seriously diminished. In humans, biotin or biotinidase deficiency causes sensory, motor and cognitive symptoms and other untreatable changes as deafness, blindness and developmental delay.
The importance of biotin in the brain suggests its storage and transport in the central nervous system. Here we report a novel biotin-containing subcellular structure located in myelinated axons and dendrites of neurons of different vertebrate species. We confirmed its biotin content with two different methods: (strept)avidin-based labeling using three different chromogens (fluorophores, gold and DAB) and also an anti-biotin antibody. We named this structure a biotin body (BB). BBs are found mostly in the lower brain regions of adult zebra finch, primarily thalamus, midbrain, brainstem and cerebellum, and also in the grey matter of the spinal cord. BBs are ~200-500 nm thick, with median length 5.1µm. Electron microscopic examination revealed that BBs are formed by parallel ~5-nm thick filaments. BBs do not possess any visible membrane component. In most cases BBs contact several mitochondria. In some cases, biotin signal appears in the internal cisternae of the mitochondria juxtaposed to BBs but not in the cisternae of other mitochondria. This suggests that a biotin containing compound could be exchanged between mitochondria and BBs.
BBs in zebra finch brain are also labeled by an antibody generated against mammalian p63 protein; we have not, however, been able to confirm the identity of the antigen using western blot data.
We propose that the BB, a novel subcellular structure in neurons, contributes to the storage and distribution of biotin or biotin containing enzymes and/or represents an enzymatic hotspot

supporting mitochondrial function or localization in distant neuronal processes. Dysregulation of BBs could thus cause serious deficits in brain function.

Disclosures: A.L. Bodor: None. **D.J. Perkel:** None.

Poster

895. Comparative Anatomy

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Topic: D.19. Comparative Anatomy and Evolution

Support: NIH grant NS-73134

NIH grant HD-38051

Title: Asymmetry of insular grey matter in the chimpanzee brain and autonomic behavioral correlates.

Authors: *C. CANTALUPO[1], S. SEASE[1], W. D. HOPKINS[2]
[1]Psycholgy, Clemson Univ., Clemson, SC; [2]Georgia State Univ., Atlanta, GA

Abstract: The insular cortex in humans is known to be implicated in a very wide range of functions, from primary sensory and motor control functions, autonomic functions and emotional processing, to higher-order functions including language, attention, memory, decision making and possibly self-awareness. Much less is known about the possible functional involvement of different insular region in the great ape brain. To address this lack of data we computed left-right asymmetry measures of the anterior and posterior insular grey matter from MRI scans of a sample of 70 chimpanzee brains. Overall, we found evidence that asymmetry of the posterior insular region predicts which side of the body the chimpanzees tend to scratch when experiencing heightened arousal. In particular, we found that chimpanzees showing a rightward asymmetry of the posterior insula tended to scratch the left side of their own bodies in conditions of heightened arousal. To the best of our knowledge this is the first evidence of autonomic behavioral correlates of insular asymmetry in the chimpanzee, and the implications for theories on the evolution of insular function are discussed.

Disclosures: C. Cantalupo: None. **S. Sease:** None. **W.D. Hopkins:** Research Grant; NIH.

Poster

895. Comparative Anatomy

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Program#/Poster#:895.10/QQ1

Topic: D.19. Comparative Anatomy and Evolution

Support: LabEx CORTEX

ANR-11-BSV4-0051

Grant from McDonnell Center for Systems Neuroscience

Title: Influence of brain size on cortico-cortical connectivity: Comparison of the interareal network properties in mouse and monkey

Authors: *A. GAMANUT[1,2], N. MARKOV[3], M.-M. ERCSEY-RAVASZ[4], K. KNOBLAUCH[1], Z. TOROCZKAI[4], A. BURKHALTER[5], H. KENNEDY[1]
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Abstract: Ongoing studies show that individual cortical areas in mouse and monkey are connected to specific sets of areas. Each connection has a specific weight and the set of connected areas and their weight values constitute the connectivity profile of the area. In both species the weights span a wide range of values (orders of magnitude, mouse: 2-3; monkey: 5-6 orders) and have a log normal distribution. The density of interareal graph in the monkey is 70% and 90% in the small mouse brain. Here we are investigating if the differences in brain size in these two species correlate with other specificities in the interareal network. Injection of retrograde tracers in a target area leads to the density of labeled neurons in source areas that fall off with distance according to an exponential distance rule that is correlated with the size of the brain in both mouse and monkey. Random networks that are constrained according to this distance rule exhibit 3-node motif distributions that are similar to those observed in the cortex, suggesting that weight-distance relationships determine the specific architectural features of the cortex.

Disclosures: A. Gamanut: None. **N. Markov:** None. **M. Ercsey-Ravasz:** None. **K. Knoblauch:** None. **Z. Toroczkai:** None. **A. Burkhalter:** None. **H. Kennedy:** None.

Poster

895. Comparative Anatomy

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Topic: D.19. Comparative Anatomy and Evolution

Support: Botterell Foundation at Queen's University

Ontario Graduate Scholarship

Banting and Best Scholarship from CIHR

Title: A comparative examination of biomarkers for olfactory ensheathing cells in hamsters, rabbits, pigs, non-human primates, and mice

Authors: *K. S. RAWJI[1], S. X. ZHANG[2], Y.-Y. TSAI[2], L. J. SMITHSON[1], M. D. KAWAJA[1]
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Abstract: Olfactory ensheathing cells (OECs) are the chief glial population of the mammalian olfactory nervous system, residing in the olfactory mucosa and at the surface of the olfactory bulb. We investigated the neurochemical features of OECs in a variety of mammalian species (including hamsters, rabbits, non-human primates, mice, and pigs) using three biomarkers, glial fibrillary acidic protein (GFAP), S100β, and α-smooth muscle actin (αSMA). Mucosal and bulbar OECs from all five of the aforementioned mammalian species express S100β. Mucosal OECs, but not bulbar OECs, also express GFAP in adult hamsters and non-human primates; adult mice, by comparison, have only a sparse population of OECs expressing GFAP. Both mucosal and bulbar OECs of non-human primates express αSMA; only bulbar OECs of hamsters and only mucosal OECs of rabbits express αSMA as well. Though αSMA immunostaining was not detected in the OECs of adult mice, we postulated that this structural protein may be expressed at earlier developmental periods. In adult mutant mice lacking αSMA expression, mucosal OECs displayed fewer cytoplasmic processes extending among the hundreds of olfactory axons in the olfactory nerve fascicles, and many bulbar OECs had perturbed nuclear morphology. In sum, these findings (and our previous findings in rats, guinea pigs, and cats) highlight the efficacy of S100β and αSMA as biomarkers for mammalian OECs *in vivo*. In addition, this study emphasizes the importance of empirically testing these and other

biomarkers when assessing mucosal and bulbar OECs from various mammalian species.

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Poster

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South African National Research Foundation (P.R.M.; FA2005033100004)

Title: Neuromorphology of giraffe (Giraffa camelopardalis) visual and motor cortices

Authors: T. HARLAND[1], D. KENNEDY[1], N. JOHNSON[1], B. WICINSKI[2], P. R. HOF[2], C. C. SHERWOOD[3], P. R. MANGER[4], M. SCHALL[1], *B. G. JACOBS[1]
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Abstract: The morphology of neurons in the giraffe (Giraffa camelopardalis) neocortex remains largely unexplored. The present study investigates giraffe primary visual and motor cortices removed from the perfusion-fixed brains of three sub-adults and stained with a modified rapid Golgi technique. Neurons (N = 269) were quantified on a Neurolucida computer-assisted microscopy system (MBF Bioscience), which allowed examination of both dendritic and spine systems. Qualitatively, giraffe neocortex contained an array of complex spiny neurons that reflected both “typical” pyramidal neuron morphology (i.e., pyramidal neurons, n =143; magnopyramidal neurons, n = 10; Betz-like neurons, n = 6; extraverted neurons, n=35) and “atypical” spiny neurons in terms of morphology and/or orientation (i.e., horizontal pyramidal neurons, n = 17; and crab-like neurons, n = 4). In general, the neocortex appeared to exhibit a vertical columnar organization of apical dendrites. Although there was no significant difference in dendritic complexity for pyramidal neurons between primary visual (n = 78) and motor cortices (n = 65), there was a significant difference in dendritic spine density (motor cortex > visual cortex). Giraffe aspiny neurons appeared to be morphologically consistent with those of other eutherian mammals. For cross-species comparisons, giraffe pyramidal neurons were

compared to those quantified with the same methodology in elephants and cetaceans (e.g., bottlenose dolphin, minke whale, humpback whale). Across species, elephant exhibited widely bifurcating apical dendrites compared to cetaceans and giraffes. Quantitative dendritic measures (e.g., total dendritic length) showed that the elephant and humpback whale had more extensive dendrites than giraffes, whereas minke whale and bottlenose dolphin had smaller dendritic arbors. Spine measures (i.e., dendritic spine number) were highest in the giraffe, perhaps due to high quality perfusion fixation. The morphology of neurons in giraffe neocortex thus seems generally consistent with what is known about neuromorphology in cetartiodactyls.

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Title: Postnatal developmental changes in excitatory and inhibitory postsynaptic protein content and the ratio of excitatory-to-inhibitory synapses in chimpanzee cerebral cortex

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Abstract: The present study continues our attempt to understand the ultrastructural and neurochemical alterations in chimpanzee cerebral cortex that accompany postnatal development.

Here, we applied morphological analysis to describe postnatal changes in the number of excitatory and inhibitory synapses across several areas of the chimpanzee cerebral cortex [the primary somatosensory (area 3b), primary motor (area 4), prefrontal (area 10), and prestriate visual cortex (area 18)] captured with transmission electron microscopy and quantitative image analysis. Using electron microscopy we found that there is an increase in the density of excitatory and inhibitory synapses in prefrontal cortex of chimpanzees between birth to 2 years of age, especially in number of asymmetric synapses. We also quantified the developmental patterns of expression for two key scaffolding proteins, the postsynaptic density (PSD) proteins PSD-95 and gephyrin. These proteins are critical for dictating the ratio of excitatory-to-inhibitory synaptic contacts. Significant changes in the
expression level of scaffolding protein PSD-95, related to excitatory,
glutamatergic transmission, were observed. The expression of PSD-95 was significantly increased in area 10 of chimpanzee during development (by 310% in the 12-24 month age group compared to the 0-12 month age group). Then, from 2 years after birth, a plateau is reached, and a steady decline in the expression level of PSD-95 occurs with age. The expression level of gypherin, associated with PSD of symmetric synapses did not appear to have significant change over the course of postnatal chimpanzee development. The differential expression of the PSD proteins at excitatory and inhibitory synapses likely accounts, at least in part, for the differences in the ultrastructural appearance of the postsynaptic densities in the two types of synapses. During chimpanzee postnatal development, marked changes in PSD-95 expression occur, presumably
reflecting changes in functional properties of excitatory networks.

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The Leakey Foundation

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Title: Exceptionally rapid brain growth in wild Virunga mountain gorillas (Gorilla beringei beringei)

Authors: *S. K. BARKS[1], S. C. MCFARLIN[1], M. W. TOCHERI[2], J. S. MASSEY[3], A. B. ERIKSEN[4], K. A. FAWCETT[5], P. R. HOF[6], T. G. BROMAGE[7], A. MUDAKIKWA[8], M. R. CRANFIELD[9], C. C. SHERWOOD[1]
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Abstract: Understanding the life history correlates of ontogenetic differences in the brain growth of humans and apes requires information from a diversity of hominoid species. At present, however, data on how brain size changes over the course of development are only available from chimpanzees and humans. This study describes brain growth derived from necropsy reports and measurements of endocranial volume from wild Virunga mountain gorillas. The youngest individual in our sample was an 11-day-old neonatal male with a brain mass of 208 g, which represents 42% of the adult male average. This suggests that mountain gorillas have relatively mature brains at the time of birth. Furthermore, the rate of postnatal brain growth in mountain gorillas is extraordinarily rapid, achieving maximum adult-like brain mass by two years of age. By contrast, adult brain mass in chimpanzees is not attained until age 4-5 years of age. In the skeletal sample of mountain gorillas we found that endocranial volume reaches adult size by the time of the emergence of the first permanent molars. These findings demonstrate that brain growth is accelerated in mountain gorillas in a manner that matches other life history characteristics of this species.

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Poster

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McDonnell Foundation

Title: Humans and great apes share layer- and area-specific increases in cortical neuropeptide Y innervation

Authors: *M. RAGHANTI[1], J. SUDDUTH[1], T. BOHUSH[1], J. M. ERWIN[2], P. R. HOF[3,4], C. C. SHERWOOD[2]
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Abstract: Neuropeptide Y (NPY) is a chemical messenger that plays a role in a variety of basic physiological functions, but has also been implicated in higher cognitive functions such as learning and memory. The density of cortical NPY-expressing axons is decreased in a variety of neuropathological processes, including depression, bipolar disorder, schizophrenia, and Alzheimer's disease, potentially contributing to the associated deficits in cognitive functions. The goal of the present analysis was to determine if there was an evolutionary shift in cortical NPY innervation in primate phylogeny. The study sample included humans, chimpanzees, gorillas, baboons, moor macaques, pigtailed macaques, capuchins, and squirrel monkeys. Stereologic methods were used to obtain the ratio of NPY axon length density to neuron density (ALv/Nv) in layers III and V/VI of cortical areas 10, 24, 44, and 22 (Tpt) and varicosity density to neuron density (Vv/Nv) in layers III and V/VI of areas 10 and 24. NPY Vv/Nv was significantly correlated with ALv/Nv in each layer and area (Pearson's r, all p's < 0.01). Among-species differences were not detected within the taxonomic groups of New World monkeys, Old World monkeys, and hominoids for any of the measures. Thus, species were collapsed into these categories for further analyses. The results of the mixed-model ANOVAs of ALv/Nv revealed significant main effects of taxonomic group and layer for each cortical area. The interaction of layer by taxonomic group was significant for all areas except 22. Post hoc analyses demonstrated that humans and great apes (hominoids) had significantly higher ALv/Nv in layers III and V/VI of area 10 and 44 relative to New and Old World monkeys. In area 24, hominoids had higher Alv/Nv in layer III. In layers V/VI, hominoids were significantly different from Old World, but not New World monkeys. In area 22, hominoids were different from New World monkeys in layer III and Old World monkeys in layers V/VI. No significant differences were detected between Old World and New World monkeys in any of the areas or layers examined here. These

results indicate that humans and great apes share a significant increase in cortical NPY innervation that is area- and layer-specific. This increase in cortical NPY innervation may contribute to the higher behavioral and cognitive flexibility observed in hominoids relative to other primates.

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Title: The molecular evolution of NMDA receptors in the human lineage

Authors: *J. L. BAKER[1,3], T. DUKA[4], T. MAYNARD[4,5], B. WOOD[2,6], P. R. HOF[7], J. J. ELY[8], W. B. BAZE[9], S. J. SCHAPIRO[9], M. RAGHANTI[10], A. LEWANDOWSKI[11], D. E. WILDMAN[12,13], C. C. SHERWOOD[4,3]
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Abstract: The evolution in humans of an extended juvenile period, altered patterns of brain glucose utilization, neuroplasticity, and increased longevity are associated with the emergence of encephalization, but how these coevolving features are related to one another remains unclear. In the present work we focused on *GRIN3A*, a gene implicated as playing a central role both in

memory and synaptic plasticity. *GRIN3A* encodes a subunit of the NMDA receptor (NMDAR) family of glutamate-regulated ion channels. By modulating NMDAR activity *GRIN3A* may be involved in the development of dendritic spines and the evolution of human brain plasticity. *GRIN3A* is enriched in the postsynaptic plasma membrane and densities in the cerebral cortex and subcortical regions. Given the importance of postsynaptic proteins, especially the NMDA receptors, in synaptic plasticity, it is of great interest to understand the molecular evolution of the postsynaptic complex. To better understand the evolution of synaptic plasticity we performed three experiments. First, to study the molecular evolution of the NMDA receptors we examined multiple alignments of coding sequences and promoter regions to identify phylogenetically conserved and derived *cis*-regulatory elements in human, chimpanzee, gorilla, orangutan, macaque, baboon, marmoset, tarsier, and mouse lemur. We found that much of the *GRIN3A* promoter is conserved among primates, and conserved *cis*-elements include CREB- and PAX-binding sites. Moreover, conserved POU elements suggest a potential role for *GRIN3A* in dopaminergic neurons. The conservation of *cis*-elements in this gene suggests important functional constraints on the regulation of *GRIN3A* throughout primate evolution. Second, using real-time quantitative PCR we examined the expression of *GRIN3A* in different species and found a decrease in *GRIN3A* expression, which may be inversely correlated with brain size across the primate lineage. Our third experiment examined protein expression using quantitative Western blot analysis in a chimpanzee developmental series. Preliminary data suggest that chimpanzees downregulate GluN3A (NR3A) in a slightly different pattern from that seen humans. By investigating the relationship between the molecular evolution of *GRIN3A* and gene and protein expression, our results contribute to the understanding of glutamate receptor biology across the primate lineage.

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Poster

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Title: Organization of the chimpanzee temporal lobe: Localization of auditory core and the area MT+ complex using diffusion tensor imaging.

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Abstract: Chimpanzee are the animals most closely related to humans, and therefore understanding how the two species resemble, and differ from, one another is crucial for understanding the evolutionary specializations of the brain. Chimpanzee neuroanatomy is very poorly known, however - in part because, like humans, they cannot be studied with invasive techniques. The development of noninvasive neuroimaging, and in particular diffusion-tensor imaging (DTI), which can track fiber pathways, has made direct studies of chimpanzees possible and, consequently, more detailed comparisons of humans, chimpanzees, and other nonhuman primates. The acuity of these comparisons depends on how well one can track cortical connections and localize cortical areas in chimpanzees with DTI. To evaluate the potential of DTI, we performed probabilistic tractography on in vivo chimpanzee DTI scans to localize two major cortical landmarks: 1) the auditory core, a primary sensory area with major inputs from the thalamus; and 2) the MT+ complex, a multimodal association area. Based on recent comparative myeloarchitectonic studies (Glasser et al., 2011, Soc. Neurosci. Abstr. 290.05; Hackett et al., 2001, J. Comp. Neurol. 441:197-222), we hypothesized that 1) the auditory core of the chimpanzee would be localized in Heschl's gyrus; and 2) the MT+ complex would be localized in the posterior part of the superior temporal sulcus (STS). Methods. We drew region-of-interest (ROI) masks that included the hypothesized locations of the two landmark regions on MT+ on T1-weighted images with 0.8-mm isotropic resolution. ROI masks were also drawn on known inputs to the auditory core (medial geniculate nucleus) and area MT (intraparietal sulcus (IPS), primary visual cortex (V1), and area V2. Probabilistic tractography software within FSL (www.fmrib.ox.ac.uk/fsl) was used to track cortico-cortical and subcortical connections in diffusion-weighted scans acquired with 1.8 mm isotropic resolution with 60 gradient directions and b values of 0, 1000s/mm2. Results. First, MGN projections to cortex were localized in Heschl's gyrus, indicative of the auditory core. Second, tracts from IPS and V1/V2 converged in a small gyrus buried in the posterior STS, indicative of a macaque-like position of area MT+ rather than in a human-like position, corroborating MT+ localization by Glasser and co-workers (2011). We conclude that DTI and probablistic tractography can contribute to the construction of a detailed map of chimpanzee cortical organization.

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Poster

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Title: Density of total cells and neurons across the cortical sheet of Old World monkeys and a chimpanzee (Pan troglodytes)

Authors: *C. E. COLLINS[1], N. A. YOUNG[1], J. H. KAAS[1], F. AWORUNSE[1], D. K. FLAHERTY[2]
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Abstract: Previously published data from Old World baboons and macaque monkeys, a New World owl monkey and prosimian galagos illustrated a typical pattern of cell and neuron density distribution in the cerebral cortex (Collins et al., 2010b) with primary sensory areas having the highest neuron densities and of those, primary visual cortex having the highest density of neurons and total cells compared to all other cortical areas. A more than 5-fold variation in neuron density was demonstrated across the cortical expanse and significant variation was demonstrated within functional areas. In the present study, we have flattened and dissected the entire cortical sheet of a macaque monkey, a baboon and a chimpanzee and dissected each first into identifiable cortical areas, then into tiny cubes. Each tissue piece is assigned a set of coordinates on the cortical area map, weighed then homogenized into a suspension of cellular nuclei. Small aliquots from each suspension are stained for a neuron-specific marker to determine the percentage of neurons in the sample (Collins et al., 2010a). Total cell number is determined from a different aliquot from each suspension stained with the DNA stain, DAPI, and evaluated for total cells using flow cytometry (Young et al., 2012). The pattern of distribution of cell and neuron density previously described for the cortex of prosimian, New World and Old World primates appears to be conserved in the chimpanzee cortex, though the increased number of cell density transitions in the chimpanzee cortex is suggestive of an expanded number of cortical areas.

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Poster

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Topic: D.19. Comparative Anatomy and Evolution

Support: G. Harold and Leila Y. Mathers Foundation

Title: Cell and neuron numbers in cortical areas of New World and Prosimian primates

Authors: *N. A. YOUNG, C. E. COLLINS, F. AWORUNSE, L. DOLL, J. KREBS, J. H. KAAS
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Abstract: Previous studies have reported total numbers of neuronal and non-neuronal cells for the entire cerebral cortex as a whole in non-human primates. This study was undertaken to determine if there is a predictable and non-uniform pattern of distribution in the cortical sheet of New World and Prosimian primate species. Hemispheres from squirrel monkeys (Saimiri sciureus), an owl monkey (Aotus trivirgatus) and Prosimian galagos (Otolemur garnettii) were separated from subcortical structures and the pia was removed. The sulci were opened to manually flatten the cortex. Cortical areas were accurately identified by backlighting flattened cortex using a light box to visualize densely myelinated areas. Flattened hemispheres were dissected into 5 x 5 mm pieces. Each piece was weighed and assigned to a cortical area or region. Tissue pieces were processed using the flow fractionator method to rapidly and accurately quantify the neuronal populations. Preliminary results indicate that cortical areas in New World primates differ in neuron densities and proportions of neurons. Primary visual cortex (V1) and adjoining extrastriate visual areas are the most neuron dense, followed by non-visual primary sensory areas (S1 and A1), while motor (M1) and non-primary sensory areas are the least neuron dense. These differences were more pronounced in diurnal squirrel monkeys than in nocturnal owl monkeys and galagos.

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Topic: D.19. Comparative Anatomy and Evolution

Title: NADPH-diaphorase-positive neurons in the human claustrum

Authors: L. EDELSTEIN[1], D. HINOVA-PALOVA[2], *F. J. DENARO[3], B. LANDZHOV[2], L. MALINOVA[2], M. MINKOV[2], A. PALOFF[2], W. OVTSCHAROFF[2]
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Abstract: Introduction: NADPH-diaphorase (NADPHd) is a marker for neurons producing nitric oxide, somatostatin and neuropeptide Y, whose absence has been implicated as a causative agent in the excitotoxic damage prevalent in both Alzheimer's disease and Huntington's disease.
Objective: The objective of this study was to assess the distribution and morphological characteristics of NADPHd-positive cells in the human claustrum.
Sample/Methods: Samples of claustrum were obtained from the brains of two females (39 and 48 years of age) and one male (63 years of age) at autopsy. The brains did not show any overt signs of pathology or trauma. The delay from death until fixation was 6-12 hours. NADPHd histochemistry was employed, along with light and electron-microscopic analysis.
Results: NADPHd-positive neurons were irregularly distributed, often in clusters of 10 to 15. Very few positive neurons were observed adjacent to or in close proximity of the extreme and external capsules, or in the claustrum's most dorsal or caudal aspect. Reaction product diffusely filled the cytoplasm of positive neurons, while their nuclei remained stain-free. In many instances, cell staining was so intense that they resembled Golgi-impregnated neurons. These neurons were present throughout the claustrum, primarily in three sizes.
[1] Large neurons were 25-35µm in diameter, displaying multipolar, pyramidal, fusiform, elliptical, irregular, and oval soma. Each had 2-6 primary dendrites which branched into secondary and tertiary dendrites. The tertiary dendrites had an undulating course, radiating 700-900 µm from the soma. Their dendrites spread in all directions, some running parallel to the fibres of the extreme and external capsules. As a rule, they were aspiny, with the axon emanating from the perikaryon with a distinct hillock.
[2] Medium neurons were 19-25 µm in diameter, less common than the large type, and displayed round, multipolar or irregular soma, each with 3-4 dendrites with primary dendrites bifurcated or trifurcated into diverging secondary dendrites.
[3] Small neurons were 14-18 µm in diameter, with oval or elliptical soma, each with 3-4 dendrites. Primary dendrites were noted as being thin and varicose.

Conclusions: Our results provide additional insight into the distribution of NADPHd within the human claustrum. Given the critical importance of NADPHd in the context of neural transmission and its relationship to the etiology and sequelae of both Alzheimer's and Huntington's disease, it behooves us to investigate its presence in the claustrum, which has also been implicated in the development of these life-threatening disorders.

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Title: Neuronal nitric oxide synthase immunoreactivity in the human claustrum: A light- and electron-microscopic investigation

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Abstract: Introduction: Neuronal nitric oxide synthase (nNOS) is a relatively unique neurotransmitter which participates in many physiological and pathological processes, including the development of the nervous system and cell communication. Nevertheless, little is known about the nNOS-immunoreactive (nNOS-ir) neurons and fibers in the human claustrum, which has been the subject of growing interest from the standpoint of multisensory integration/binding, cross-modal processing and spike coincidence detection.
Objectives: The objectives of this study were to confirm the existence of nNOS-ir neurons and fibers in the human claustrum, employ light microscopy to describe their morphology and distribution, employ electron microscopy to describe and analyze their ultrastructure and synaptic architecture, verify whether they comprise a specific subpopulation of neurons, and establish whether they present with a specific pattern of organization.
Sample/Methods: Samples of claustrum were obtained from the brains of two females (39 and 48 years of age) and one male (63 years of age) at autopsy. The brains did not show any overt signs of pathology or trauma. The delay from death until fixation was 6-12 hours. Immunoreactivity to nNOS was assessed via the Avidin-Biotin Complex method.

Results: Light-microscopic analysis confirmed the presence of nNOS-ir neurons and fibers in all areas of the human claustrum, with neurons commonly found within a network of immunostained fibers. Cell bodies varied in shape and size, and were divided into three groups we've categorized as: small (35% of the sampled population, ‹19μm, but typically 13-17μm in diameter), medium (45%, 19-25μm in diameter), and large (20%, ›25μm in diameter). The density of immunostaining varied both within and between cell types, with some neurons staining more darkly or lightly than others. Electron-microscopic analysis confirmed the presence of nNOS immunoproduct within neurons, dendrites and terminal boutons, with neurons further defined by their ultrastructural features. Three types of nNOS-ir synaptic boutons were noted. Additional information will be presented.
Conclusions: There are different types of nNOS-ir neurons within the human claustrum. The large- and medium-sized cells most likely correspond with claustrocortical projection neurons, while the small-sized cells appear to be inhibitory interneurons. It is our hope that these results contribute to a better understanding of the claustrum's function in both health and disease, given its relationship with the development and sequelae of autism, schizophrenia, Alzheimer's disease, Parkinson's disease and Huntington's disease.

Disclosures: L. Edelstein: None. **D. Hinova-Palova:** None. **B. Landzhov:** None. **L. Malinova:** None. **M. Minkov:** None. **A. Paloff:** None. **W. Ovtscharoff:** None.

Poster

895. Comparative Anatomy

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:895.22/QQ13

Topic: D.19. Comparative Anatomy and Evolution

Support: NIH P01 AG026423-03

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Title: Improved cortical myelin maps in humans, chimpanzees, and macaques allow identification of putative areal homologies

Authors: *M. F. GLASSER[1,2], T. M. PREUSS[3,4,6], G. NAIR[7], J. K. RILLING[4,8,9], X. ZHANG[5], L. LI[7], D. C. VAN ESSEN[2]

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Abstract: Cortical myelin maps based on T1w/T2w MRI images have been used previously to identify cortical areas in humans (Glasser and Van Essen 2011), chimpanzees, and macaques (Glasser et al SFN 2011). Here, we mapped the ratio of T1w/T2w in 69 humans, 29 chimpanzees, and 19 rhesus macaques to their cortical surfaces and averaged across subjects with surface-based registration. We used customizations of FreeSurfer, including species-specific templates and improvements to white and pial surface positioning, to reconstruct the cortical surfaces automatically in all three species. With these better surfaces, we generated better myelin maps in all three species than we obtained previously (Glasser et al SFN 2011). For humans and macaques, these myelin maps can be directly compared to histological cortical surface parcellations (Van Essen et al 2011a, Van Essen et al 2011b). Putative areal homologies can be identified in the intra-parietal sulcus (IPS) across the three species. In the macaque, there are three bilateral patches of heavier myelination in the IPS and parieto-occipital sulcus (POS). The anterior patch is within area 5, just posterior to area 2 on the medial bank of the IPS. The middle patch is centered on the lateral bank of the IPS and includes LIPv, LOP, and possibly VIPm. The posterior patch includes areas PO and PIP. In the chimpanzee, three bilateral patches of heavier myelination are also present in the IPS, somewhat shifted compared to the macaque. The anterior patch lies in the post central sulcus, the middle patch is near the fundus of the IPS, and the posterior patch is at the IPS/POS juncture. Human parietal cortex also has three heavily myelinated patches, but they are further shifted relative to sulcal landmarks. The anterior patch is fully in the post-central sulcus, and the middle and posterior patches are on the medial bank of the IPS. Evolutionary expansion of lightly myelinated lateral parietal cortex in humans and great apes is associated with a shift in heavily myelinated IPS regions in the three species, but these three distinct heavily myelinated patches are still identifiable and represent putative homologues. Improved myelin maps also allow better cross-species comparison of medial cortex, which has a largely similar pattern of myelin content in medial frontal, cingulate, paracentral lobular, and retrosplenial regions across species. However, the lightly myelinated region between the retrosplenial cortex and primary visual cortex is expanded in chimpanzees and humans relative to macaques. T1w/T2w MRI-based myelin maps offer a way to compare cortical architecture across species, which is helpful in understanding cortical evolution.

Disclosures: M.F. Glasser: None. **T.M. Preuss:** None. **G. Nair:** None. **J.K. Rilling:** None. **X. Zhang:** None. **L. Li:** None. **D.C. Van Essen:** None.

Poster

895. Comparative Anatomy

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:895.23/QQ14

Topic: D.19. Comparative Anatomy and Evolution

Title: Comparative quantitative analysis reveals preserved structural connectivity patterns in the human and macaque brain.

Authors: *A. GOULAS[1], M. BASTIANI[2,3], G. BEZGIN[4], P. STIERS[1], A. ROEBROECK[2]
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Abstract: Decades of research on the primate brain have revealed a mosaic of distinct regions characterized by properties such as unique structural connectivity with the rest of the brain. Evidence suggests that the function of a region is constrained by its structural connections (Passingham et al., 2002). Hence, regions with similar connectivity patterns can carry out similar functions.
Since methodological and ethical issues constrain our knowledge on connectivity of the human brain, the connectivity of the macaque brain often functions as a model. However, the accuracy of the extrapolation of findings on the macaque brain to humans has been questioned since direct quantitative comparisons of the connectivity of the brain of the 2 species are scarce.
In the current study we directly compared the whole brain structural connectivity patterns of the macaque and the human brain. We used an a priori whole brain parcellation, called the Regional Map (Bezgin et al., 2012), which consists of assumed homologues in the human and the macaque brain. Information on macaque structural connectivity, as revealed through tracing studies, was extracted from the CoCoMac database (cocomac.g-node.org). Structural connectivity was estimated in 2 human subjects in vivo with diffusion imaging and probabilistic tractography. This procedure resulted in one connectivity matrix for each species describing their whole brain structural connectivity. We employed a network metric, i.e. matching index (Hilgetag et al., 2002), in order to quantify all pairwise similarities of connectivity patterns among the brain regions of each species. The similarity of the whole brain connectivity patterns was statistically significant between the species ($r=0.50$ $p<0.0001$, permutation statistics). Additional region-wise analysis revealed that the connectivity patterns of the subdivisions of the cingulate and parietal cortex were not preserved between the species ($p>0.05$, Bonferroni correction, permutation statistics). Interestingly, these subregions have been expanded during evolution (Hill et al., 2010). Moreover, cingulate subdivisions in the human brain are related to self-referential/introspective processes. Hence, the “rewiring” of these regions might be related to processes that are unique to humans.

Our approach offers rigid quantification of interspecies structural connectivity and provides new insights into connectivity changes and preservations during primate evolution.

Disclosures: A. Goulas: None. **M. Bastiani:** None. **G. Bezgin:** None. **P. Stiers:** None. **A. Roebroeck:** None.

Poster

895. Comparative Anatomy

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Program#/Poster#:895.24/QQ15

Topic: D.19. Comparative Anatomy and Evolution

Support: DFG GR 833/9-1: Architecture of fiber tracts of the cerebellum: Studies on the compartmentalization and connectivity using diffusion tensor imaging (DTI)

Title: Lateralization Effects in the Human thalamus

Authors: *V. KUMAR, W. GRODD
Dept. of Psychiatry, Psychotherapy and Psychosomatics, RWTH, Univ. Clin., Aachen, Germany

Abstract: Introduction:
The thalamus acts as the central relay station of the brain and links all information from the sensory systems and the cerebellum to the cortex. However, in vivo identification of thalamic substructures are hampered by the low intrinsic tissue contrast in conventional MRI. Consequently, no valid human in vivo data exists on the variability of thalamic substructures. However, with the advent of diffusion tensor imaging (DTI) several studies (1-3) have demonstrated the ability of an anatomical parcellation. In the present study a DTI approach was used to parcellate thalamic substructures into 21 DTI clusters (3,4). Here we report on the variability of these clusters in a group of healthy subjects.
Material & Methods:
Subjects: Data were collected from 63 right-handed volunteers (32 male and 31 female) aged 20-40 years (male: 26.3 years; female: 25.5 years) were recruited. *DTI Acquisition:* 1.5 T (Siemens) EPI sequence (TR= 6700 ms; TE= 82 ms, 52 axial slices (b-value 800; 12 directions), resolution 2 x 2 x 2.5 mm. P*rocessing*: Motion correction, realignment and normalization to MNI space including b-matrix transformation. After tensor estimation each voxel was assigned to one reference direction. Volume from resulted clusters was calculated and a group statistic performed. For evaluation only clusters, greater than 60 voxels were chosen resulting in eight remaining clusters.

Results:
All 21 clusters showed a considerable individual variability in size and location. The group results in addition revealed significant hemispheric differences in five clusters # 1, 3, 6, 7, 15 accounting for ± 26 % (males) resp. ±28 % (female) differences of the whole thalamic volume
Discussion:
The hemispheric differences most probably reflect the thalamic correspondence of the structural and functional differences between both hemispheres. However, the clusters did not exhibit a clear anatomical correspondences with thalamic anatomy provided in histological atlases (6, 7). One reason might be that the available atlases do not consider the myeloarchitecture and therefore cannot reflect differences in the directionality of the underlying fiber pathways.
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Disclosures: V. Kumar: None. **W. Grodd:** Research Grant; DFG.

Poster

895. Comparative Anatomy

Location: Hall F-J

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Program#/Poster#:895.25/QQ16

Topic: D.19. Comparative Anatomy and Evolution

Title: Non-invasive three dimensional analysis of the human insula reveals a variable gyral pattern

Authors: *A. ROSEN[1,2], K. DAVIS[3,2,4], M. HODAIE[3,2]
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Abstract: Background
The detailed anatomy of the insular cortex has been difficult to study in-vivo due to its hidden location under the operculae. Prior anatomical studies have designated the presence of 6 insular gyri divided by a central insular sulcus (CIS). Since the majority of anatomical studies of the insula have been post-mortem, variation in insular anatomy has not been explored in detail. We performed cortical surface reconstruction to non-invasively assess the insula in a large group of healthy controls. The aim of the novel application of this tool was the study the precise gyral/sulcal anatomy and normal anatomical variations.
Methods
High resolution MRI images were obtained from 60 healthy subjects (33F, 27M; 38 +/- 12.8 years old) using a 3T MR axial fast spoiled gradient (FSPGR) sequence (slice thickness=1mm, slice number=160, TR=12msec, TE=5msec, 20° flip angle, matrix=256x256, FOV=24, inversion time=300msec). The University Health Network Research Ethics Board approved the procedure. FreeSurfer software (http://surfer.nmr.mgh.harvard.edu) was used to obtain pial renderings of the cortical surface of each hemisphere. The operculae were manually removed and the resulting 120 insulae were analyzed for structural integrity. The conspicuity of each of the six main gyri and the CIS were rated either “not seen,” “poorly seen” or “well seen” in each individual image.
Results
The visualization method reliably recreated the topologic structure of the insula with a high yield, allowing us to study 94% of processed images. The CIS, anterior short (ASG), anterior long (ALG) and posterior short (PSG) gyri were consistently seen in over 80% of cases. However, the accessory (AG), middle short (MSG) and posterior long (PLG) gyri were “well seen” in less than 35% of cases. Conspicuity of the MSG was greater in males than females, and in the left hemisphere compared to the right hemisphere ($p<0.05$). Within-subject analyses demonstrated increased conspicuity of the MSG in the left hemisphere compared to the right hemisphere of the same individual ($p<0.05$).
Conclusion
These data suggest that insular structures such as the CIS, ASG, ALG and PSG are consistently visualized, while others including the AG, MSG and PLG are highly variable. The anatomical variability of these insular structures provides a context to understand the diversity of functions attributed to such as pain perception, taste and viscerosensation.

Disclosures: A. Rosen: None. **K. Davis:** None. **M. Hodaie:** None.

Poster

895. Comparative Anatomy

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Topic: D.19. Comparative Anatomy and Evolution

Support: NIH Grant RC2MH089921

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Title: A comprehensive digital atlas for adult human brain: Modern neuroanatomical parcellation based on a combined cyto- and chemo-architectonic analysis

Authors: *S.-L. DING[1], J. J. ROYALL[1], B. A. C. FACER[1], S. M. SUNKIN[1], L. NG[1], A. PLAYER[2], B. R. MCMURRAY[1], P. G. LESNAR[1], C. LAU[1], D. B. SANDMAN[1], A. R. JONES[1], B. FISCHL[2], M. HAWRYLYCZ[1], E. S. LEIN[1], J. G. HOHMANN[1]
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Massachusetts Gen. Hospital; Harvard Med. Sch., Boston, MA

Abstract: A rapidly increasing number of neuroimaging studies of human brains have resulted in renewed interest in human brain anatomy and an urgent need of more precise and comprehensive human brain atlases. The few currently available human brain atlases including the widely used Brodmann's map are mainly based on classic Nissl and myelin stains, which are not precise enough to allow detailed parcellation of some brain regions (e.g., Ding et al, J Comp Neurol 514:595-623, 2009; Ding and Van Hoesen, Hum Brain Mapp 31:1359-1379, 2010). The Allen Institute for Brain Science, as part of a larger human brain mapping project, has produced a series of digital atlases of the developing human brain. In this study we have combined classic Nissl and some modern immunocytochemical (ICC) stains, as well as high-resolution imaging and informatics techniques to generate a more precise and comprehensive digital adult human brain reference atlas. MRI and DTI imaging data were obtained from the specimen, following which entire brain was sectioned coronally. Closely adjacent sections were stained for Nissl substances (200 um interval) and SMI-32 and calcium-binding proteins such as parvalbumin (400 um interval for each ICC stain). Both cortical gyri and histologically identified areas such as Brodmann's areas were included in cortical area mapping. Using a new microscopy system, the full coronal Nissl and ICC stained sections are scanned at high resolution with images clearly showing individual neurons and their dendrites and axons. Digital images of the stained sections from the entire brain including cerebral cortex, striatum, basal forebrain, amygdala, thalamus, hypothalamus, brainstem and cerebellum are annotated, with structures outlined with polygons and color-coded for easy identification of the extent of specific structures and for great usability by end-users. This reference atlas is freely accessible as part of the BrainSpan Atlas of the

Developing Human Brain, which is available via the Allen Brain Atlas data portal at www.brain-map.org or directly at www.brainspan.org. In summary, this comprehensive digital human brain reference atlas provides an important tool for medical and neuroanatomical education, comparative brain research with other species, human brain imaging studies, and diagnostic localization of brain lesions among other uses.

Disclosures: S. Ding: None. **J.J. Royall:** None. **B.A.C. Facer:** None. **S.M. Sunkin:** None. **L. Ng:** None. **A. Player:** None. **B.R. McMurray:** None. **P.G. Lesnar:** None. **C. Lau:** None. **D.B. Sandman:** None. **A.R. Jones:** None. **B. Fischl:** None. **M. Hawrylycz:** None. **E.S. Lein:** None. **J.G. Hohmann:** None.

Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:896.01/QQ18

Topic: E.03. Behavioral Neuroendocrinology

Support: Immunostar Corp gift

Title: Distribution of the 5HT2a receptor based on receptor specific antibody binding and functional distribution by agonist induced fos

Authors: R. J. FOREMAN[1], A. MITZEY[2], *M. S. BROWNFIELD[3]
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Abstract: The purpose of this study was to map the distribution of the 5HT2A receptor by two methods: specific antibody binding and by specific agoninst-induced fos expression. The antibody was generated against a 5HT2a [22-41] multiple antigenic peptide and affinity purified against 5HT2a[22-41] covalently bound to an aminolink (Pierce Chemical Co.) gel column. The antibody was determined to be selective by Western blotting and its immunodistribution in our lab and by comparative immunodistribution with agonist-induced fos expression using the potent and selective 5HT2a agonist TCB-2, and by radioreceptor binding done by other labs. This antibody was also evaluated using 5HT2a knockout animals and other criteria by Weber and Andrade, 2010. Their conclusion was that our antibody is the only selective antibody available at this time.
Immuno- and agonist-induced fos localizations were determined by avidin-biotin immunocytochemistry against the receptor and against fos in the Sprague-Dawley rat cortex, thalamus, hypothalamus, caudal brainstem and spinal cord. The extensive distribution in each

study was scored by cell and fiber density and tabulated. These parallel studies showed a close correlation between immunoreactive 5HT2a cell bodies and TCB-2 induced fos expression.

Disclosures: R.J. Foreman: None. **A. Mitzey:** None. **M.S. Brownfield:** Employment; University of Wisconsin. Other Research Support; Immunostar Corporation. Ownership Interest; None. Consultant/Advisory Board; Immunostar Corp.

Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:896.02/QQ19

Topic: E.03. Behavioral Neuroendocrinology

Support: NHMRC (Australia)

Title: Neuroanatomical relationships between raphe nuclei 5-HT and relaxin-3 neurons, and promotion of arousal and social interaction via RXFP3 activation in mice

Authors: *C. M. SMITH[1], B. CHUA[1], A. BLASIAK[2], C. ZHANG[1], A. YANG[1], A. W. WALKER[1], D. E. GANELLA[1], S. LAYFIELD[1], F. SHABANPOOR[1], J. D. WADE[1], R. A. D. BATHGATE[1], A. L. GUNDLACH[1]
[1]Neuropeptides, Florey Neurosci. Inst., Melbourne, Australia; [2]Neurophysiol. and Chronobiology, Jagiellonian Univ., Krakow, Poland

Abstract: Relaxin-3 is a highly conserved neuropeptide expressed by small populations of brainstem/midbrain neurons, and which acts via its cognate G-protein coupled receptor, RXFP3. Relaxin-3-positive neurons project to RXFP3-rich regions throughout forebrain, including limbic, hypothalamic and septohippocampal circuits that control modalities such as arousal, emotion/mood, and stress responses; and the ability of relaxin-3/RXFP3 to modulate these behaviours has been demonstrated in a range of in vivo functional studies (see [1]). Here, we present findings from three experimental studies which explore the anatomy and function of relaxin-3/RXFP3 in adult male mice. Study 1: The neuroanatomical distribution of relaxin-3 immunoreactive soma and proximal processes was mapped relative to a range of neuronal markers revealing that relaxin-3-positive cell bodies/fibres in the nucleus incertus and paramedian raphe closely 'flank' more medial 5-HT neurons in the inferior dorsal raphe and median raphe, respectively. Study 2: The ability of RXFP3 activation to modulate social behaviour was tested. C57BL/6J mice were acutely injected with a selective RXFP3 agonist (RXFP3-A2, 1.0 nmol, icv) and subjected to a 3-chamber social preference test, which revealed that agonist-treated mice spent more than twice as much time interacting with novel mice than

vehicle controls (450 vs. 210 s in a 1200 s trial, $p < 0.05$, $n = 7$-10). Study 3: The effects of chronic activation of RXFP3 were assessed using a lentiviral construct that drives secretion of an RXFP3 agonist and accumulation of GFP in transduced cells. Icv infusion of this lentivirus resulted in GFP expression within cells of the choroid plexus and ventricle wall for up to 6 weeks, and mice displayed reduced habituation (i.e. prolonged high levels of locomotor activity, $p < 0.05$, $n = 12$-18) during a 30 min exposure to a novel environment 8-9 days post surgery compared to mice infused with GFP-only control lentivirus. Furthermore, local infusion into the hypothalamic paraventricular nucleus (PVN) strongly transduced cells within this region, prompting studies currently underway to investigate subsequent effects on HPA-axis and physiological stress responses. In conclusion, these studies further define the anatomy of the relaxin-3 system and demonstrate an ability of RXFP3 activation to modulate arousal and social behaviours, highlighting the potential of this signalling system as a target for therapeutic exploitation. Supported by NHMRC (Australia).
1. Smith CM et al. (2011) J Chem Neuroanat 42, 262-275

Disclosures: C.M. Smith: None. **B. Chua:** None. **A. Blasiak:** None. **C. Zhang:** None. **A. Yang:** None. **A.W. Walker:** None. **D.E. Ganella:** None. **S. Layfield:** None. **F. Shabanpoor:** None. **J.D. Wade:** None. **R.A.D. Bathgate:** None. **A.L. Gundlach:** None.

Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:896.03/QQ20

Topic: E.03. Behavioral Neuroendocrinology

Support: RO1 NS049166

Title: Local infusion of NMDA antagonists into prefrontal cortex rescues spatial working memory deficits in gonadectomized adult male rats

Authors: *M. N. LOCKLEAR, M. F. KRITZER
Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: Gonadectomy (GDX) in adult male rats significantly increases extracellular dopamine (DA) levels in the prefrontal cortex (PFC). Recent studies show that this is due in part to an exaggerated NMDA-mediated drive on the PFC pyramidal cells that project to the ventral midbrain and control firing rates of mesoprefrontal DA neurons (Aubele & Kritzer, Cerebral Cortex, 2011). The data presented here suggest that this same intracortical glutamatergic dysregulation also produces the striking deficits in DA-dependent PFC behaviors, e.g., spatial

working memory, behavioral flexibility, that are also seen in GDX rats. Specifically, we found that GDX rats' impaired performance in Barnes maze testing was rescued by bilateral intraPFC infusion of the selective NMDA antagonist APV (5µg, 15 min before the first trial). The Barnes maze is an elevated circular platform 4 ft across with 12 evenly spaced holes along its outer edge; one of the holes leads to a recessed chamber. Spatial working memory is measured in this non-rewarded task in the time it takes rats to find the secluded goal and in the number of secondary errors- repeat visits to non-goal holes, that animals make in a series of four 3 min trials (15 min inter-trial interval). We previously showed that trial-by-trial, GDX rats take significantly longer than controls (CTRL) to locate the goal and make more secondary errors and take more circuitous routes in doing so. Here we reasoned that by blocking NMDA receptors in the PFC we would lower the abnormally high PFC DA levels of GDX rats and improve their performance. This would also be expected to elevate PFC DA levels in the CTRL rats and impair their goal-finding ability. Both of these predictions were supported in our findings. For example, both GDX rats infused with APV and CTRL rats infused with saline demonstrated steep overlapping learning curves; across the four trials, latency to find goal dropped precipitously and secondary errors were few to none by the second or third trial. In comparison, the performance of CTRL rats infused with APV and GDX rats infused with saline was notably worse; these rats took significantly longer to find the goal and continued to make secondary errors on all four trials. Assessments of path and pathlength also demonstrated inefficiencies in their spatial strategies relative to CTRL and APV-treated GDX rats. These findings suggest new roles and routes whereby circulating gonadal hormones impact PFC function. These may serve as novel targets for alleviating the cognitive deficits in schizophrenia where sex differences in incidence and severity have long suggested hormone involvement (e.g, Leung and Chue et al, 2000, Acta. Psychiatr. Scand).

Disclosures: M.N. Locklear: None. **M.F. Kritzer:** None.

Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

Location: Hall F-J

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Program#/Poster#:896.04/RR1

Topic: E.03. Behavioral Neuroendocrinology

Support: FAPESP 2010/14469-2 and 2010/52068-0

Title: Endocrine and functional aspects of melanin-concentrating hormone (MCH) expression during lactation

Authors: *R. D. ALVISI[1], J. C. BITTENCOURT[1], E. ZIMBERKNOPF[2], L. F. FELICIO[1]
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Abstract: Melanin-concentrating hormone (MCH) is a neuropeptide widely distributed in the brain of mammals, synthesized by lateral hypothalamic area (LHA) and incerto-hypothalamic area (IHy). MCH has been largely implicated in the control of food intake, body weight and energy homeostasis. Moreover lactation is an important physiological model to study hypothalamic integration to peripheral sensory signals, such as suckling stimulus and also those related to energy balance. Concentrations of mRNA for MCH increase during lactation in the medial preoptic area (MPOA) and hypothalamic anterior paraventricular nucleus, especially around the 19th day when this hormone reveals its highest peak of expression, and decrease after weaning. The physiological significance of this phenomenon is unclear. In this sense, the aim of the present study is to contribute to the investigation of sensory and endocrine factors influencing in the expression of MCH and its relationship to neuroendocrine and behavioral changes involving the end of lactation, weaning and perpetuation reproductive cycle. Wistar female rats (n= 8) were submitted to a chronic jugular vein catheterization on 13th day of lactation. Blood samples were obtained in 15, 16, 17, 18, 19, 20 and 21 days before and after suckling stimulus. Seric estrogen, progesterone, corticosterone, prolactin and MCH concentrations were measured by radioimmunoassay. Another group of subjects (n= 56), divided in subgroups of four animals, were sacrificed every day from 15th to 21st day of lactation, before and after suckling stimulus. MCH expression was evaluated in the LHA, MPOA and IHy by immunohistochemistry. On the other hand, results have confirmed that expression of MCH expression strengthens around the 19th day of lactation. This result is similar to that previously observed in Sprague-Dawley rats. The causal relation between the end of lactation and the increased expression of MCH is to be determined.

Disclosures: R.D. Alvisi: None. **J.C. Bittencourt:** None. **E. Zimberknopf:** None. **L.F. Felicio:** None.

Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

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Program#/Poster#:896.05/RR2

Topic: E.03. Behavioral Neuroendocrinology

Support: FAPESP 2010/06774-0

Title: Role of μ and κ opioid receptors of rostro-lateral periaquedutal gray in behavioral selection during lactation

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Abstract: It is known that peripheral morphine disrupts maternal behavior in advance of predatory hunting and that periaqueductal gray (PAG) plays an important role in this behavioral selection. Nevertheless, the specific role for each opioid receptor in this context is to be determined. The aim of this study was to evaluate the role of μ and κ opioid receptors in behavioral selection between maternal behavior and predatory hunting. 72 Wistar female lactating rats with a guide cannula implanted in rostro-lateral PAG (rlPAG) were separated in 2 experiments with 4 groups (n=12/group) in each one: Experiment 1 - 10 nmol of μ receptor agonist, DAMGO (μ), 10nmol of κ opioid agonist, U69593 (κ), 7.91nmol of morphine sulfate (MOR) and control group (CS) which received 0.6μL of saline; Experiment 2 - 10nmol of μ receptor antagonist, CTAP plus 7.91nmol of morphine sulfate (NμM), 10nmol of κ opioid antagonist, nor-Binaltorphimine, and morphine sulfate (NκM), 7.91nmol of morphine sulfate (MOR) and control group (CS). The behavioral test was on lactation days 5 or 6. The test started 30 minutes after the drug/saline injection. Lactating females were placed in the experimental cage with their respective 8 pups and 5 cockroaches. In Experiment 1, in relation to predatory hunting, there was no difference in the number of insects captured, latency to capture each insect and no difference in the percentage of animals that captured each insect. This suggests that an acute single activation of PAG μ or κ opioid receptors does not interfere with the expression of this behavior. On the other hand, animals that received morphine injection presented an inhibition of maternal behavior. Morphine-treated animals (MOR) showed longer latencies to retrieve pups and smaller percents of animals capturing 1, 5 or 8 pups. In Experiment 2, blocking PAG μ or κ opioid receptors in the presence of morphine did not modify the morphine-induced inhibition of maternal behavior. However, the blockage of μ opioid receptor in the presence of morphine (NμM) modulated the expression of predatory hunting in morphine-treated dams. Those animals captured less insects, took longer time to catch the cockroaches and the number of animals capturing insects was decreased. Accordingly, the activation of more than one type of opioid receptor may be required to inhibit maternal behavior expression. Also, this study suggests that μ opioid receptor might play a role in expression of predatory hunting.

Disclosures: M.O. Klein: None. **A.M. Cruz:** None. **N.S. Canteras:** None. **L.F. Felicio:** None.

Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

Location: Hall F-J

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Title: Rapid inflow of thyroid hormone induced by visual imprinting primes later learning.

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Abstract: Newly hatched chicks follow the first conspicuous moving object they see. This learning, called filial imprinting is restricted to a sensitive period for a few days after hatching. We have investigated determining factors for the start of the sensitive period. We have recently found that rapid inflow of the thyroid hormone 3,5,3′-triiodothyronine (T_3) accompanying imprinting training determines the opening of the sensitive period.
Intravenous injection of T_3 facilitated visual imprinting on day 1. In addition, after the sensitive period closed on day 4, chicks injected with T_3 30 min before the training became imprintable, indicating that T_3 reopened the sensitive period.
Since T_3 regulates the beginning of the sensitive period in imprinting, we examined whether injection of T_3 could extend the sensitive period of imprinting. Chicks intravenously injected with T_3 on day 1 became imprintable on day 6 or 8. This suggests once the potential of imprinting is acquired, it lasts for long period independent of visual experience. Even after the sensitive period closed on day 4, T_3 injection enabled imprinting on day 8. We call this potential given by the wave of T_3 “memory priming” (MP). Furthermore, we found the first increase in T_3 could confer MP to learning other than imprinting. That is, T_3 injection on day 1 facilitated the correct choice ratio in reinforcement learning of a color discrimination pecking task on day 4. Taken together, these data suggests once MP is conferred, the sensitive period remains open and subsequent learning can be driven. We propose that MP which originates from T_3 induced by imprinting may be followed by cascading layers of later learning.

Disclosures: N. Aoki: None. **S. Yamaguchi:** None. **T. Kitajima:** None. **T. Matsushima:** None. **K.J. Homma:** None.

Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:896.07/RR4

Topic: E.03. Behavioral Neuroendocrinology

Title: Thyroid hormone determines the start of the sensitive period of visual imprinting

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Abstract: Filial imprinting in precocial birds has been studied intensively. Newly hatched chicks and ducklings follow the first conspicuous moving object they see. This is the mother under natural conditions. However, under experimental conditions it can be any other object. While following, the birds learn the color and shape of the object and become attached to it. This learning behavior, called filial imprinting, is restricted to a sensitive period which lasts only a few days in the case of chicks and ducklings. There are several theories concerning the control of the time course of this sensitive period about which very little was known, including the existence of a determining factor. Here we show for the first time that the thyroid hormone 3,5,3'-triiodothyronine (T_3) determines the opening of the sensitive period.
We attempted to identify the genes differentially expressed at an earlier phase of imprinting. Immediately after imprint training, whole brains were collected and used for cDNA microarray analysis and subjected to quantitative RT-PCR. We identified 18 genes, including Dio2, Type 2 iodothyronine deiodinase, that upregulated accompanying imprinting and its expression was enriched in brain capillaries. These results imply that Dio2 converts thyroxine (T_4) to T_3 in endothelial cells of brain capillaries and provides T_3 to brain cells for imprinting. Indeed, we found that T_3 in brain peaked around hatching, and imprinting caused an additional T_3 increase through enhanced conversion in the endothelial cells of brain capillaries, enabling imprinting via a transient non-genomic action. On the other hand, four days after hatching, T_3 levels fell to background level, at the same time that imprinting faded among dark-reared chicks. However, we found that the injecting T_3 on day 4 enabled imprinting once again. Our findings indicate that T_3 determines the start of the sensitive period of imprinting and plays a critical role in imprinting.

Disclosures: S. Yamaguchi: None. **N. Aoki:** None. **T. Kitajima:** None. **T. Matsushima:** None. **K.J. Homma:** None.

Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:896.08/RR5

Topic: E.03. Behavioral Neuroendocrinology

Support: FAPESP

CNPq

CAPES

Title: The role of oxytocin on sodium appetite after low-sodium diet

Authors: *A. S. MECAWI, SR[1], T. VILHENA-FRANCO[1], L. MARGATHO[1], F. V. FONSECA[2], L. C. REIS[2], L. L. K. ELIAS[1], J. ANTUNES-RODRIGUES[1]
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Abstract: The control of water/sodium acquisition and loss is crucial for maintaining life in terrestrial environment. However, up to now, literature data on the oxytocin (OT) involvement in sodium appetite seems to be controversial. Thus, the current work has been developed aiming to investigate the changes induced by the sodium restriction diet over the oxytocinergic system, as well as the involvement of OT in the control of sodium appetite in this condition. Male Wistar rats were submitted to a normal-sodium diet (1% NaCl) or a low-sodium (NaCl < 0.1%) during four days. While on sodium restriction diet, the animals presented a significant reduction in water intake (~21%, p < 0.01), urinary sodium excretion (~83%, p < 0.01) and urinary volume (~30%, p < 0.01), associated to blood volume reduction observed by the hematocrit increasing (~8%, p < 0.05) as well as total plasma protein (~21%, p < 0.05), resulting in decrease in arterial pressure (~4%, p < 0.05) and body weight gain (~20%, p < 0.05), whereas no significant changes were observed in the plasma sodium concentration. After offering hypertonic saline (0.3 M NaCl) the sodium-restricted animals presented a significant increasing in saline intake (~190% or 3 times, p < 0.001), related to a lower increase in water ingestion (~74%, p < 0.001), which resulted in the enhance of preference for sodium (~66%, p < 0.001). We also observed a reduction in the OT plasma concentration (~38%, p < 0.05), without significant differences in the neurohypophyseal OT concentration in sodium-restricted animals. Further, animals which were on low-sodium diet presented a reduction in OT mRNA expression in the paraventricular (PVN) and supraoptic nuclei observed through real time PCR (PVN ~23% e SON ~32%, p <0,05) and in situ hybridization (PVN ~55% e SON ~33%, p

<0,05). The sodium diet restriction has induced a reduction in co-localization of Fos related antigens and OT in PVN magnocellular medial region (~50%, p < 0,05) and parvocellular posterior region (~40%, p < 0.05), demonstrating a diminution of the oxytocinergic neurons basal activity after low-sodium diet. Additionally, OT icv microinjection led to the inhibition of both hypertonic saline intake (~100%, p < 0.05) and water (~100%, p < 0.05) further to the low-sodium diet. On the other hand, it was not observed significant effect for the oxytocinergic receptor antagonist (vasotocin) over the fluids ingestion. The current data has shown that four-day-sodium-diet leads to an inhibition of OT synthesis and secretion, related to a reduction in the oxytocinergic system basal activity in both PVN and SON nucleus, which is related in its turn to the induction of sodium appetite.

Disclosures: A.S. Mecawi: None. **T. Vilhena-Franco:** None. **L. Margatho:** None. **F.V. Fonseca:** None. **L.C. Reis:** None. **L.L.K. Elias:** None. **J. Antunes-Rodrigues:** None.

Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:896.09/RR6

Topic: E.03. Behavioral Neuroendocrinology

Support: Swedish medical research council grant 9459

Title: The effect of growth hormone on memory and gene expression in intact rats

Authors: *A. GRÖNBLADH, J. JOHANSSON, F. NYBERG, M. HALLBERG
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Abstract: Growth hormone (GH) is a polypeptide hormone with a wide range of functions in the periphery and this hormone also has an important role in the brain. Especially the role of GH on cognition has received much attention during recent decades, demonstrating reduced cognitive capabilities in patients with growth hormone deficiency (GHD) that can be alleviated by GH replacement therapy. We have earlier demonstrated that GH in hippocampal cells may counteract apoptosis induce by opioids. In addition, GH has also been suggested to induce neurogenesis in the brain and to be involved in neuroprotection. Hence, GH has the potential of being able to improve drug-induced impairments in the brain. Anabolic androgenic steroids (AAS) are drugs known to affect the central nervous system (CNS) and has been reported to affect CNS-related behaviors such as aggression, anxiety and cognitive impairments. The aim of the present study was to examine the effects of recombinant human GH (rhGH) on memory and gene expression in several brain areas in rats pretreated with AAS. Male Wistar rats were treated with the

anabolic androgenic steroid nandrolone decanoate (15 mg/kg) or peanut oil every third day during three weeks, subsequently the rats were treated with rhGH (1.0 IU/kg) or saline daily during ten days. The Morris water maze was used to test the spatial learning and memory of the rats during the GH treatment. Quantitative real-time PCR (qPCR) was used to examine the gene expression in several brain regions, including hippocampus and hypothalamus. The results demonstrated that GH improves spatial memory in intact rats. Effects on gene expression in several brain areas were seen. In conclusion, rhGH treatment can improve memory in intact rats and the underlying mechanism may involve alterations in the GH/IGF-1 axis.

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Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

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Support: Investigator Initiated Research Grant-Shire Pharmaceuticals

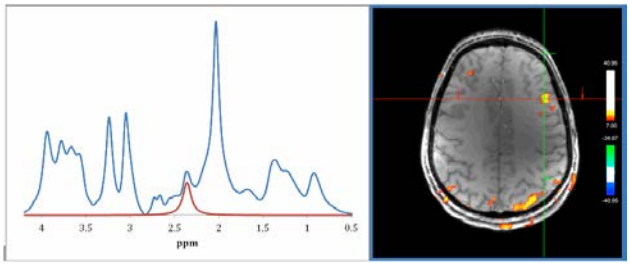
K24 DA30301

Title: Psychostimulant treatment effects on prefrontal brain activation, glutamate concentration and executive function in menopausal women with memory complaints

Authors: S. SHANMUGAN[1], R. P. R. NANGA[2], H. HARIHARAN[3], M. ELLIOTT[3], J. BRADLEY[4], C. SCHINSTINE[4], R. REDDY[3], *C. N. EPPERSON[5]
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Abstract: Background: Hypogonadal women frequently report a subjective decline in executive function. The psychostimulant lisdexamfetamine (LDX) may offset the effect of estrogen loss by enhancing dopamine and modulating glutamate function in the prefrontal cortex.
Methods: Eleven healthy menopausal women with cognitive complaints have been enrolled in this on-going, double-blind, placebo controlled, crossover study investigating the efficacy of LDX in improving executive functions, measured by self report on the Brown Attention Deficit Disorder Scale (BADDS) and performance on the continuous performance task (CPT) and N-back. A subset of women have and will undergo fMRI and ^{1}H-MRS at baseline and at the end of

each 4-week treatment arm. Left dorsal lateral prefrontal cortex (DLPFC) glutamate concentrations are measured prior to performance of the N-back task while BOLD signal is measured during task performance. All scans are conducted with a Siemens 7 Tesla whole body scanner. NIFTI brain templates are used along with MP-RAGE MRI images to aid in MRS voxel placement. Absolute glutamate concentrations are calculated from MRS data using water MRS spectra as reference using in-house developed curve fitting programs.
Results: Eight women have completed the clinical trial and three additional women have undergone baseline fMRI/^{1}H-MRS session. Baseline DLPFC glutamate levels are 8.0mM +/- 0.5mM. Fig. 1 depicts DLPFC ^{1}H-MRS data with fitted glutamate peak and fMRI data overlaid with anatomy data for the N-back task from one subject. Mean ± SD BADDS scores at Baseline and during the Placebo arm are 24.1±9.6 and 26.5±11.9, respectively. BADDS scores (18.5 ± 15.5) during the LDX treatment arm are trending lower. Likewise, there is a trend for improved performance on the CPT (p=0.08) in the LDX versus placebo condition.
Conclusions: LDX may improve subjective and objective measures of executive functioning during menopause. Multimodal brain imaging provides a more direct assessment of LDX effects in brain regions subserving executive functions.



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Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

Location: Hall F-J

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Topic: E.03. Behavioral Neuroendocrinology

Support: P20RR01647012 & 8P20

GM103475-12.

Title: Exercise-induced hippocampal-specific activation of fatty acid synthase and accumulation of stearate is mediated by insulin receptor signaling: Impact on spatial learning and memory

Authors: *N. E. CHORNA[1,2,3], I. J. SANTOS-SOTO[1], A. VAZQUEZ-MONTES[1,3], J. L. MORALES[1], J. DE LA NUEZ[1], N. M. CARBALLEIRA[4], A. P. CHORNYY[5], S. PENA DE ORTIZ[1,2,3]
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Abstract: In neurons, endogenously synthesized fatty acids (FA) are esterified predominantly into phospholipids that stimulate membrane production or are used for lipid-based post-translational modification of membrane proteins. In addition, FA and their derivatives are important second messengers triggering signal transduction pathways that may result in regulation of transcription factors. Since running is known for its beneficial effects in learning and memory, we hypothesized that different life styles, voluntary exercise versus sedentariness, would differ in specific lipid-related pathways associated with improved cognition. Non-targeted neurolipidomic profiling of selective brain regions of middle-aged C57BL/6 mice identified hippocampus-specific increases in stearate, associated to voluntary exercise. Administration of streptozotocin (STZ), an established diabetogenic agent known to block insulin receptor (IR) intracellular signaling, specifically inhibited exercise-induced accumulation of stearate in the hippocampus. Accordingly, voluntary exercise induced hippocampus-specific increases in the expression of fatty acid synthase (FASN) but not elongase 1 or elongase 6 -enzymes involved in stearate biosynthesis via elongation from palmitate, an effect that was also blocked by STZ. Importantly, intracerebroventricular (i.c.v.) injections of C75, an irreversible FASN inhibitor, or STZ, where used to inhibit exercise-induced forebrain FASN activity or upregulation, respectively. Results indicate that directly blocking running associated FASN enzymatic activity with C75 or blocking its exercise-induced upregulation impairs spatial learning and memory in the Barnes Maze. Both treatment increased the escape latency, caused animals to shift from spatial to non-spatial searching strategies, and impaired spatial discrimination memory. Interestingly, in all our behavioral experiments results indicated that both C75 and STZ interfered with the exercise mediated improvements in learning and memory. Overall, these studies identified a machinery of exercise-induced hippocampal FA regulation, involving FASN and IR-mediated intracellular signaling, relevant for the beneficial effects of exercise in spatial learning and memory

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Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

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Title: Adult mice maintained on a high-fat diet exhibit object location memory deficits and aberrant hippocampal gene expression

Authors: *F. D. HEYWARD[1], R. G. WALTON[2], M. S. CARLE[1], M. A. COLEMAN[1], W. T. GARVEY[2], J. D. SWEATT[1]
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Abstract: Mounting evidence has established that diet-induced obesity (DIO) is associated with deficits in hippocampus-dependent memory. The bulk of research studies dealing with this topic have utilized rats fed a high-fat diet as an experimental model. To date, there has been a paucity of research studies that have established whether the memory deficits exhibited in DIO rats can be recapitulated in mice. Moreover, the majority of experiments that have evaluated memory performance in rodent models of DIO have utilized memory tests that are essentially aversive in nature (i.e., Morris water maze). The current study sought to fill an empirical void by determining if mice maintained on a high-fat diet exhibit deficits in two non-aversive memory paradigms: novel object recognition (NOR) and object location memory (OLM). Here we report that mice fed a high-fat diet over 23weeks exhibit intact NOR, albeit a marked impairment in hippocampus dependent OLM. We also determined the existence of corresponding aberrations in gene expression within the hippocampus of DIO mice. DIO mice exhibited significant reductions in both SIRT1 and PP1 mRNA within the hippocampus. Our data suggest that mice maintained on a high-fat diet present with impaired hippocampus-dependent spatial memory and a corresponding alteration in the expression of genes that have been implicated in memory consolidation.

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Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant GM060665

NIH Grant RR 03037

CUNY Collaborative #18

Title: Multiparous rats: A model of better neural aging?

Authors: S. HUSBAND[1,2], F. LEMA[1], K. MONDE[1,2], J. L. GOMEZ[1,2], N. CARMONA[3], M. FRANKFURT[4], *V. N. LUINE[1,2]
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Abstract: Some evidence shows that rats undergoing multiple bouts of pregnancy, birth and pup rearing (multiparous rats) show less anxiety and stress responsivity, and better cognitive function than virgin rats (nulliparous rats). However, few experiments included young rats in the studies in order to determine whether the multiparous rats age differently than nulliparous rats. We compared young virgin (2-4 mo. old), middle-aged virgin (12-15 mo. old) and middle-aged multiparous (12-15 mo. old) Fisher 344 rats for activity on the open field, anxiety on the elevated plus maze (EPM) and recognition memory (object recognition, OR and object placement OP). Young virgins were more active than middle-aged virgin and multiparous females on the open field suggesting that aging but not parity affects activity. On the EPM, no differences were found between the groups for number of open arm entries or time spent in the open arms which indicates neither age nor parity effects on anxiety. In OR memory tests with a 2h or 4 h inter-trial delay between the sample and recognition trial, no differences were found between groups which suggests that object memory is also not affected by aging or parity. In OP memory tests with a 4 h inter-trial delay, the young virgins were significantly different from the middle-aged virgins but not the multiparous females suggesting an effect of both aging and parity. Behavioral results suggest that multiple bouts of motherhood (parity) do not affect age-related decreases in overall activity. For anxiety, no age or parity effects were noted at middle-age. Likewise, object recognition was not affected by age or parity at middle-age. For spatial memory, as assessed by OP, multiparous females were not different from young virgins while the performance of middle aged-virgins was impaired suggesting that parity mitigates the effects of aging on spatial

memory. Current studies are measuring spine density in the hippocampus and medial prefrontal cortex to determine whether parity effects on memory may be related to changes in these areas. Overall, our results suggest that specific behavioral domains are affected differently by aging and by motherhood in female rats.

Disclosures: S. Husband: None. **F. Lema:** None. **K. Monde:** None. **J.L. Gomez:** None. **N. Carmona:** None. **M. Frankfurt:** None. **V.N. Luine:** None.

Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

Location: Hall F-J

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Program#/Poster#:896.14/RR11

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH 1R15AT003586-01A1

Title: Impairment of spatial memory by sub-chronic administration of genistein to adult male rats

Authors: *C. C. WRENN[1], M. J. BURR[2], E. R. BALL[3], B. D. WOLFE[2]
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Abstract: Genistein is a phytoestrogen found in soy that may act as an endocrine disruptor due to its ability to bind estradiol receptors. Previous work in our laboratory has shown that when female rats consume genistein during both gestation and lactation, their male offspring have impaired spatial learning. These data suggest that genistein exerts a deleterious organizational effect on brain structures involved in spatial cognition. In the present study we tested the hypothesis that genistein also exerts an activational effect on spatial cognition by implanting genistein releasing pellets subcutaneously in adult male Long Evans rats. Pellets released genistein over a 21 day period at various doses (50, 100 and 150 mg). On the 13th day after pellet implantation, training to find a hidden platform in the Morris water maze began. Genistein had no effect on latency to find the platform across 8 days of training. A probe trial on day 9 found that rats receiving the lowest dose of genistein did not prefer to swim in the quadrant that had contained the platform during training indicating impaired memory of that location. Rats receiving the placebo pellets or the two higher doses of genistein preferred the platform quadrant. These data show that genistein impacts spatial cognition through an activational mechanism, but that the relationship between this effect and dose is non-monotonic.

Disclosures: C.C. Wrenn: None. **M.J. Burr:** None. **E.R. Ball:** None. **B.D. Wolfe:** None.

Poster

896. Hormones and Cognition III: Serotonin and Other Transmitters, TH, experience

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:896.15/RR12

Topic: E.03. Behavioral Neuroendocrinology

Support: UNAM-DGAPA

Title: Reversible suppression of eyestalk communication in crayfish

Authors: *B. BARRERA-MERA[1], B. CALVAELVIRA[2], V. MORALESJJ[2]
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Abstract: Visual influences as a mechanism of endocrine control for the metabolic, reproductive as well as adaptive abilities is necessary to respond to enviroment. This require the eyestalk neural and vascular communication. And the experimental suppression of such communication can be achieved from a few hours to several days leading many metabolic changes of the animal.

Disclosures: B. Barrera-Mera: None. **B. Calvaelvira:** None. **V. Moralesjj:** None.

Poster

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Topic: E.04. Autonomic Regulation

Support: NIH Grant 70830

P01-04277441

Jacob Neil Boger Foundation

Title: 14-3-3-protein analysis of the cerebrospinal fluid in the human infant: A mass spectrometry-based feasibility study

Authors: *K. G. BROADBELT[1], E. A. HAAS[2], H. F. KROUS[2], H. C. KINNEY[3], H. STEEN[4]
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Abstract: Impaired brainstem responses to homeostatic challenges during sleep are postulated to result in sleep-related sudden death of infants dying of the sudden infant death syndrome (SIDS). Our laboratory has garnered substantial evidence that a serotonergic (5-HT) disorder of regions of the medulla oblongata (lower brainstem) critical to homeostatic control is associated with a neural subset of SIDS. We have also reported that the signal transduction protein 14-3-3 family, known to play role in 5-HT regulation, is deficient in the one component of the medullary 5-HT system analyzed (gigantocellularis) with broad-based proteomics and western blotting. Ultimately we would like to determine if potentially altered cerebrospinal fluid (CSF) levels of 14-3-3 serve as a biomarker of abnormal 14-3-3 levels in the medulla of SIDS infants. As a step towards this goal, we undertook a feasibility study of the proteome in CSF obtained at autopsy in four human infants in the SIDS age range (1-5 postnatal months, median=3 months) as well as two SIDS infants (1.5-3 months, median=2.5). In the 6 cases, we applied mass-spectrometry based proteomics to: 1) create an infant postmortem CSF proteome profile; and 2) develop a targeted mass spectrometry assay to identify and quantitate the different 14-3-3 isoforms (using both spectral counting and stable isotope labeling) and confirm with western blotting. The obtained peptides were analyzed on an LTQ-Orbitrap mass spectrometer. All MS/MS data were analyzed using Protein Pilot (AB SCIEX). Protein identifications were accepted if established at greater than 50.0% probability, and if they contained at least 2 identified peptides. These values resulted in an overall peptide false discovery rate (FDR) of 1% and protein FDR of 1%. In 2 non-SIDS cases (n=2) with completed analysis to date, we identified 666 proteins in the CSF of which 46% were proteins related to cellular regulation, 19% were related to the cytoskeleton, and 11% were related to development, among other categories. We identified all but one of the 7 isoforms of the 14-3-3 family; sigma was the exception. In the single SIDS case analyzed to date we saw comparable protein number, identifying 633 proteins, and comparable assigned spectra. All 6 isoforms of the 14-3-3 proteins identified in the controls were also identified in the SIDS case. We conclude that the use of our approaches is feasible in human infant CSF at autopsy, and hold potential for the future delineation of a CSF biomarker related to 14-3-3 protein pathology in SIDS. The developmental information about the human CSF proteome in early life is of value towards understanding the role of CSF in brain function and development.

Disclosures: K.G. Broadbelt: None. **E.A. Haas:** None. **H.F. Krous:** None. **H.C. Kinney:** None. **H. Steen:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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First Candle/SIDS Alliance

CJ Foundation for SIDS

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Title: Analysis of copy number variations in sudden infant death syndrome

Authors: *D. S. PATERSON[1], F. RAHIMOV[3], K. SCHMITZ ABE[3], E. A. HAAS[4], H. F. KROUS[4], H. C. KINNEY[2], K. MARKIANOS[5]
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Abstract: The sudden infant death syndrome (SIDS) is the leading cause of postneonatal infant mortality in the United States today with an incidence of 0.57/1,000 live births. The pathogenesis of SIDS is proposed to be a multifactorial, involving a combination of environmental and genetic risk factors. While multiple environmental risk factors, including prone sleep position and maternal cigarette and alcohol use during pregnancy have been identified, genetic studies have been less successful in identifying genes associated with increased SIDS risk. In this study, we analyzed the genome of 50 Caucasian SIDS cases (36 male, 14 female) for copy number variations (CNVs) using comparative genomic hybridization (CGH) with the Agilent SurePrint G3 4x180 array in order to determine if deletions or duplications of chromosomal regions are important in SIDS. Array hybridization was successful for 49/50 SIDS cases; we identified a total of 384 CNVs (0.6% of the genome), 96 of which were unique non-overlapping CNVs. CNVs were then screened and candidates selected for further analysis. A CNV was selected if it was: 1) located in a candidate gene previously associated with SIDS; 2) a deletion(deletions are more likely to be harmful than duplications); 3) novel, i.e., not listed in the 1258 Hapmap

samples using the same CNV pipeline, the Database of Genomic Variants (DGV) and University of California Santa Cruz (UCSC) Genome Browser (previously unidentified CNVs are more likely to be specifically associated with SIDS); 4) large (over 100kb) (bigger deletions are more likely to be pathogenic); and 5) observed in multiple cases (the more cases with the CNV the greater the probability that it is associated with SIDS). Based upon these criteria, we selected 5 CNVs with copy number 0 (CNV0), representing complete deletion of the region from the genome (loss of region from both chromosomes), for further analysis, including 3 novel CNVs of 56, 121 and 147 kilobases in chromosomes 2, 4, and 6, respectively. The CNV in chromosome 6 was identified in 14/50 (28%) SIDScases. We are currently in the process of confirming the presence of these CNVs in the original cases plus an expanded cohort of 150 SIDS cases and 150 ethnicity matched controls using qPCR assays (Applied Biosystems). This study has the potential to identify novel candidate genes and pathways that may contribute to the pathogenesis of SIDS, as well as provide potential markers for use in a screening test to identify subsets of infants at increased risk of SIDS.

Disclosures: D.S. Paterson: None. **F. Rahimov:** None. **K. Schmitz Abe:** None. **E.A. Haas:** None. **H.F. Krous:** None. **H.C. Kinney:** None. **K. Markianos:** None.

Poster

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NIH R01HD052772

Title: Fetal nicotine blunts pH chemosensitivity of 5-HT neurons *in vitro* and the ventilatory response to CO2 of WT but not Lmx1b CKO mice *in vivo*

Authors: J. AVRAAM, K. L. PROCH, Y. WU, *G. B. RICHERSON
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Abstract: ° SIDS is linked to brainstem 5-HT abnormalities and to maternal smoking. Given that medullary 5-HT neurons are chemoreceptors and fetal nicotine exposure can impair postnatal respiratory chemoreception, it is possible that prenatal nicotine impairs the function of 5-HT neurons. An osmotic minipump that supplied 0, 6 or 60 mg/kg/day of nicotine was implanted in pregnant mice at E10 (prenatal). After pups were born, neuronal cell cultures were prepared from

the medullary raphé and fed with culture medium containing 0, 0.05 or 0.5 μM nicotine (postnatal). Neurons were placed in a recording chamber and bathed in aCSF with 5% CO_2 (pH 7.4). Patch clamp recordings were made from 5-HT neurons until firing rate (FR) stabilized at 1 Hz. They were then exposed to two episodes of 9% CO_2 (pH 7.2) each with wash out. This was repeated with aCSF containing 10 μM nicotine. Neurons exposed to prenatal nicotine, with or without postnatal nicotine, exhibited a reduced response to acidosis and to acute nicotine. At 12-21 and >22 days, neurons given the higher dose of prenatal plus postnatal nicotine had a 45% and 54% lower response to acidosis, respectively. At >22 days, the response to acute nicotine was 56% smaller. At >22 days, neurons given the lower dose of prenatal, or prenatal plus postnatal, nicotine also exhibited a significant reduction in the response to acidosis (39%) and to acute nicotine (48%). The percentage of neurons that were responsive to acidosis and acute nicotine was also significantly reduced. At 12-21 days 25% fewer neurons exposed to the higher dose of prenatal plus postnatal nicotine responded to acidosis, and at >22 days 40% fewer of these responded to acute nicotine.
° Separate experiments were performed using plethysmography on WT and $Lmx1b^{f/f/p}$ mice (which lack >99% of central 5-HT neurons) to examine the role of 5-HT neurons in mediating the effects of prenatal nicotine on the ventilatory response to CO_2. A 4 week minipump was implanted into pregnant mice at E10 supplying 60 mg/kg/day of nicotine. Litters were studied at P7, P15 and P25. Preliminary data (n=3-6 pups per group) showed that the ventilatory response to 7% CO_2 of untreated $Lmx1b^{f/f/p}$ mice was 37% of untreated WT mice at P25. At this age there was no difference in the response of untreated vs nicotine exposed $Lmx1b^{f/f/p}$ mice. However, nicotine treated WT pups had a response to 7% CO_2 that was only 15% of untreated WT pups.
° Prenatal nicotine impaired pH sensitivity of 5-HT neurons, and this may be responsible for severe blunting of chemoreception that was seen *in vivo*. These results provide a possible cellular mechanism by which maternal smoking might increase the risk of SIDS.

Disclosures: J. Avraam: None. **K.L. Proch:** None. **Y. Wu:** None. **G.B. Richerson:** None.

Poster

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Topic: E.04. Autonomic Regulation

Support: NIH Grant P01HD36379

NIH Grant R01HD052772

Title: Isoflurane eliminates serotonin (5-HT) neuron chemosensitivity *in vitro* and markedly depresses the hypercapnic ventilatory response *in vivo*.

Authors: *C. A. MASSEY, Y. WU, G. B. RICHERSON
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Abstract: 5-HT neurons in the brainstem have been proposed to be central chemoreceptors, responsible for detecting hypercapnia and subsequently stimulating respiratory areas to properly maintain pH homeostasis. However, there have been inconsistent results when studies have examined the response of 5-HT neurons to increases in CO2 in vivo. Some of these studies utilized inhalational anesthetics, including isoflurane and halothane, which are potent agonists for 2-pore domain K+ (TASK) leak channels. 5-HT neurons have one of the highest levels of TASK channel expression, thus making them prime targets for these anesthetics. Here we used perforated patch-clamp recordings from cultured medullary ePet1-YFP mouse neurons (n=8) and found that isoflurane nearly completely silenced the baseline firing of 5-HT neurons in vitro and also abolished their chemosensitivity. On average 5-HT neurons tripled their firing rate in isocapnic acidosis (0.47 Hz in pH 7.4 to 1.51 Hz in pH 7.15). In isoflurane, baseline firing dropped to 0.01 Hz, and in response to acidosis increased to only 0.11 Hz (pH 7.15). Both of these effects occurred rapidly, and reversed with washout of isoflurane. Furthermore, using whole animal plethysmography we found that isoflurane markedly decreased the hypercapnic ventilatory response (HCVR) in vivo by 75% (n=4). Minute ventilation (Ve) in male wildtype (WT) C57/Bl6 mice increased by 400% (from 2.36 ml g-1 min-1 to 12.54 ml g-1 min-1) in 21% O2, 7% CO2, balance N2; in 1% isoflurane the response was reduced, as Ve increased by only 100% (from 2.48 ml g-1 min-1 to only 5.01 ml g-1 min-1). In order to isolate the effects of isoflurane on central chemoreception, we first examined the HCVR in 50% O2 to silence peripheral chemoreceptors in male WT C57/Bl6 mice. Our preliminary data (n=3) showed that Ve in these mice increased by 275% (from 3.13 ml g-1 min-1 to 11.88 ml g-1 min-1) when CO2 was increased from 0% to 7%. In 50% O2, 1% isoflurane severely reduced the HCVR in these mice, as Ve increased by only 40% when CO2 increased from 0% to 7% (from 2.21 ml g-1 min-1 to 3.16 ml g-1 min-1). These preliminary results indicate that isoflurane silences 5-HT neurons, blocks 5-HT neuron chemosensitivity, and severely blunts respiratory chemoreception, preventing any conclusions about chemoreceptors from experiments using this anesthetic. These data also support the conclusion that 5-HT neurons are central pH/CO2 chemoreceptors.

Disclosures: C.A. Massey: None. **Y. Wu:** None. **G.B. Richerson:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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Support: FONDECYT Grant 1090375

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Title: Prenatal nicotine exposure reduces the activity of Raphe Obscurus neurons from P3 mice.

Authors: *V. CERPA[1,2], E. BRAVO[2], G. B. RICHERSON[1,3], J. EUGENIN[2]
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Abstract: Mouse neonates prenatally exposed to nicotine show hypoventilation and reduced response to CO2 in vivo. In addition, isolated brainstem-spinal cord preparations from nicotine exposed P3 mice show reduced respiratory responses to acidosis and respiratory cycles shorter and more irregular than those observed in controls. Since raphe neurons contribute to the respiratory chemosensory drive, we studied whether they are involved in prenatal nicotine induced effects on respiratory rhythm.
Osmotic minipumps were implanted subcutaneously into pregnant mice (1-5 days CF1) to deliver nicotine ditartrate 60 mg Kg-1 day-1 until the end of gestation. We studied electrophysiological properties and the response to CO2 of Raphe Obscurus (ROb) neurons using whole-cell patch-clamp recording in slices from P3 control and nicotine-exposed mice. Immunohistochemistry, to detect co-expression of tryptophan hydroxylase (TpH, serotoninergic marker) and c-fos protein (neuronal activity marker), as well as immunofluorescence to detect 5-HT1A receptors were done.
The rostral part of ROb from nicotine-exposed mice presented lesser TpH+ neurons than controls (35.1 ± 2 and 41.6 ± 6, respectively; $p < 0,05$, ANOVA, post test Bonferroni). Also, TpH+ and TpH- neurons expressed less c-Fos than controls, suggesting they were hypoactives in basal conditions. This hypoactivity was confirmed in slice recordings, in which ROb neurons of nicotine-exposed mice showed reduced firing rate under basal conditions in presence or absence of glutamatergic or gabaergic synaptic currents ($p < 0.05$ and $p < 0.01$, respectively, Mann-Whitney U Test). No other differences were detected in the electrophysiological properties (Vm, RI, AHP, Cm) or in response to CO2. In addition, 5-HT1A receptors were over-expressed in ROb of nicotine-exposed mice.
In conclusion, our results show that perinatal nicotine exposure reduced the number and activity of ROb neurons at P3. Hypoactivity could be explained by an over expression of 5-HT1A autoreceptors. These findings have relevant implications for the understanding of the mechanisms underlying nicotine-induced deficit in respiration due to the key role that the raphe nuclei play in respiratory drive, chemoreception and generation of respiratory output.

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Poster

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Topic: E.04. Autonomic Regulation

Support: NIH 2U54NS041069-06A1

Alaska INBRE 5P20RR016466

Title: Intermittent hypercapnia enhances ventilatory CO2/PH chemosensitivity to overcome serotonergic dysfunction

Authors: *B. MOSHER[1], M. B. HARRIS[2]
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Abstract: Serotonergic dysfunction is thought to enhance vulnerability to Sudden Infant Death Syndrome (SIDS) by compromising critical homeostatic reflexes such as ventilatory CO_2/pH chemosensitivity. We test the hypothesis that exposure to mild intermittent hypercapnia (IHc) during postnatal development will enhance chemosensitivity to overcome consequences of serotonergic deficit. Rat pups were exposed to IHc (9 consecutive cycles of 5 min 5% CO_2 in air, followed by 10 min air; constant air control) each day for 5 days beginning at postnatal day P12, and were subsequently assessed for CO_2/pH chemosensitivity using an unanesthetized juvenile rat *in situ* perfused decerebrate brainstem preparation. Chemosensitivity in the air control group was abolished by antagonism of 5-HT_{2A} receptors, confirming our previous observation that this sensitivity is critically dependent on 5-HT_{2A} receptor-mediated mechanisms. Chemosensitivity was greatly enhanced by IHc. Enhanced sensitivity was attenuated by 5-HT_{2A} receptor antagonism, but residual sensitivity was comparable to that of untreated air controls. These data demonstrate that IHc enhances a non-5-HT_{2A} receptor mediated processes capable of maintaining chemosensitivity under conditions of serotonergic dysfunction normally abolishing such sensitivity. We have proposed a “push-pull” model of raphé contributions to central chemosensitivity involving both 5HT and γ-aminobutyric acid (GABA) synthesizing neurons. We postulate that IHc enhances GABA neuron contributions resulting in preserved chemosensitivity when 5-HT neuron contributions are compromised.

Disclosures: B. Mosher: None. **M.B. Harris:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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Title: Probing a local chemosensory network in the medullary raphé

Authors: *M. B. HARRIS[1,2], K. E. ICEMAN[1], T. E. DICK[3], D. M. BAEKEY[4]
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Abstract: The modulation of respiratory activity in the brainstem is, at least in part, dependent on the medullary raphé. The organization of raphe circuitry involved has not yet been described. We have previously confirmed the presence of CO2/acidosis-stimulated serotonin synthesizing (5-HT) neurons, and CO2/acidosis inhibited γ-aminobutyric acid (GABA) synthesizing neurons within the rat medullary raphé. Recently, we have identified an additional population of raphé CO2/acidosis-stimulated cells we are terming “raphé chemosensory amplifier” (RCA) interneurons. We hypothesize that raphé chemosensitivity is due to, or enhanced by, an intra-raphé network where RCA neurons receive input from both serotonergic and GABAergic neurons creating a “push-pull network” underlying raphé contributions to central chemosensitivity. To determine the relationships of the identified populations, multiple individual raphé neurons were recorded in the in situ perfused rat preparation, characterized with respect to phrenic and/or vagal activity, and further through responses to 4% hypercapnic challenges. We have simultaneously recorded representatives of each using a 16 channel multielectrode extracellular array with independent depth adjustments to allow maximal yield of individual neurons. Preliminary results illustrate relationships during baseline conditions, changes in individual neuron firing frequency and variability while altering chemosensory drive, and coordinated changes among the populations during hypercapnic stimulation. The results support our push-pull network model of chemosensory influence in the medullary raphé.

Disclosures: M.B. Harris: None. **K.E. Iceman:** None. **T.E. Dick:** None. **D.M. Baekey:** None.

Poster

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Topic: E.04. Autonomic Regulation

Support: NIH 2U54NS041069-06A1

Alaska INBRE 5P20RR016466

Title: Influence of isoflurane on CO_2 sensitive and insensitive raphé neurons

Authors: *S. L. JOHANSEN[1], K. E. ICEMAN[1], G. B. RICHERSON[3], M. B. HARRIS[2]
[1]Biol. & Wildlife, [2]Inst. of Arctic Biol., Univ. of Alaska Fairbanks, Fairbanks, AK; [3]Neurol., Univ. of Iowa, Iowa City, IA

Abstract: Raphé neurons contribute to the hypercapnic ventilatory response, but their identity as central chemoreceptors is debated. *In vitro* raphé serotonin (5-HT) and ɣ-aminobutyric acid (GABA) synthesizing neurons are activated and inhibited by CO_2/acidosis, respectively. We have previously demonstrated that in the unanesthetized juvenile rat *in situ* perfused decerebrate brainstem preparation these neurons are similarly chemosensitive. Studies conducted using anesthetized rats *in vivo* have not illustrated these chemosensitive neuron types. We test the hypothesis that chemosensitivity of 5-HT and/or GABA raphé neurons is masked by the anesthetic isoflurane. To determine the impact of isoflurane on neuron chemosensitivity, raphé extracellular recordings were made in the unanesthetized *in situ* brainstem preparation during a 4% hypercapnic challenge, both before and during exposure to isoflurane. Juxtacellular labeling and subsequent immunohistochemistry identified neurotransmitter phenotypes of electrophysiologically characterized neurons. Results indicate that 5-HT neurons either lose chemosensitivity or are silenced when exposed to isoflurane, in a dose dependent manner. We conclude that chemosensitive raphé 5-HT neurons identified *in vitro* and *in situ* are masked by isoflurane and potentially other anesthetics *in vivo*.

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Poster

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NIH Alaska INBRE 5P20RR016466

Title: Identifying a medullary "raphé chemosensory amplifier" (RCA) neuron mediated by serotonin and GABA *in situ*

Authors: *K. E. ICEMAN[1], H. M. TALLAN[1], M. B. HARRIS[2]
[1]Biol. and Wildlife, [2]Inst. of Arctic Biol., Univ. of Alaska Fairbanks, Fairbanks, AK

Abstract: Neurons within the rat medullary raphé are intrinsically sensitive to CO_2/pH change *in vitro*. Serotonin synthesizing (5-HT) neurons are activated by CO_2/acidosis, while γ-aminobutyric acid (GABA) synthesizing neurons are inhibited. We have previously demonstrated that these neurons are similarly chemosensitive *in situ.* Here we hypothesize that a previously uncharacterized pool of raphé interneurons also exhibit chemosensitivity, and that sensitivity of these medullary "raphé chemosensory amplifier" (RCA) interneurons is due to intra-raphé network input from 5-HT and GABAergic neurons in an intact system. We use extracellular recording of individual raphé neurons in the unanesthetized juvenile rat *in situ* perfused decerebrate brainstem preparation to assess chemosensitivity of putative RCA neurons before and after pharmacological antagonism of 5-HT_{2A} and $GABA_A$ receptors. We show that CO_2-stimulated RCA neurons are present in the medullary raphé and that antagonism of $GABA_A$ receptors abrogates RCA neuron chemosensitivity. Blockade of $GABA_A$ receptors increases RCA neuron baseline firing indicating removal of tonic inhibitory input from GABA neurons. In contrast, blockade of 5-HT_{2A} receptors decreases RCA neuron baseline firing, suggesting removal of tonic excitatory input from 5-HT neurons. Results demonstrate that $GABA_A$ receptor-mediated input is necessary for RCA neuron chemosensitivity and 5-HT_{2A} receptor-mediated input is necessary for RCA tonic drive. Juxtacellular labeling and immunohistochemistry indicate that RCA cells are not serotonergic, but are closely apposed to 5-HT and GABAergic neurons. Results support our "push-pull network" model of raphé contributions to central chemosensitivity. Thus, we have characterized a new type of CO_2–stimulated medullary raphé neuron, the RCA interneuron, which is mediated by intra-network inputs from serotonergic and GABAergic neurons.

Disclosures: K.E. Iceman: None. **H.M. Tallan:** None. **M.B. Harris:** None.

Poster

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Title: Neuroanatomical relationship between CO_2 chemosensitive noradrenergic neurons and nitric oxide synthase immunoreactive cells in locus coeruleus

Authors: S. M. JOHNSON[1], *O. DEHKORDI[2], J. ALLARD[1], S. IRAVANI[2], R. M. MILLIS[1], A. JAYAM TROUTH[3]
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Abstract: Locus coeruleus (LC) in the dorsal pons is an important component of the central chemosensitive network participating in the ventilatory response to hypoxia and hypercapnia. Recent studies have demonstrated the involvement of LC noradrenergic neurons in hypoxia and hypercapnia induced hyperventilation. Moreover, nitric oxide (NO) mechanisms appear to regulate the noradrenergic cells of LC and to modulate the hypoxic ventilatory responsiveness of LC neurons. However, the neuroanatomical relationships between LC noradrenergic neurons and NO expressing cells in the LC remain unclear. Therefore, we hypothesized that the neurochemical profile of noraderenergic LC cells might include neuronal NO synthase (nNOS). We evaluated the expression of nNOS with respect to noradrenergic cells of LC by studying Prp57 transgenic mice which express green fluorescent protein (GFP) in their LC noradrenergic cells. We detected nNOS at sites medial to LC, in a small number of cells in LC, as well as in dendritic and axonal processes within LC. However, none of the GFP-positive noradrenergic cells in LC demonstrated NOS-like immunoreactivity. The presence of nNOS immunoreactivity in dendritic and axonal processes in the vicinity of GFP-positive noradrenergic cells confirms the results of previous functional studies concerning involvement of L-arginine and NO signaling mechanisms in the regulation of noradrenergic LC neurons.

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Poster

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Topic: E.04. Autonomic Regulation

Support: MRC, UK

Title: Pattern of connexin 26 expression in medulla oblongata

Authors: X. ZHANG[1], G. KOENTGES[1], *N. E. DALE[2]
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Abstract: The medulla oblongata contains the chemosensors that impart the sensitivity of breathing to changes in PCO_2. We have previously shown that hemichannels of the gap junction protein connexin 26 (Cx26) are sensitive to changes in PCO_2 around the physiological norm of 40 mmHg. Cx26 is expressed in the chemosensitive areas at the ventral surface of the medulla. To identify more precisely the types of cells involved in the central CO_2 chemosensing, we have studied in detail the expression patterns of Cx26 by immunohistochemistry and in situ hybridization.
Cx26 has three main components of expression in the ventral medulla. Firstly, it is highly expressed in the meninges, specifically in the leptomeningeal cells rather than the endothelial cells of the blood vessels. Secondly, it is expressed in the glial limitans but at a much lower level than in the meninges. In the glial limitans, Cx26 is expressed in the endfeet of glial cells that have cell bodies deep within the medulla. Cx26 is also present in cells on the very ventral surface that have long thin cell bodies in an orientation that parallels the surface. In addition Cx26 is expressed in C-shaped cells that line the entrance of blood vessels into the brain parenchyma. All of these cells in the glial limitans are positive for GFAP. Thirdly, Cx26 is expressed on cells that line blood vessels within the parenchyma. Cx26 puncta are present at the outer surface of the blood vessels between the endothelial cells and the pericytes and glia that ensheath the vessels. Precise knowledge of the cells expressing Cx26 obtained via double in situ staining is required so that we can selectively alter the expression of Cx26 to determine the specific cell types that sense CO_2 in the medulla.

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Poster

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Topic: E.04. Autonomic Regulation

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BBSRC UK

Title: Connexin 26 hemichannels: Receptors for CO2 that contribute to central chemosensitivity

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Abstract: When CO_2 reacts with H_2O it produces HCO_3^- and H^+. Consequently the partial pressure of CO_2 (PCO_2) closely controls the pH of blood and other bodily fluids. CO_2 homeostasis, through regulation of breathing, is therefore a vital function that helps to control physiological pH. CO_2-dependent ATP release from the chemosensory areas of the ventral medulla plays a key role in triggering adaptive respiratory responses (Gourine et al 2005, Nature 438, 108-111). Our previous work has demonstrated that several β connexin hemichannels respond to changes in CO_2 in the absence of a pH change (Huckstepp et al 2010, J Physiol 588, 3921-3931). Connexin 26 (Cx26) is highly localised to the ventral surface of the medulla oblongata, an important CO_2 chemosensory area and thus binding of CO_2 to Cx26 (and the subsequent opening of Cx26 hemichannels) provides a route for the CO_2-dependent release of ATP (Huckstepp et al 2010, J Physiol 588, 3901-3920). Here we investigate the nature of the interaction of CO_2 with Cx26 to determine whether it is indeed direct and to understand how this interaction leads to opening of the hemichannel.
We have used heterologous expression of various connexins in HeLa cells that have little or no endogenous connexin expression to test the CO_2 sensitivity of connexin hemichannels via CO_2-dependent loading of carboxyfluorescein and whole cell patch-clamping. We found that Cx31, a closely related member of the β connexin family, was insensitive to CO_2. This demonstrates that CO_2 sensitivity is not a property of all β connexins and is instead characteristic of a specific subgroup, comprising connexins 26, 30 and 32. Using sequence comparison of the β connexins, we identified a conserved region which is present in all of the CO_2 sensitive connexins but absent from Cx31. We mutated the Cx31 gene to include this conserved region (mCx31) and found that mCx31 now exhibited sensitivity to CO_2 as determined both by dye loading experiments and patch clamp recordings. We used an elastic network model based simulation designed using the published X-ray structure of Cx26 to investigate the first 25 normal modes for the Cx26 hemichannel and the effect of CO_2-binding on these modes. Binding of CO_2 to the conserved region dramatically altered the contribution of select normal modes to overall hemichannel motion when compared to the unbound state, making the open state more prevalent and greatly

reducing time in the closed state. We suggest CO_2 binding biases the Cx26 hemichannels towards an open state, allowing ATP release until CO_2 is removed.

Disclosures: L. Meigh: None. **T.L. Rodgers:** None. **M.J. Cann:** None. **N. Dale:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:897.13/SS5

Topic: E.04. Autonomic Regulation

Support: NS076201

HL095731

Title: Contribution of glia-neuron interactions to central control of breathing

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Abstract: Central chemosensory mechanisms maintain homeostasis of blood and brain pH and partial pressure of carbon dioxide by regulating respiration. Recent studies suggest that glia play an instrumental role in central chemoreception. However, detailed mechanisms of the interaction between glia and neurons within chemosensory brain regions are only partially known. Glial cells express a high density of glutamate transporters, thereby regulating the time course of excitatory synaptic transmission, glutamate spillover across synapses, and ambient concentration of glutamate. Since glutamate transporters are electrogenic, the glial Vm can regulate the activity of these transporters. Here, we test the hypothesis that acidification regulates excitatory neurotransmission within the nucleus of the solitary tract (NTS) by depolarizing glia and, as a result, compromising their ability to uptake glutamate. In accordance, we find that glia in the NTS respond to mild acidification (from pH 7.4 to pH 7.0) with a depolarization (11.2 ± 2.9 mV). This depolarization is accompanied by a decrease in a K+ selective membrane conductance which is occluded by1 mM Ba2+. Moreover, voltage-clamp recordings from glia show that focal application of D-aspartate in the presence of blockers of ionotropic glutamate receptors elicits a voltage sensitive inward current mediated by glutamate transporters. Concurrently, through recordings from NTS neurons, we find that repetitive stimulation (20 pulses at 10 Hz) at/near the solitary tract leads to an AMPA and NMDA receptor mediated slow excitatory potential (SEP; amplitude: 6.55 ± 0.87 mV, Tau decay: 4.3 ± 0.5 sec), likely due to a long-lasting buildup in the extracellular glutamate concentration. Accordingly, the SEP is potentiated by (D,L)-TBOA, a

blocker of glutamate transporters. Interestingly, mild extracellular acidification (from pH 7.4 to 7.0) also potentiates the SEP in a subset of NTS neurons. Together, these experiments suggest that acidification can regulate synaptic transmission within the NTS and provide a potential cellular mechanism involved in central chemoreception.

Disclosures: R. Huda: None. **D.R. McCrimmon:** None. **M. Martina:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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Program#/Poster#:897.14/SS6

Topic: E.04. Autonomic Regulation

Support: CURE Epilepsy Foundation

NIH HL104101

NS073981

Title: KCNQ and SK channels contribute to intrinsic excitability of chemosensitive neurons in the retrotrapezoid nucleus.

Authors: X. CHEN[1], J. M. HAWRYLUK[1], A. V. TZINGOUNIS[1], *D. K. MULKEY[2]
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Abstract: The retrotrapezoid nucleus (RTN) contains a population neurons (i.e., chemoreceptors) that provide a CO_2/H^+-dependent excitatory drive to respiratory centers to regulate depth and frequency of breathing. Despite the importance of these cells, little is known regarding molecular determinants of their intrinsic excitability or CO_2-responsivness. We have recently determined that RTN chemoreceptors express voltage-gated KCNQ channels and Ca^{2+}-activated small conductance K^+ (SK) channels. Here, we use electrophysiological techniques and pharmacological approaches to explore contributions of these channels to firing rate behavior and CO_2-sensitivity of RTN chemoreceptors in brain slices (300 µM) from neonatal rat pups (P7-12). In the loose-patch current clamp configuration, application of XE991 (10 µM, a selective KCQN channel blocker) increased firing rate by 1.7 ± 0.2 Hz but had no effect on CO_2-sensitivity. In contrast, application of apamin (100 nM, selective SK channel blocker) had no effect on either baseline firing activity or CO_2-sensitivity. Interestingly, the combined application of apamin and XE991 tended to increase burst-like behavior and robustly increased the firing

rate response to CO_2. These results suggest that KCNQ and SK channels are expressed by RTN chemoreceptors, and confer the ability to differentially regulate repetitive firing behavior and CO_2-responsivness. A computational model has been implemented to describe these differentiated effects.

Disclosures: **X. Chen:** Employment; University of Connecticut. **J.M. Hawryluk:** Employment; University of Connecticut. **A.V. Tzingounis:** Employment; University of Connecticut. Research Grant; CURE Epilepsy Foundation, NS073981. **D.K. Mulkey:** Research Grant; CURE Epilepsy Foundation, NIH HL104101.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

Location: Hall F-J

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Program#/Poster#:897.15/SS7

Topic: E.04. Autonomic Regulation

Support: NIH grant HL104101

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FAPESP grant 11/13462-7

Title: P2Y1-receptors are expressed by C1 cells and regulate peripheral chemoreceptor modulation of breathing and blood pressure

Authors: *I. C. WENKER[1], D. K. MULKEY[1], T. S. MOREIRA[2], A. C. TAKAKURA[3], C. R. SOBRINHO[2]
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Abstract: Central and peripheral chemoreceptors sense changes in CO_2/H^+ and/or O_2 and communicate this information to cardiorespiratory centers to regulate breathing and sympathetic outflow to ensure adequate ventilation-perfusion matching in tissues. Despite the importance of this reflex, the cellular and neurotransmitter basis for integration of this information in the brainstem is unclear. It has been shown that excitatory drive from peripheral chemoreceptors is relayed through the retrotrapezoid region (RTN) located in the ventrolateral medulla, a region

also known to contribute to central chemoreception. Evidence also suggests that purinergic signaling in this region can influence both respiratory and sympathetic drive, possibly by activation of P2Y1-receptors (P2Y1-R). Therefore, we hypothesize that P2Y1-R represent the molecular basis for central and peripheral chemoreceptor integration at the level of the RTN region. Here, we use *in vivo* and *in vitro* recording techniques to examine the role of P2Y1-R's expressed by RTN region neurons in central and peripheral chemoreception. *In vivo*, unilateral injection of MRS2365, a P2Y1-R specific agonist, into the RTN region of anesthetized adult rats increased mean arterial pressure by 23 ± 1 mmHg (compared to 4 ± 1 mmHg in control experiments) and increased phrenic nerve amplitude and frequency by 59 ± 6% and 53 ± 5%, respectively. Conversely, application of the specific P2Y1-R antagonist MRS2179 decreased peripheral chemoreceptor mediated activation of breathing and blood pressure but did not affect CO2-responsivness. *In vitro*, loose-patch recordings of neurons in slices from rats (P7-12) show that baseline activity and CO_2/H^+-sensitivity of chemoreceptors was unaffected by MRS2179 or MRS2365. Conversely, MRS2179 decreased baseline activity of non-chemosensitive neurons by 42% and MRS2365 increased activity of these cells by 630%. After recording, a subset of CO_2-insensitive P2Y1-expressing cells were filled with biocytin for later immunohistochemical characterization; 4 of 6 cells were immunoreactive for tyrosine hydroxylase and the transcription factor phox2b, suggesting they are C1 cells. These data indicate that i) P2Y1-R are preferentially expressed by C1 cells and contribute to peripheral chemoreceptor modulation of breathing and blood pressure; ii) P2Y1-R are not expressed by ventral surface chemoreceptors and do not contribute to CO2-responsivness in vivo or in vitro; iii) P2-R targeted by CO2-evoked ATP release remain undetermined. These results suggest that purinergic signaling contributes to cardiorespiratory integration at the level of the RTN region.

Disclosures: I.C. Wenker: None. **D.K. Mulkey:** None. **T.S. Moreira:** None. **A.C. Takakura:** None. **C.R. Sobrinho:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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Program#/Poster#:897.16/SS8

Topic: E.04. Autonomic Regulation

Support: FAPESP 11/13462-7

FAPESP 10/19336-0

Title: The role of purinoceptors and astrocytes of the retrotrapezoid nucleus in the chemosensory control of breathing

Authors: C. SOBRINHO[1], A. TAKAKURA[2], V. ANTUNES[1], D. MULKEY[3], *T. S. MOREIRA[1]
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Abstract: A brainstem region called the retrotrapezoid nucleus (RTN) contains a population of CO2/H+-sensitive neurons that appear to function as important chemoreceptors (i.e., neurons that regulate breathing in response to tissue CO2/H+). Evidence also indicates that CO2-evoked adenosine 5'-triphosphate (ATP) release from RTN astrocytes modulates activity of RTN chemoreceptors in vitro. However, mechanism(s) by which RTN astrocytes sense changes in CO2 in vivo are unknown, and their contribution to central chemoreception remains controversial. The goals of this study are to determine the extent to which ATP contributes to RTN chemoreception in vivo, and whether purinergic drive to RTN chemoreceptors relies on astrocytes. To test the hypothesis that RTN astrocytes contribute to chemoreception by a purinergic mechanism, we used fluorocitrate (FC) to selectively depolarize astrocytes while measuring respiratory output. Mean arterial pressure (MAP) and phrenic nerve activity (PNA) were recorded in urethane-anaesthetized, vagotomized and artificial ventilated male Wistar rats (n=6/group). Unilateral injection of fluorocitrate (1 mM in 50 nl) into the RTN increased PNA amplitude (54 ±5% of baseline) and frequency (38 ±2% of baseline), without changes in MAP (115 ±6, vs saline: 118 ±7 mmHg). Bilateral injections of pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate (PPADS, a P2-receptor antagonist) into the RTN decreased the PNA amplitude (37 ± 3%, vs saline: 54 ± 5%) and PNA frequency (30 ± 1%, vs saline: 38 ± 2%) response elicited by FC. We also use immunocytochemical techniques to show that exposure to hypercapnia resulted in cFos-immunoreactivity in glial fibrillary acidic protein (GFAP)-positive RTN astrocytes. Our results further suggest that RTN astrocytes sense changes in CO2 and release ATP to activate RTN chemoreceptors.

Disclosures: C. Sobrinho: None. **A. Takakura:** None. **V. Antunes:** None. **D. Mulkey:** None. **T.S. Moreira:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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Program#/Poster#:897.17/SS9

Topic: E.04. Autonomic Regulation

Support: CURE Epilepsy Foundation

NIH HL104101

NS073981

AHA 11PRE7580037

Sao Paulo Research Foundation

Title: KCNQ channels determine serotonergic modulation of ventral surface chemoreceptors and respiratory drive

Authors: *J. M. HAWRYLUK[1], I. C. WENKER[1], A. C. TAKAKURA[2], T. S. MOREIRA[3], A. V. TZINGOUNIS[1], D. K. MULKEY[1]
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Abstract: Chemosensitive neurons in the retrotrapezoid nucleus (RTN) regulate breathing in response to CO2/H+ changes. Their activity is also sensitive to neuromodulatory inputs from multiple respiratory centers, and thus they serve as a key nexus of respiratory control. However, molecular mechanisms that control their activity and susceptibility to neuromodulation are completely unknown. Here, we show in vitro and in vivo that KCNQ channels are critical determinants of RTN neural activity. In brainstem slices (300 µm thick) isolated from rat pups, bath application of XE991 (10 µM, a selective KCNQ channel blocker) increased basal activity by 1.4 ± 0.2 Hz and decreased serotonin responsiveness by ~50%. In contrast, inhibition of SK channels with apamin (100 nM) had no effect on basal activity or serotonin-sensitivity. Unexpectedly, neither XE991 nor apamin had any effect on other raphe transmitters. Further, we show that blocking KCNQ channels in the RTN in vivo blunts the ventilatory response to serotonin in awake and anesthetized animals. Together, these results indicate that i) KCNQ channels regulate tonic activity and serotonin-modulation of RTN neurons in vitro; ii) KCNQ channels are effectors of serotonergic-modulation in RTN neurons in vivo; iii) KCNQ channels contribute to respiratory drive in anesthetized and awake animals. Given that serotonergic dysfunction may contribute to respiratory failure, our findings suggest a new therapeutic avenue for respiratory complications associated with multiple neurological disorders.

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NS073981. **D.K. Mulkey:** Employment; University of Connecticut. Research Grant; CURE Epilepsy Foundation, NIH HL104101.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:897.18/SS10

Topic: E.04. Autonomic Regulation

Support: FAPESP 10/15692-7

FAPESP 10/19336-0

Title: Pontine Kölliker-Fuse neurons are important for chemosensory control in conscious rats

Authors: R. S. DAMASCENO[1], *A. T. TAKAKURA[2], T. S. MOREIRA[1]
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Abstract: The pontine nuclei, particularly the Kölliker-Fuse region (KF) and the lateral parabrachial nucleus (LPBN) have been implicated in the maintenance of cardiorespiratory control. However, most of the experiments showing the role of pontine KF region were done in anesthetized animals. Here we evaluated the involvement of KF region and LPBN in the cardiorespiratory responses elicited by chemoreceptor activation in conscious rats.Male Wistar rats (280-300g, n=5-6) with bilateral stainless steel guide-cannulas implanted into the KF region or LPBN were used. In conscious rats, injection of muscimol (120 pmol/100nl) into the KF decreased resting ventilation (979 ± 108, vs. saline: 1402 ± 108 ml/kg/min) and the increase in ventilation produced by hypoxia (8% O2) (1827 ± 61, vs. saline: 3179 ± 335 ml/kg/min) or hypercapnia (7% CO2) (1488 ± 277, vs. saline: 3130 ± 427 ml/kg/min). Bilateral injection of muscimol into the KF did not change resting MAP (4 ± 3, vs. saline: 5 ± 2 mmHg) and resting HR (7 ± 4, vs. saline: 8 ± 3 bpm), however, reduced the hypotension (1 ± 2, vs. saline: -13 ± 4 mmHg) and the tachycardia (58 ± 9, vs. saline: 95 ± 12 bpm) elicited by hypoxia. Bilateral injection of muscimol into the LBPN did not change resting ventilation (1389 ± 55, vs. saline: 1399 ± 32 ml/kg/min), nor the increase in ventilation elicited by hypoxia (2826 ± 61,vs. saline: 3012 ± 44 ml/kg/min) or hypercapnia (3124 ± 78, vs. saline: 3088 ± 88 ml/kg/min). The injection of muscimol into the LPBN increased resting MAP (11 ± 2, vs. saline: 4 ± 2 mmHg), without changing resting HR (3 ± 3, vs. saline: 6 ±4 bpm). In addition, muscimol into the LBPN reduced the hypotension (-5 ± 3, vs. saline: -15 ± 4 mmHg) but did not change the tachycardia (91 ± 12, vs. saline: 87 ± 11 bpm) elicited by hypoxia in conscious rats. The results suggest that

KF region, but not LPBN, have mechanisms to control the ventilation in resting, hypoxic or hypercapnic conditions in conscious rats.

Disclosures: R.S. Damasceno: None. **A.T. Takakura:** None. **T.S. Moreira:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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Topic: E.04. Autonomic Regulation

Support: International Rett Syndrome Foundation (JMB)

NIH (GM)

Rett Syndrome Research Trust (GM)

Title: Depletion of methyl-CpG-binding protein 2 (MeCP2) in astrocytes depresses chemosensitivity

Authors: *J. M. BISSONNETTE[1], S. K. GARG[2], D. T. LIOY[2], S. J. KNOPP[1], G. MANDEL[2]
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Abstract: The respiratory response to CO_2 is dampened in mouse models of Rett syndrome (RTT) (Am J Physiol 301:C729, 2011; FASEB J 26:894.6, 2012), which may contribute significantly to the frequent apnea observed in the syndrome. Astrocytes on the ventral surface of the medulla respond to changes in CO_2 with an increase in intracellular calcium and secretion of adenosine triphosphate that in turn stimulates adjacent neurons to increase ventilation (Science 329:571, 2010). Additionally astrocytes play a pivotal role in the respiratory disorders of RTT (Nature 475:496, 2011). Here we show that depletion of MeCP2 confined to astrocytes markedly depresses chemosensitivity. Studies were carried out in male mice bearing a tamoxifen-responsive Cre-recombinase under the control of a human glial fibrillary acidic protein (*hGFAP*) promotor and in which loxP sites flanked exon III of *Mecp2* (GFAP-Cre/Flox). CO_2 response was determined by measuring minute ventilation (VE) in 1, 3 and 5% CO_2. Baseline CO_2-response was performed at P40 followed by a 7-day tamoxifen (TAM) regime (100mg/Kg/day) to eliminate the expression of MeCP2 in astrocytes. Respiratory pattern in GFAP-Cre/Flox mice breathing air was not affected by TAM treatment. VE in CO_2, however, was depressed by 61.8±9.5, 60.8±11.7 and 49.9±11.4% at 1, 3 and 5% CO_2 compared to pre-TAM recordings.

Immunohistochemistry of the retrotrapezoid nucleus showed that Phox2b positive, tyrosine hydroxylase negative neurons were not compromised for MeCP2 expression. Although, neurons in the respiratory network ultimately determine the ventilatory response to CO_2, this study demonstrates that loss of MeCP2 in astrocytes alone is sufficient to generate the depressed CO_2 phenotype arguing for a critical role of MeCP2 in glia in regulation of respiratory chemosensitivity.

Disclosures: J.M. Bissonnette: None. **S.K. Garg:** None. **D.T. Lioy:** None. **S.J. Knopp:** None. **G. Mandel:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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Program#/Poster#:897.20/SS12

Topic: E.04. Autonomic Regulation

Title: Pre- and postsynaptic cholinergic modulation of locus coeruleus neurons in wild-type and mecp2-null mice

Authors: *M. F. OGINSKY[1], N. CUI[1], C. M. JOHNSON[1], G. S. CYMBALYUK[2], C. JIANG[1]
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Abstract: Rett Syndrome (RTT) is a sex-linked disease which affects 1 in 10,000 live female births and occurs because of mutations to the MECP2 gene. In addition to autistic manifestations, people with RTT have abnormalities in breathing and sleep patterns, which may be attributed to defects in the cholinergic and noradrenergic systems. To understand how cholinergic signaling affects neuronal activity in the locus coeruleus (LC), the major noradrenergic center in the brain, we studied pre- and postsynaptic cholinergic modulation of LC neurons using whole-cell patch clamp in mouse brain slices. Activation of postsynaptic nicotinic receptor caused depolarization, an increase in firing frequency and a decrease in input resistance. Presynaptic cholinergic modulation was examined by analysis of sEPSCs and sIPSCs. Blocking muscarinic acetylcholine receptors increased glutamatergic sEPSC frequency and amplitude. We found that there are two populations of GABAergic input that differentially modulates LC neurons. Nicotinic receptor activation showed an increase in sIPSC frequency and amplitude in one group, whereas the other group showed a decrease in frequency with no change in sIPSC amplitude. sIPSCs were measured from LC neurons of Mecp2-null and wild-type littermates during bath-application of mecamylamine, a nictotinic receptor antagonist. We found that cholinergic modulation was enhanced in Mecp2-null mice compared to wild-type. The decrease in sIPSC frequency was

greater in Mecp2-null mice than in wild-type mice. There was no change in amplitude. These data suggest that the Mecp2 knockout causes a defect in presynaptic modulation of LC neurons involving GABA and cholinergic systems. Lastly, as a first step towards understanding how the dysregulation of these neurons affects breathing and sleep in Mecp2-null mice, we developed a mathematical model of an LC neuron. The model possesses key ionic currents, introduced with Hodgkin-Huxley formalism, and reproduces experimentally observed spiking properties.

Disclosures: M.F. Oginsky: None. **N. Cui:** None. **C.M. Johnson:** None. **G.S. Cymbalyuk:** None. **C. Jiang:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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Program#/Poster#:897.21/SS13

Topic: E.04. Autonomic Regulation

Title: GABAergic modulation of locus coeruleus neurons in a mouse model of Rett syndrome

Authors: *X. JIN, W. ZHONG, N. CUI, C. JIANG
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Abstract: Rett Syndrome is an autism spectrum disorder caused by disruption in the Mecp2 gene. This neurodevelopmental disease has multiple neurobiological defects, especially the abnormalities in norepinephrinergic and GABAergic systems. However, the relationship between these two systems is unclear. Therefore, we performed studies to investigate how the Mecp2 knockout affects the GABAergic synaptic neurotransmission in neurons of the locus coeruleus (LC), the prominent norepinephrinergic nucleus in the central nervous system. Experiments were conducted in brain slices obtained from the wild-type and Mecp2-null mice in whole-cell voltage clamp. In comparison to the wild-type, LC neurons from mutant mice showed significant decreases in both frequency and amplitude of spontaneous $GABA_A$-ergic IPSCs. Both the frequency and amplitude of the IPSCs were significantly reduced, while the reduction of the frequency was more obvious than that of the amplitude, suggesting that the LC neurons in mutant mice are deficient in presynaptic GABAergic input. In wt LC neurons, the GABAergic IPSCs were strongly inhibited by the $GABA_B$ agonist baclofen, likely to be mediated by GABAergic auto-receptors. Such a feedback regulation of GABA release from presynaptic terminals seems intact in LC neurons of mutant mice, although it was slightly stronger than that of wild-type mice. These results suggest that the decrease of GABAergic input in LC neurons

may contribute to abnormalities of the norepinephrinergic function seen in the Mecp2-null mice as well as people with Rett Syndrome.

Disclosures: X. Jin: None. **W. Zhong:** None. **N. Cui:** None. **C. Jiang:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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Topic: E.04. Autonomic Regulation

Support: NIH Grant HL28066

Title: Antagonism of orexin receptors reduces the exaggerated respiratory response to hypercapnia observed in conscious spontaneously hypertensive rats

Authors: *A. LI[1], E. NATTIE[2]
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Abstract: Orexins (A & B), neuropeptides located in the lateral hypothalamus, are important in the regulation of arousal, food intake, and energy balance, as well as blood pressure and respiration. Studies from our lab have demonstrated that the orexin system is important in central chemoreception and acute, specific antagonism of both orexin receptors by an orally administered dual orexin receptor antagonist, Almorexant (Almxt), significantly reduces the respiratory response to hypercapnia (CO_2 response or central chemoreflex) in wakefulness in the dark period of unanaesthetized, freely moving normotensive rats (*Li & Nattie., J. Physiol 2010*). The spontaneously hypertensive rat (SHR) has in addition to increased blood pressure and sympathetic nerve activity, behavioral hyperactivity and increased prepro-orexin expression in the hypothalamus. We hypothesized that SHR rats may have an exaggerated central CO_2 chemoreflex compared to normotensive Wistar-Kyoto rats (WKY) and this augmented response may be modulated by orexin. Here in conscious SHR rats we found an increased ventilatory (~30%) response to 7% CO_2 in both wakefulness and sleep. Treatment with Almxt reduced the exaggerated CO_2 response to the same level as in WKY rats. Our results link the greater ventilatory response to CO_2 in SHR rats to orexin and suggest a role for orexin in the pathophysiology of the SHR rat.

Disclosures: A. Li: None. **E. Nattie:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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Topic: E.04. Autonomic Regulation

Support: NIH Grant HD36379

Title: Refining the serotonergic contribution to central chemosensitivity

Authors: R. D. BRUST[1], *A. E. CORCORAN[2], R. S. RAY[1], E. E. NATTIE[2], G. B. RICHERSON[3], S. M. DYMECKI[1]
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Abstract: The involvement of brain serotonergic neurons (5HT neurons) in a remarkably wide range of physiological functions and disorders motivates a deeper examination of their heterogeneity with respect to developmental origin, functional properties, and molecular expression. We are using these characteristics to subdivide 5HT neurons and study the involvement of molecularly defined subtypes in different functions. Here, we are interested in the role of 5HT neurons in central respiratory regulation, particularly in the respiratory chemoreflex (defined as an increase in ventilation in response to elevated CO_2 to restore blood gas and pH levels to normal). To test this more definitively, we developed a transgenic mouse strain capable of inducibly and reversibly inhibiting 5HT neuron activity in the awake and behaving mouse, building on the Di system developed by Bryan Roth and colleagues (Armbruster et al 2007). We have found that suppressing neuron activity in all 5HT neurons causes a decreased ventilatory response to breathing 5% CO_2 (Ray et al 2011). We now continued to apply this approach toward silencing subtypes of 5HT neurons in order to identify those that contribute most to the CO_2 respiratory chemoreflex. Using a dual recombinase (Cre and Flpe)-based intersectional genetic approach we have labeled and manipulated (via Di expression) subtypes of 5HT neurons defined by developmental gene expression differences. Present findings highlight a subset defined by having a history of expression of the transcription factor Krox20 (Egr2) and arising from hindbrain rhombomere 5. In vivo plethysmography confirms the importance of these particular neurons in the ventilatory response to hypercapnia. Using patch clamp electrophysiology we find that this same population (identified by eGFP labeling) is chemosensitive in vitro (increase firing rate in response to a decreased bath pH caused by elevating bath CO_2, a property that is not shared among all 5HT neurons). Further, immunohistochemical studies demonstrate projections from eGFP labeled Krox20 5HT neurons

to other brainstem nuclei known to be involved in cardiorespiratory control, including other non-5HT neurons implicated in central chemosensitivity. This work defines a molecular and developmental 5HT neuron subtype involved in CO_2 chemosensitivity and extends our understanding of 5HT neuron biology and its role in a vital homeostatic neuronal network.

Disclosures: R.D. Brust: None. **A.E. Corcoran:** None. **R.S. Ray:** None. **E.E. Nattie:** None. **G.B. Richerson:** None. **S.M. Dymecki:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:897.24/SS16

Topic: E.04. Autonomic Regulation

Support: RO1 NS18400

Title: Effects of metabolic acidosis on different cell types inside and outside the central nervous system

Authors: A. I. SALAMEH[1], V. RUFFIN[1], *W. F. BORON[2]
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Abstract: Metabolic acidosis (MAc), a decrease in extracellular pH (pH_o) produced by a decrease in [HCO_3^-] at fixed CO_2, usually induces a large drop in intracellular pH (pH_i) in medullary raphé (MR) neurons. Previous studies have shown that most cell types subjected to MAc exhibit $\Delta pH_i/\Delta pH_o$ that ranges from 20-30% in the non-chemosensitive cells up to 75-80% in the chemosensitive cells. However it is difficult to compare these studies results because they utilized different protocols to induce MAc and different methods to measure pH_i changes. Here we examined the effects of MAc on different cell types outside the central nervous system (CNS) and compared the results with MR neuron responses. We focused our study on two parameters **1)** the cells ability to defend themselves (i.e., have small $\Delta pH_i/\Delta pH_o$ in a first MAc pulse) and **2)** the cells ability to adapt (i.e., have less $\Delta pH_i/\Delta pH_o$ in a second MAc pulse compared with the first MAc pulse). We reduced pH_o from 7.4 to 7.2 by dropping the extracellular [HCO_3^-] from 22 mM to 14 mM at fixed 5% CO_2. Using fluorescence imaging with the pH-sensitive dye 2'-7'-bis(carboxyethyl) 5(6) –carboxy fluorescein (BCECF) to measure pH_i changes, we found that cells have wide range of responses. The skeletal muscle cell line (C2C12) and the freshly isolated guinea pig ventricular myocytes showed a strong defense in the first MAc pulse ($\Delta pH_i/\Delta pH_o$ = 2% for both) and they were able to maintain it in the second MAc pulse (5% and 0%, respectively). Cells from a colon cancer cell line (CT26) had somewhat strong defense in the

first MAc pulse (14%); however, in the second MAc pulse they were not able to adapt (32%). Primary cultures of mouse macrophages had poor defense in the first MAc pulse (46%) and they showed an even greater decrease in their pH_i in the second MAc pulse (52%). The MR neurons had large drop in their pH_i in the first MAc pulse (58%) and they showed small adaptation in the second MAc pulse (52%); however, it was not statistically significant. When we compared the cells from outside the CNS with the MR response to the first and second MAc pulses, we found statistical difference between MR neurons and C2C12, Cardiac, and CT26 cells. There was no statistical difference between MR neurons and macrophages in their responses to the first and second MAc pulses.

Disclosures: A.I. Salameh: None. **V. Ruffin:** None. **W.F. Boron:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

Location: Hall F-J

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Program#/Poster#:897.25/SS17

Topic: E.04. Autonomic Regulation

Support: NIH/NIAID contract N01-AI-30063

NIH/NIAID grant 1U54 AI-065357-04

Title: Respiratory insufficiency is the likely mechanism of death in West Nile virus (WNV) disease and possibly for other viral encephalitides

Authors: *J. D. MORREY[1], H. WANG[1], V. SIDDHARTHAN[1], R. M. HARPER[2]
[1]Utah State Univ., LOGAN, UT; [2]Dept. of Neurobiology, David Geffen Sch. of Med., Univ. of California at Los Angeles, Los Angeles, CA

Abstract: The mechanism of death is unknown for fatal WNV disease. Since respiratory insufficiency has a poor-prognosis in human WNV disease, we addressed the hypothesis that WNV infects neurological tissues to cause fatal respiratory failure, and that other viral encephalitides may have a similar mechanism of death. WNV infection of rodents suppressed diaphragmatic and intercostal electromyography (EMG) activities, which correlated with medullary infection, in contrast to spinal cord, lung, diaphragm, or other extra-neurologic tissues. Direct ventrolateral medullary injection of viral solution caused rapid and robust loss of diaphragmatic EMG. To determine if this electrophysiological suppression results in respiratory insufficiency, plethysmography patterns in mortally WNV-infected mice were compared to surviving WNV-infected and sham-infected mice. All respiratory parameters measured,

including minute volume, frequency, and peak inspiratory and expiratory volumes, were suppressed in mice that died from infection, but not in surviving WNV-infected or sham-infected mice. Suppression of diaphragmatic EMG was correlated with suppression of plethysmography parameters. SaO_2 values were significantly lower in WNV-infected compared to sham-infected mice. Among respiratory "drives," chemoreceptor processes were specifically impaired; no alert fatally-ill WNV-infected mice improved ventilation to a 7% CO_2 challenge, whereas, 60% of the sham-infected mice responded. Most of the histopathology and WNV-infected cells were located in the ventral medulla. Remarkably, most of the WNV-stained neurons in the rostral ventral medulla co-stained for somatostatin, a neuropeptide with respiratory roles. Histopathology and WNV-infected cells also appeared in the retrotrapezoid nucleus, identified with Phox2b staining. Similar ventilatory suppression also occurred in other viral encephalitic infections, including Japanese encephalitis virus. The likely mechanism of death in WNV, and possibly other viral encephalitides includes viral infection of the ventral medulla leading to fatal respiratory insufficiency.

Disclosures: J.D. Morrey: None. **H. Wang:** None. **V. Siddharthan:** None. **R.M. Harper:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:897.26/SS18

Topic: E.04. Autonomic Regulation

Support: NIH grant HL028785

Title: C1 and RTN neuron stimulation produces cortical arousal in sleeping rats

Authors: *S. B. ABBOTT, M. B. COATES, R. L. STORNETTA, P. G. GUYENET
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Abstract: Sleep disordered breathing is associated with repeated bouts of hypoxia and hypercapnia leading to respiratory, autonomic and cortical arousal. Phox2b-expressing neurons of the retrotrapezoid nucleus (RTN) and C1 adrenergic population in the rostral ventrolateral medulla (RVLM) are important for the cardiorespiratory response to hypoxia and hypercapnia and innervate brain regions that control wakefulness. In this study, we evaluate if selective stimulation of Phox2b-expressing neurons is sufficient to produce sleep to wake transitions during non-rapid eye movement sleep (NREMS) and rapid eye movement sleep (REMS) in rats instrumented for recordings of cortical EEG and neck EMG in a whole-body plethysmography

chamber. Channelrhodopsin2 (ChR2) was restricted to C1 and RTN neurons using a lentivirus vector carrying a Phox2b-dependent promoter. Photostimulation (2-20 Hz, 10 ms pulses) produced frequency dependent transitions from NREMS to wakefulness in 72% of trials ($P<0.001$, N=8). Stimulation during REMS produced transitions to wakefulness in 34% of trials ($P<0.05$, N=7). Photostimulation during NREMS increased tidal volume (TV: +34%) and breathing frequency (fR: +57%), whereas only TV was increased (+24%) during REMS (fR: +5%). The arousal and ventilatory effects of photostimulation were significantly correlated during NREMS and REMS. Photostimulation trials causing cortical desynchronization were associated with single augmented breaths/sighs in 59% of NREM trials and 46% of REM trials. On average, EEG desynchronization preceded sigh breaths by 4.8 ± 1.0 s. This study demonstrates that photostimulation of RVLM Phox2b neurons produces cortical arousal in sleeping rats and facilitates sigh breaths. This work shows that the activation of RVLM Phox2b neurons is sufficient to produce stereotypic cardiorespiratory and cortical arousal responses to hypoxia and hypercapnia during sleep.

Disclosures: S.B. Abbott: None. **M.B. Coates:** None. **R.L. Stornetta:** None. **P.G. Guyenet:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:897.27/SS19

Topic: E.04. Autonomic Regulation

Support: FAPESP

CNPq

Title: Purinergic receptors of the lateral parabrachial nucleus are involved on compensatory responses to hypoxia

Authors: *P. M. DE PAULA, M. F. MENEZES, M. T. FAVERO, J. V. MENANI
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Abstract: The lateral parabrachial nucleus (LPBN), a pontine structure located dorsal-laterally to the superior cerebellar peduncle, is part of the hindbrain circuitry involved in the respiratory control. Hypoxia triggers cardiorespiratory responses, including increased pulmonary ventilation (VE) that depends on the peripheral chemoreceptor projections to the nucleus of the solitary tract (NTS) an area strongly connected to the LPBN. Adenosine-5'-triphosphate (ATP) is considered

an important central neurotransmitter and purinergic receptors are present in the LPBN. However, the involvement of purinergic mechanisms of the LPBN in the control of VE during hypoxia is still unknown. In the present study, we investigated the effects of alpha, beta- methyl ATP (a selective P2X purinergic receptor agonist) injected into the LPBN on cardiorespiratory responses to hypoxia in awake rats. Male Holtzman rats (290-310 g, n=5-8/group) with stainless steel cannulas implanted into the LPBN were used. A polyethylene tubing (PE-10 connected to a PE-50) was inserted into abdominal aorta through femoral artery for recording mean arterial pressure (MAP) and heart rate (HR) in awake rats. Respiratory frequency (fR), tidal volume (VT) and VE were recorded by whole-body plethysmography. Rats received bilateral injections of alpha, beta- methyl ATP (2 nmol/200 nl) or saline into the LPBN 5 min before starting the hypoxia. Hypoxia (7% O2 for 60 min) reduced MAP (92 ± 2 mmHg, vs. normoxia: 103 ± 9 mmHg, $p<0.05$), and increased HR (498 ± 9 bpm, vs. normoxia 389 ± 7 bpm; $p<0.01$) and VE (1131 ± 110 ml/min/kg, vs. normoxia 512 ± 33 ml/min/kg, $p<0.01$). Bilateral injections of alpha, beta- methyl ATP into the LPBN enhanced the increase of VT produced by hypoxia (Δ 4.0 ± 0.3 mL/kg, vs. saline 2.8 ± 0.2 mL/kg, or 42% of increase, $p = 0.013$), without significantly changing hypoxia-induced tachypnea (Δ 51 ± 2 cpm, vs. saline 55 ± 9 cpm, or 8% of reduction, $p=0.721$) or VE (Δ 794 ± 68 mL/kg/min, vs. saline 619 ± 110 mL/kg/min, or 22% of increase, $p=0.122$). Bilateral injections of alpha, beta- methyl ATP into the LPBN did not change hypotension (Δ -8 ± 2 mmHg, vs. saline: -11 ± 2 mmHg) and tachycardia (Δ 129 ± 12 bpm, vs. saline 109 ± 9 bpm) during hypoxia. The results showed that the activation of purinergic receptors in the LPBN enhanced the increase of tidal volume produced by hypoxia. However, hypoxia-induced hyperventilation was not significantly increased by the same treatment, probably as a consequence of the reduced, although not significantly, increase in respiratory frequency.

Disclosures: P.M. De Paula: None. **M.F. Menezes:** None. **M.T. Favero:** None. **J.V. Menani:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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Program#/Poster#:897.28/SS20

Topic: E.04. Autonomic Regulation

Support: CIHR, Grant MT-15563

Collaborative Discovery Grant from Eli Lilly and Company

Title: The loss of upper airway muscle activity in sleep is prevented by potassium channel blockade at the hypoglossal motor pool

Authors: *K. P. GRACE[1], R. L. HORNER[2]
[2]Med., [1]Univ. of Toronto, Toronto, ON, Canada

Abstract: Obstructive sleep apnoea (OSA) is a growing public health burden, characterized by repeated episodes of airway narrowing and closure during sleep. Attempts to treat OSA pharmacologically have been unsuccessful because they fail to address the root cause of pharyngeal hypotonia during sleep: the state-dependent inhibition of motoneurons that rhythmically activate the pharyngeal musculature. Increasing potassium conductance is a potential convergent mechanism by which state-dependent neuromodulators reduce motoneuron excitability. Therefore, we determined if potassium channel blockade is an effective strategy to reactivate pharyngeal musculature during sleep. Using reverse microdialysis in 31 rats chronically instrumented for recording of sleep-wake states and respiratory muscle activity, we locally microperfused pharmacological agents at the hypoglossal motor pool to block potassium channels of the three major classes: voltage-gated, inwardly rectifying, and two-pore domain leak channels. Local blockade of voltage-gated and inwardly rectifying potassium channels at the hypoglossal motor pool restored respiratory and tonic pharyngeal motor activity in non-REM and REM sleep to 133-300% of waking values. Importantly, such a complete reversal of airway hypotonia during sleep, particularly in REM sleep, is not possible with other re-activation strategies including blockade of fast-synaptic inhibition or local monoaminergic, glutamatergic, and nicotinic receptor stimulation. Recent efforts to develop OSA pharmacotherapy have focused on two-pore domain type TASK (TWIK-related acid sensitive potassium) channels which are highly expressed at hypoglossal motor neurons. However, we show that activating effects of local TASK channel blockade on pharyngeal motor activity are largely absent in sleep and occur only in wakefulness. Targeting hypoglossal TASK channels may therefore have limited therapeutic potential for the treatment of OSA. Our results show that potassium channel blockade at the hypoglossal motor pool is the most effective strategy identified to date for the preservation of upper airway motor activity during sleep. Potassium channel subtypes, other than TASK, having high hypoglossal expression (i.e. Inwardly rectifying potassium channel 2.4) represent the most significant therapeutic targets for OSA pharmacotherapy.

Disclosures: K.P. Grace: None. **R.L. Horner:** None.

Poster

897. Respiratory Regulation: Central Respiratory Chemoreception

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Program#/Poster#:897.29/TT1

Topic: E.04. Autonomic Regulation

Support: NIH Grant HL111598

NIH Grant HL80209

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NIH Grant T32 HL007654

Title: Severe intermittent hypoxia causes systemic inflammation and impairs respiratory plasticity

Authors: *A. G. HUXTABLE, S. M. SMITH, T. PETERSON, J. J. WATTERS, G. S. MITCHELL
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Abstract: Inflammation is prevalent in many diseases and disorders that challenge ventilatory control (eg. lung disease, spinal injury, neurodegenerative diseases and obstructive sleep apnea). Although inflammation can enhance and undermine plasticity in various neural systems, little is known concerning the impact of inflammation on plasticity in respiratory motor control. Here, we study the impact of systemic inflammation induced by 8 hrs of severe intermittent hypoxia (sIH, 2 min, 10.5% O2 separated by 2 min normoxia), a model which simulates hypoxic episodes experienced during obstructive sleep apnea. We tested the hypotheses that this model of sIH: 1) upregulates inflammatory genes in the cervical spinal cord; and 2) undermines an important model of spinal respiratory plasticity, phrenic long-term facilitation (pLTF) induced by acute intermittent hypoxia (AIH, 3X5 min hypoxia; 5 min intervals). Preliminary data suggest that sIH significantly reduces pLTF from 33±9% (n=7) in normoxic controls vs. -1±5% (n=6). However sIH had no detectable effect on the short term hypoxic phrenic response. Treatment with the non-steroidal anti-inflammatory drug ketoprofen appears to restore pLTF after sIH (45%, n=1). Spinal gene expression showed elevated IL-1β in isolated microglia (n=8) immediately after sIH, but there were no detectable changes in microglial COX-2 or TNFα. Understanding the impact of systemic inflammation on respiratory plasticity is important since inflammation may undermine the capacity for respiratory compensation during pathological states and the ability to harness respiratory plasticity as a therapeutic strategy in the treatment of devastating breathing disorders, such as cervical spinal injury or motor neuron disease.

Disclosures: A.G. Huxtable: None. **S.M. Smith:** None. **T. Peterson:** None. **J.J. Watters:** None. **G.S. Mitchell:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:898.01/TT2

Topic: E.05. Stress and the Brain

Title: Nutrient availability defines the expression and localization of SIRT1 in PC12 cells

Authors: K. FUJINO, Y. HASHIMOTO, *T. NEDACHI
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Abstract: Nutrient availability is one of the most important signals that regulate cellular fates including cell growth, differentiation, and death. Accumulated evidence suggested NAD^+-dependent histone deacethylase, sirtuin 1 (SIRT1) plays prominent role on connecting between changes in nutritional availability and regulation of cellular functions. SIRT1 was initially identified as a senescence-regulating enzyme; however, recent studies suggested that SIRT1 not only regulates senescence of cells but also the other cellular functions, including skeletal muscle differentiation and metabolic regulation in hepatocytes. SIRT1 expression is also observed in the nerve cells; however, the role of SIRT1 and/or expressional regulation of SIRT1 are not fully understood. The aim of present study is that to elucidate whether and how the glucose availability affects the expression and localization of SIRT1 using PC12 cells.
Initially, we found the total expression level of SIRT1 in PC12 cells was reduced in response to increasing in glucose availability. The gene expression of SIRT1 was also produced similar changes. Next, we checked whether the intracellular localization of SIRT1 was changed by glucose availability. Upon increasing in glucose availability, SIRT1 localization was gradually changed from nucleus to cytoplasm. During this high glucose-dependent locational change, SIRT1 appeared to be glycosylated. Currently, the impact of this glycosylation on SIRT1 function was unclear; however, it could be suggested that the modification of SIRT1 protein and the regulation of SIRT1 amounts cooperatively control acethylation of intracellular proteins, thereby cellular functions. We finally confirmed physiological relevance of this SIRT1 regulation by high glucose condition because SIRT1 overexpression is known to stimulate neurite outgrowth. As expected, high glucose condition attenuated neurite outgrowth induced by NGF.In conclusion, in this study, we successfully identified several mechanisms of glucose-dependent SIRT1 regulation. Because SIRT1 is now considered as neuroprotective proteins, further studies on this issue may provide methodological advance against neurodegenerative diseases.

Disclosures: K. Fujino: None. **Y. Hashimoto:** None. **T. Nedachi:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

Location: Hall F-J

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Topic: E.05. Stress and the Brain

Support: NIH Grant R21MH080500-01A2

Title: Fluoxetine alters the neurogenomic profiles and anxiety-related behavior in wild-derived zebrafish

Authors: *R. Y. WONG[1], S. OXENDINE[2], X. YAO[2], J. GODWIN[2]
[1]Biol., North Carolina State Univ., Raleigh, ; [2]Biol., North Carolina State Univ., Raleigh, NC

Abstract: Anxiety disorders account for a large proportion of mental illnesses with over 40 million people in the U.S. being diagnosed with an anxiety-disorder. Studies have shown that in humans and animals, treatment with selective serotonin reuptake inhibitors (SSRIs; e.g. fluoxetine) can reduce anxiety and anxiety-like behaviors. Yet treatments with SSRIs in humans can vary in their efficacy and have many side effects due to the wide ranging influences of serotonin. To better understand the molecular consequences of fluoxetine treatment, we chronically administered fluoxetine to zebrafish (*Danio rerio*), which is an emerging model system for the study of anxiety-related behaviors, and characterize the brain transcriptome using RNA-Sequencing. Both racemic and stereoismeric fluoxetine treatment had anxiolytic effects on anxiety-related behaviors in our wild-derived strains using the Novel Tank Diving Test paradigm. We identified 637 genes that were differentially expressed between racemic fluoxetine treatment and control. Gene ontology (GO) analyses reveal that over half of the genes that are over-enriched belong to Biological Processes with metabolic functions. We observed similar GO categorization patterns when separately examining genes significantly up-regulated in fluoxetine (352) and control (285) treatments. Further we observed some consistent patterns of expression in putative rodent neurochemical biomarkers of fluoxetine efficacy and stress response. Overall, we describe the influences of fluoxetine on the transcriptome and point to other potential targets and effects of the drug.

Disclosures: R.Y. Wong: None. **S. Oxendine:** None. **X. Yao:** None. **J. Godwin:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

Location: Hall F-J

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Program#/Poster#:898.03/TT4

Topic: E.05. Stress and the Brain

Title: Sp1 serves pivotal role in negative regulation of PAC1 gene expression by involvement of transglutaminase 2.

Authors: A. MIURA, Y. KAMBE, K. INOUE, T. KURIHARA, *A. MIYATA
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Abstract: Pituitary adenylate cyclase activating polypeptide (PACAP) functions as a pleiotrophic neuropeptide through three types of G protein-coupled receptors (GPCRs), PAC1, VPAC1, and VAPC2. PAC1 binds exclusively to PACAP with higher affinity than VIP. Recently, we reported that nerve growth factor augmented PAC1 gene expression through the activation of Sp1 via Ras/MAPK pathway in PC12 cells (Miura A, et al., 2012). On the other hand, it was reported that global ischemia induced a persistent reduction in the hippocampal PAC1 expression, which is moderately localized in the CA1 pyramidal cell layer and highly in the granule cell layer (Riek-Burchardt M, et al., 2010). Further, we also observed that PAC1 expression in Neuro2a cells was transiently suppressed during in vitro ischemic condition, oxygen-glucose deprivation (unpublished data). Since endoplasmic reticulum (ER) stress is induced by ischemia, we attempted to clarify whether Tunicamycin (TM), which induces ER stress, affects on the expression of PAC1. As a result, TM potently suppressed the expression of PAC1 in addition to induction of apoptosis. Salubrinal, a selective inhibitor of the ER stress-induced pancreatic ER kinase (PERK) signaling pathway, cancelled its suppression. Then we attempted to elucidate the molecular mechanism of negative regulation of PAC1 expression due to ER stress. Luciferase reporter assay demonstrated that the mutated vectors for each 5 Sp1 site at -314/-305, -282/-273, -116/-107, +184/+188 or +227/+236bp cancelled the suppression due to ER stress. Further western blot analysis showed that protein level of Sp1 was significantly decreased, although its mRNA level did not changed. Recently it was reported that transglutaminase 2 (TG2), which is a cross-linking enzyme and implicated in apoptosis, is induced in the nuclei by ER stress, resulting in crosslinking and inactivation of Sp1 (Tatsukawa et al., 2009). Then we pretreated the cells with cystamine, which is a TG activity inhibitor, before TM treatment. As a result, suppression of PAC1 gene expression was significantly cancelled as well as Salubrinal. These suggest that Sp1 might be a crucial transcriptional element in negative regulation of PAC1 gene expression with involvement of TG2 in ER stress.

Disclosures: A. Miura: None. **Y. Kambe:** None. **K. Inoue:** None. **T. Kurihara:** None. **A. Miyata:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

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Topic: E.05. Stress and the Brain

Support: NSF IOS 0922085 (MO)

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Title: Localization of the corticosterone-sensitive organic cation transporter 3 (OCT3/Slc22a3) mRNA and protein in the male rat brain via *in situ* hybridization and immunohistochemistry

Authors: *J. S. TALBOOM[1,2], J. MOLINARO[1], H. C. TOMPKINS[1], L. MUMAW[1], C. A. LOWRY[3], K. J. RENNER[4], M. ORCHINIK[1]
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Abstract: The organic cation transporter 3 (OCT3/Slc22a3) is a low affinity and high capacity polyspecific monoamine transporter found in the human and rat brain. OCT3 is believed to clear extraneuronal monoamines (e.g., serotonin, norepinephrine, and dopamine), thereby helping to terminate their signal after release. OCT3 may complement other specific (e.g., SERT, DAT, and NET) or polyspecific (e.g., PMAT, OCT1, and OCT2) transporters in regions of the brain that are innervated by monoaminergic terminals. However, OCT3 is unique in that it is the only known monoamine transporter inhibited by physiological levels of corticosterone (CORT). Among other stress-related neural processes, CORT-mediated inhibition of OCT3 may modulate the hypothalamic-pituitary-adrenocortical (HPA) axis via alterations in monoaminergic neurotransmission. We hypothesize that OCT3 functions in hierarchical systems regulating the HPA axis, including areas of the hippocampus, the amygdala, and the hypothalamus, to increase extracellular monoamine concentrations during the acute stress response. Previous studies determining the location of OCT3 mRNA in the rat brain have been contradictory. Therefore, we determined the location of OCT3 mRNA and protein through *in situ* hybridization (ISH) and immunohistochemistry (IHC). In order to examine OCT3 mRNA in the brains of young male Sprague-Dawley rats, digoxigenin (DIG)-labeled OCT3 riboprobes were *in vitro* transcribed in a process starting with RNA isolated from the rat brain. These riboprobes were then used to perform ISH in rat brain sections following a standard DIG ISH protocol. Separate brain sections were processed for OCT3 IHC following a standard chromogenic protocol. Our results indicated that OCT3 mRNA is found in the hippocampus, amygdala, and hypothalamus. Furthermore, the OCT3 mRNA distribution corresponded to the OCT3 protein distribution in these brain regions.

Collectively, these data support the conclusion that CORT-sensitive OCT3 is expressed in brain regions that mediate behavioral and neuroendocrine stress responses, which may lead to a better understanding of CORT-mediated regulation of the HPA axis.

Disclosures: J.S. Talboom: None. **J. Molinaro:** None. **H.C. Tompkins:** None. **L. Mumaw:** None. **C.A. Lowry:** None. **K.J. Renner:** None. **M. Orchinik:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:898.05/TT6

Topic: E.05. Stress and the Brain

Title: Integrative adaptive responses to mild mitochondrial uncoupling emphasize arc/arg3.1 signaling and tsc2-associated mtor inhibition

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Abstract: At the organismal level, mild energetic stress (e.g., dietary restriction and exercise) enhances neuroplasticity and increases the resistance of the nervous system to injury and disease (Stranahan and Mattson, *Nat. Rev. Neurosci.* 2012; 13: 209-216). At the cellular level, mild mitochondrial uncoupling enhances energy expenditure by dissipating energy production in mitochondria. In the present study we examined the effects of mild energetic stress on molecular signaling networks related to bioenergetics in cultured rat cerebral cortical neurons and in the brains of adult mice. Exposure of cultured cortical neurons to subtoxic levels of the mitochondrial uncoupling agent 2, 4-DNP elicited a stress response as indicated by enhanced glucose uptake and NADH oxidation. C57BL6 mice were exposed to 2, 4-DNP at a dose that was previously shown to be neuroprotective, and gene microarray analysis was performed on RNA samples isolated from cortical tissue after 2, 4-DNP administration. Analysis of bioenergetics-related signaling pathways and network linkage analysis revealed prominent up-regulation of TSC2 (tuberous sclerosis complex), which is linked to inhibition of mammalian target of rapamycin (mTOR) complex mTORC1 and mTORC2/Rheb signaling. Genes related to insulin receptor signaling pathway and PI3K/pAkt signaling were mostly down-regulated. Genes encoding BDNF and Arc/Arg3.1, two important stress- and activity- responsive proteins that influence neuronal network activity and energy metabolism were also up-regulated. Changes in the levels of several of the key proteins encoded by the up- and down-regulated genes were

confirmed by immunoblot analysis. The increased Arc/Arg3.1 expression was associated with changes in genes involved in the regulation of cellular endocytosis. Our findings suggest that mild mitochondrial uncoupling induces a coordinated cellular network of bioenergetics- and plasticity-related stress responses in cerebral cortical neurons.

Disclosures: D. Liu: None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:898.06/TT7

Topic: E.05. Stress and the Brain

Support: NIDA Grant R01 DA016682

NIDA Grant R24 DA017298

Title: Differences in pattern of extracellular regulated-protein kinase activation in male and female C57BL/6 mice caused by stress

Authors: *M. GARG[1], A. TSENG[1], M. MILET[1], M. AZIZY[1], A. HAMID[2], P. MARQUEZ[1,2], K. LUTFY[1,2]
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Abstract: Activation of extracellular regulated-protein kinase (ERK) has been implicated in neuronal plasticity, mood disorders and drug addiction. In the present study, we assessed whether there is a gender-related difference in ERK activation, as measured by the level of phosphorylated ERK (p-ERK) following swim exposure. Male and female C57BL/6 mice were allowed to freely swim in 23°C-water for 15 min. The amount of time the animals remained immobile was recorded and used as a measure of depressive-like behaviors. At the end of swim, mice were sacrificed and their brains were removed. Different brain regions were dissected out, homogenized in RIPA buffer and assayed for protein concentration. A sample containing 25 µg of protein was blotted and assayed for phosphorylated ERK using the primary (phospho-Erk1/2Thr202/Tyr204(D13.14.4E, Rb mAb), Cell Signaling Technology; 1:1000 dilution)and secondary (anti-rabbit HRP-linked antibody (7074), Cell Signaling Technology; 1:1000 dilution) antibodies. Samples were also assayed for beta-actin using the primary (anti-actin mouse monoclonal IgG (MAB1501), Millipore; 1:2000) and secondary HRP-conjugated goat anti-mouse IgG (12-349), Millipore; 1:2000) antibodies. The level of p-ERK activation was

expressed as percent of p-ERK level in naïve animals for each gender. Immobility time appeared to be higher in female than male mice but the difference was not statistically significant ($p>0.05$). Highest levels of p-ERK were found in the cerebellum followed by the cortex, striatum, hippocampus, midbrain, hypothalamus and pons. Although p-ERK level was increased in almost all brain areas in male mice following the 15-min swim exposure, it was not altered in female mice except in the cortex (increased by about 50%) and hippocampus (reduced by about 20-30%). However, the differences in ERK activation and immobility time observed between male and female mice were not statistically significant ($p>0.05$). These results suggest that there exist no gender-related differences in immobility time or ERK activation following acute stress exposure.

Disclosures: M. Garg: None. **A. Tseng:** None. **M. Milet:** None. **M. Azizy:** None. **A. Hamid:** None. **P. Marquez:** None. **K. Lutfy:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

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Topic: E.05. Stress and the Brain

Support: MH40008

DA09082

Title: Fate of internalized corticotropin releasing factor receptors in the rat locus coeruleus in a social defeat paradigm

Authors: *B. A. REYES[1], K. VAKHARIA[1], S. K. WOOD[2], R. J. VALENTINO[2], E. J. VAN BOCKSTAELE[1]
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Abstract: Exposure to repeated stress is known to contribute to the onset of a number of psychiatric disorders. In rodents, social stress can be modeled using the social defeat paradigm. Previously, it was shown that rats exposed to social defeat cluster into two distinct subpopulations defined by the latency to assume the defeat posture; namely, short latency (SL) and long latency (LL). These subpopulations exhibit distinct endocrine and behavioral phenotypes as well as different expression levels of corticotropin-releasing factor (CRF) and

CRF receptor (CRFr) in selected brain regions. The locus coeruleus (LC) norepinephrine system is a stress-sensitive region whose neurons exhibit changes in the cytoplasmic vs plasmalemmal distribution of the CRF receptor following social defeat. In the present study, high-resolution electron microscopy using different sized immunogold-silver particles to detect CRFr and a marker of early endosomes was used to probe the fate of internalized receptors within LC neurons. Sprague-Dawley rats were subjected to repeated (5 days) social defeat or control manipulation and perfused 24 h after the last session. Sections through the LC were analyzed for the association of CRFr with early endosome antigen 1 (EEA), a marker of early endosomes (Arjonen et al., Traffic, 13: 610-625). Following social defeat, LL rats (n = 3) exhibited an increased association of CRFr with EEA as evidenced by an increased in proximity (0.155 μm) of differentially sized gold-silver particles indicative of CRFr and EEA within the cytoplasmic compartment compared to control (n = 4) and SL (n = 3) rats. These results indicate that following social defeat, CRFr is internalized and potentially recycled back to the cell surface. Additional time points are required to determine whether CRFrs return to the cell surface in LL rats. Adaptations in the distribution of CRFr in LC neurons may have important consequences on behavioral and physiological responses to stress.

Disclosures: B.A. Reyes: None. **K. Vakharia:** None. **S.K. Wood:** None. **R.J. Valentino:** None. **E.J. Van Bockstaele:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

Location: Hall F-J

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Program#/Poster#:898.08/TT9

Topic: E.05. Stress and the Brain

Support: PAPIIT grants IN-207210 and IN-205410 from Universidad Nacional Autónoma de México

Title: Changes in the brain lipid profile of rats chronically exposed to extremely low frequency electromagnetic fields and movement restriction

Authors: *J. MARTÍNEZ-SÁMANO[1], A. FLORES-POBLANO[1], P. TORRES-DURÁN[1], M. JUÁREZ-OROPEZA[1], L. VERDUGO-DÍAZ[2]
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Abstract: Diary exposition to electromagnetic fields generated by various electric devices as electrical installation, can origin a chronic exposition to extreme low frequency electromagnetic

fields (ELF-EMF). The interaction between this type of radiation and the biological systems still unclear, but there are some theories that try to explain this interaction. One of them is the half life extension of free radicals that can produce damage of tissues. The brain is particularly vulnerable to damage by free radicals due to its high metabolic rate and its low antioxidant enzymes levels. Lipids play an important role in nervous system. It is known that physical/physiological stress has an effect in the oxidative status balance, leading to oxidative stress and deterioration of lipid membranes. The aim of the present study was to evaluate the effects of chronic exposure (2 h/day, for 21 days) to ELF-EMF (60 Hz, 2.4 mT), movement restraint (MR, 2h/day, for 21 days) and both, in the brain lipid profile of adult Wistar rats. After the treatments, the rats were killed by cervical dislocation. The brains were dissected and separated into three fractions: cerebral cortex, cerebellum and subcortical structures. This procedure was made to see treatment differences in brain regions. The brain fractions were homogenized and total lipids were extracted with Folch's method. Total cholesterol, triacylglycerols, non-esterified fatty acids and phospholipids were determined in the samples. A fraction of total lipid extract was used to determine the non-saturated/saturated lipids, through gas chromatography. Preliminary results showed that, there are not statistical differences in content of triacylglycerols in any of the fractions analyzed. The cholesterol content showed a significant increase ($p<0.05$) in the cortex and cerebellum of groups exposed to EMF. The phospholipid content showed a significant increase in the groups exposed to EMF ($p<0.05$) in cerebral cortex and the group exposed to EMF and MR ($p<0.05$) in the subcortical structures. The gas chromatography showed an increase in the unsaturated fatty acids and decrease in saturated fatty acids in the cortex ($p<0.05$). These preliminary data suggest that the EMF effects are specific in brain lipid composition and for some brain regions and, deserve more investigation.

Disclosures: J. Martínez-Sámano: None. **A. Flores-Poblano:** None. **P. Torres-Durán:** None. **M. Juárez-Oropeza:** None. **L. Verdugo-Díaz:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:898.09/TT10

Topic: E.05. Stress and the Brain

Title: Novel atypical d3 dopamine receptor agonist exhibits antidepressant-like activity

Authors: *S. J. FURST[1,2], S. COTE[1], S. KORTAGERE[3], E. KUZHIKANDATHIL[4]
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Abstract: The dopamine neurotransmitter activates D1-like (D1, D5) and D2-like dopamine receptors (D2, D3, and D4). The D2-like receptors modulate adenylate cyclases, ion channels and protein kinase cascades. Previous studies have reported antidepressant-like activities of the dopamine D2/D3 receptor agonists such as pramipexole and PD128907. The D3 receptor in particular has been implicated in the pathophysiology of major depression. We have previously characterized two properties that distinguish D3 from D2 receptors. The tolerance property of the D3 receptor is the progressive decrease in receptor signaling function upon repeated stimulation by classical agonists, including dopamine. The slow response termination (SRT) property is the prolongation of time taken to terminate the signaling function of the D3 receptor, after removal of the agonist. Interestingly, while PD128907 induces severe D3 receptor tolerance and SRT, we have recently identified a novel D3 receptor agonist, ES609, that does not induce tolerance and SRT. The objective of this study was to compare the effects of ES609 and PD128907 on depression induced by prolonged restraint stress. The antidepressant efficacy of ES609 and PD128907 were compared using the Forced Swim test (FST). A novel Drd3-EGFP transgenic mouse model was utilized for these studies. FST measures induction of a depressed state by forcing mice to swim in a narrow container from which they cannot escape. A characteristic immobile posture is adopted after a brief period of vigorous activity. Depressive state is measured by duration of immobility and frequency of immobility bouts. To reduce individual variability, Drd3-EGFP mice were submitted to 6 min of pre-training in the FST on day 1. On day 2, the mice were subjected to a “mild stressor” for 2 hrs in which the animal was restrained in a 50 ml plastic tube. Subsequently, we subcutaneously administered 10 mg/kg ES609, 0.05mg/kg PD129807, or saline vehicle 30 min before the test. Each animal was then submitted to 6 min of FST and the total duration of immobility and the frequency of immobility bouts measured during the last 4 min of the test. No baseline differences were observed between the various experimental groups during the pre-training FST. ES609 produced a significant reduction in immobility as well as frequency of immobility bouts in the stressed animals compared to PD128907 and saline vehicle. This study showed that ES609 is more effective than a D3 selective dose of PD128907. The results suggest that the novel atypical D3 receptor agonists that do not induce D3 receptor tolerance and SRT might have antidepressant-like activity in a restraint stress model.

Disclosures: S.J. Furst: Other Research Support; FM Kirby Foundation. **S. Cote:** None. **S. Kortagere:** None. **E. Kuzhikandathil:** Other Research Support; FM Kirby Foundation.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:898.10/TT11

Topic: E.05. Stress and the Brain

Title: Ovarian steroids regulate the gene expression of GR co-chaperones Fkbp5 and Ppid

Authors: *C. H. BOURKE[1], S. A. MALVIYA[2], S. D. KELLY[3], C. S. HARRELL[3], G. N. NEIGH[3]
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Abstract:
A common clinical finding in patients with major depression is hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, the system that regulates the stress response. It has been suggested that alterations in the glucocorticoid receptor (GR)-mediated negative feedback prolongs the activation of the HPA axis, leading to the dysfunction observed in a significant subset of depressed patients. Previous studies from our lab show a sexually dimorphic change in the expression of molecular regulators and a reduction in GR sensitivity in female Wistar rats that were exposed to chronic adolescent stress. In addition, chronically stressed female rats displayed a more pronounced release of ovarian and stress hormones in response to an acute stressor. These changes could underlie the female vulnerability to developing depressive-life behavior (Bourke et al 2012). The present study tests the hypothesis that ovarian steroids are sufficient to induce alterations in the GR co-chaperone system . Using Real-Time PCR, we determined the extent to which serum ovarian steroids and uterine weights predicted the expression of GR and two co-regulators of GR sensitivity, *Fkbp5* and *Ppid*.Then, we assessed the impact of corticosterone (cort), estradiol (E2) and progesterone (P4) exposures on the expression of these genes *in vitro* in HT-22 mouse hippocampal neurons. Uterine weights positively correlated with the expression of *Fkbp5* and *Ppid*, but did not predict the expression of *Gr*. In HT-22 neurons, cort increased the expression of *Fkbp5* in a dose dependent manner. At the highest dose of cort tested, the expression of *Fkbp5* was 1.5 fold higher than baseline. E2 treatments administered
in conjunction with cort augmented the increase in *Fkbp5* expression. Exposure to E2 decreased the expression of *Ppid* in a dose-dependent manner, irrespective of cort exposure. P4 did not alter expression of PPID or Fkbp5 with or without cort co-exposure. Collectively, these results suggest that the expression of GR co-regulators is directly influenced by exposure to E2, providing a basis for the attenuated GR translocation observed in females with a history of chronic stress

Disclosures: C.H. Bourke: None. **S.A. Malviya:** None. **S.D. Kelly:** None. **C.S. Harrell:** None. **G.N. Neigh:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

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Program#/Poster#:898.11/TT12

Topic: E.05. Stress and the Brain

Support: Japan Society for the Promotion of Science (JSPS) Grants-in-Aid for Scientific Research (B) 18300127

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Japan Society for the Promotion of Science (JSPS) Grants-in-Aid for Young Scientists 24700385

Title: Stress induced phosphorylation of snap-25

Authors: *S. YAMAMORI[1], D. SUGAYA[1], Y. IIDA[2], H. KOKUBO[1], M. ITAKURA[1], E. SUZUKI[3], M. KATAOKA[4], H. MIYAOKA[2], M. TAKAHASHI[1]
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Abstract: SNAP-25, a t-SNARE protein, plays a crucial role in neurotransmitter release by exocytosis. Protein kinase C phosphorylates SNAP-25 at Ser^{187} and enhances hormonal release from adrenal chromaffin cells and insulin-secreting cells, however the physiological significance of SNAP-25 phosphorylation in brain function still remains unclear. In the present study, we found that the phosphorylation level of SNAP-25 increased rapidly in mouse brain following restraint stress in cold water. The basal and stress-induced phosphorylation of SNAP-25 was high in stress-related brain regions, including cerebral cortex, hippocampus and amygdala, and the extent of phosphorylation increased with increasing amounts of stress. Among seventeen

phosphoproteins tested, only three proteins including SNAP-25 were phosphorylated by cold restraint stress. Intravenous application of adrenaline elevated the phosphorylation level of SNAP-25, however stress-induced phosphorylation was still observed in adrenalectomized mice, indicating that phosphorylation of SNAP-25 is regulated through both central and peripheral mechanisms. Serum corticosterone levels of a SNAP-25 mutant mouse with a single amino acid substitution at S187A (*Snap-25*$^{S187A/S187A}$ mutant mice) displayed normal elevation after cold-restraint stress. In stressful conditions, both wild type and SNAP-25 knock-in mice exhibited a strong reduction in feeding. Although food intake gradually recovered to normal levels in wild type mice, it never recovered in *Snap-25*$^{S187A/S187A}$ mutant mice. These results indicate that phosphorylation of SNAP-25 plays important roles in stress response, possibly in the adaptation to stress.

Disclosures: S. Yamamori: None. **D. Sugaya:** None. **Y. Iida:** None. **H. Kokubo:** None. **M. Itakura:** None. **E. Suzuki:** None. **M. Kataoka:** None. **H. Miyaoka:** None. **M. Takahashi:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

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Topic: E.05. Stress and the Brain

Support: NIH R01 NS 35467

Title: Expression of mineralocorticoid receptor mRNA in response to mate pair separation in male and female zebra finches.

Authors: A. J. KESNER, *F. N. MADISON, G. F. BALL
Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Amongst vertebrates, several different mating patterns exist. Social monogomy, for example, is most prevalent in avian species. The highly social zebra finch forms and sustains lifelong monogamous pair bonds in the wild. Zebra finches also readily form pair bonds in the laboratory making them a good model to investigate the physiological consequences of mate pair separation. There is evidence that mate separation is stressful. Several studies have shown that separating zebra finch pairs elicits changes in behavior (e.g. increased song rates) and increases in the concentration of corticostere (CORT). However, the underlying neural mechanisms modulated in response to mate pair separation remain to be elucidated. In this study, we investigated possible changes in mineralocorticoid receptor (MR one of the receptors CORT

binds to) mRNA expression in response to acute mate pair separation in male and female zebra finches. Male and female zebra finches were maintained on long days (14L:10D) and established mate pairs were confirmed when behavioral criteria such as allopreening, clumping, and nest box cohabitation was observed. 16 mate pairs and 16 stimulus females (N=48) were assigned to one of six treatment groups: male and female birds were either separated from their mate, separated from the stimulus female, or handled but not separated from mate pair (control). After 48 hours in the new housing condition, brains were collected, frozen, and later micropunches from the hippocampus were analyzed via qPCR for MR mRNA. Blood was collected prior to (baseline) and at the end of the study. Plasma samples were analyzed for CORT via enzyme-immunoassay.No significant differences in MR mRNA expression in the hippocampus as a function of mating state were detected in male or female zebra finches. No differences in plasma CORT concentrations were observed in either baseline or in response to mate pair and stimulus female separation. Our study suggests that the psychosocial stress of mate pair separation was not sufficient in altering hippocampal MR mRNA, a neuroendocrine marker of the stress response, in male and female zebra finches.

Disclosures: A.J. Kesner: None. **F.N. Madison:** None. **G.F. Ball:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

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Topic: E.05. Stress and the Brain

Support: NSF Grant 0822129

Title: Circadian alterations in cytokine expression and adrenal hormone production

Authors: *C. M. HUESTON, T. DEAK
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Abstract: Acute stress exposure is associated with changes in a variety of hormonal, neural, and immune-related factors. Recent studies from our lab and others indicate that stress related changes in central interleukin-1β (IL-1) expression, in particular, vary remarkably depending on the time of day during which stress is imposed. Given that multiple hormones released in response to stress, including both progesterone and corticosterone (CORT), have been shown to constrain inflammatory processes under varying conditions, the goal of the following series of experiments was to clarify circadian fluctuations in these hormones and how they might impact the response to stress challenges imposed at the light-dark transitions. Adult male Sprague-

Dawley rats (N=16) were killed at either ZT0 (offset of active cycle) or ZT12 (onset of active cycle) and fluctuations in the levels of plasma hormones, adrenal enzymes responsible for the conversion of progesterone to CORT, and paraventricular nucleus (PVN) and adrenal gland cytokine mRNA levels were measured. Results indicate that while as expected, CORT increased at ZT12, in male rats progesterone did not appear to fluctuate across the circadian cycle. Within the PVN no changes in IL-1 or cfos mRNA expression was found between the two time points, however IL-6 expression was increased at ZT12. Within the adrenal gland, gene expression levels of housekeeper genes (β-Actin, GAPDH, cyclophilin) also showed differences as a function of circadian timing. As a result, target genes (IL-1, cfos, IL-6, TNFα, COX2, CYP21A1, CYP11B1) were analyzed independent of housekeeper expression. Commensurate with other published findings, we found that IL-1 mRNA levels were lowest at at ZT12. In addition, both CYP21a1 and CYP11b1 mRNA expression levels were suppressed at ZT12, which is surprising given the role of these enzymes in converting progesterone to CORT. However, as mRNA levels may not represent actual protein expression or enzyme activity, other measures of enzyme function are currently under examination. In addition, as the adrenal gland appears to have strong circadian rhythmicity, we aim to examine the mechanistic link between circadian clock genes and the expression of inflammatory factors within the adrenal glands.

Disclosures: C.M. Hueston: None. **T. Deak:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

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Topic: E.05. Stress and the Brain

Support: DA030177

DA025697

OD011103

Title: Extensive pre-mRNA splicing of REST/NRSF and its relevance to retinoic acid-induced differentiation of SH-SY5Y cells

Authors: *G.-L. CHEN, G. M. MILLER
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Abstract: Emerging evidence suggests that alternative pre-mRNA splicing, a major mechanism for both transcriptome and proteome diversity, extensively exists in the brain and plays a role in adaptive responses to environmental stimuli. The repressor element silencing transcription factor (REST), also known as the neuron-restrictive silencing factor (NRSF), acts as a hub for the recruitment of diverse chromatin modifiers to target gene loci important in behavior, neurogenesis and tumorigenesis. We performed a systematic and comprehensive analysis of REST pre-mRNA splicing and examined its relevance to the differentiation of human neuroblastoma SH-SY5Y cells by retinoic acid (RA). Specifically, cDNA samples from various neuronal and non-neuronal tissues and cell lines were amplified by numerous REST-specific primer sets for the detection of potential splice variants, and 5'- and 3'-ends of mRNAs were identified by RACE. With this approach we identified a total of 34, mostly cell-/tissue-specific, splice variants of REST that result from all types of splicing (e.g. exon skipping, alternative 5'- and 3'-splice site, intron retention and mutually exclusive exon), along with a number of novel alternative exons, promoters and poly(A) sites. While some splice variants without a stop codon may function as non-coding RNA, numerous splice variants with a premature stop codon are predicted to encode truncated proteins that functionally parallel REST4, a neuron-specific, truncated REST isoform lacking the C-terminal repression domain critical for transcriptional repression. Using quantitative real-time PCR, we assessed the expression of specific REST splice variants as well as some neuron-specific target genes (BDNF, SCG10 and Synaptin I) in non- and RA-differentiated SH-SY5Y cells. We found a global increase in the expression of REST splice variants and target genes in the RA-differentiated SH-SY5Y cells, implying that alteration in REST expression may underlie the activation of neuronal genes during neurogenesis. Our findings suggest that REST undergoes extensive pre-mRNA splicing, resulting in numerous REST mRNA isoforms that likely have distinct biological functions. The regulation and biological function of the newly-identified REST variants is currently under investigation.

Disclosures: G. Chen: None. **G.M. Miller:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:898.15/TT16

Topic: E.05. Stress and the Brain

Support: FP7-Marie Curie International Reintegration

Title: Microrna-mediated regulation of midbrain urocortin-1

Authors: *A. ASCHRAFI[1], J. M. VERHEIJEN[2], N. F. OLDE LOOHUIS[1], P. M. GORDEBEKE[2], T. KOZICZ[2]
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Abstract: Evidence suggests that the centrally projecting neurons in the midbrain Edinger-Westphal nucleus (EWcp) play an important role in controlling/modulating the animal's stress-response. Previous research has demonstrated stress-associated regulation of urocortin-1 (Ucn1), a centrally acting stress neuropeptide in the EWcp. However, the mechanisms controlling the expression of Ucn1 remain largely elusive. Increasing evidence points to a critical role of microRNA (miRNA) in several brain processes, including neuronal stress response. MiRNAs are a class of small non-coding RNA molecules that regulate the expression of several target genes at the post-transcriptional level. We investigated whether miRNAs can potentially modulate the expression of Ucn1, and examined possible upstream miRNAs that have the capacity to bind the 3' untranslated (3'UTR) region of Ucn1 mRNA. Sequence analysis of the 3'UTR of rat Ucn1 mRNA revealed the presence of putative binding sites for a number of brain-specific miRNAs including miR-326. Thus, miR-326 may have the capacity to modulate Ucn1 levels in neurons. Transfection of miR-326 mimics into rat primary midbrain neurons resulted in a significant decrease of Ucn1 mRNA and protein levels. Conversely, the transfection of synthetic anti-miR-326 oligonucleotides increased Ucn1 mRNA and protein levels in cultured midbrain neurons. Taken together, these results point to a molecular mechanism in which miR-326 acts as a potential upstream regulator of Ucn1 by binding to the 3'UTR of the Ucn1 mRNA, and consequently may play an important role in modulating the neural stress response.

Disclosures: A. Aschrafi: None. **J.M. Verheijen:** None. **N.F. Olde Loohuis:** None. **P.M. Gordebeke:** None. **T. Kozicz:** None.

Poster

898. Cellular Actions of Stress II: Gene Expression and Signal Transduction

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:898.16/TT17

Topic: E.05. Stress and the Brain

Support: Polish Ministry of Science and Higher Education Grant IP2011

Title: Genome-wide DNA methylation profiling in hippocampus of mice subjected to chronic social stress

Authors: *P. LISOWSKI[1], A. M. STANKIEWICZ[2], A. RONOWICZ[3], A. PIOTROWSKI[3], G. R. JUSZCZAK[2], J. GOSCIK[4], L. ZWIERZCHOWSKI[1], A. H. SWIERGIEL[2,5]
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Abstract: The role of DNA cytosine methylation, an epigenetic regulator of chromatin structure in brain remains unclear and DNA methylation is believed to be stable postnatally in adult mammals. However, recent data suggest changes in DNA methylation in the postnatal brain. Therefore, we examined by NimbleGen Mouse DNA Methylation 3x720K CpG Island Plus RefSeq Promoter Arrays the DNA methylation status at 20 404 promoters of 22 881 transcripts in hippocampus of mouse subjected to 8 and 13 days of chronic social stress procedure. We observed different levels of promoter-specific DNA methylation in the adult stressed individuals in comparison with the non-stressed animals. Genome-wide DNA methylation levels were correlated with gene expression patterns measured by quantitative real-time RT-PCR. A robust and progressive rise in DNA methylation levels across the stress procedure was observed for several loci, typically in conjunction with declining levels of the corresponding mRNAs. These hippocampal differences in DNA methylation and transcriptomic patterns of genes involved in neurodegenerative diseases are consistent with the emerging hypothesis that DNA methylation plays a dynamic, pathophysiological role in the adult brain, and is involved in programming the epigenome in response to chronic stress.

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Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.01/TT18

Topic: E.05. Stress and the Brain

Title: Translocator protein 18kDa occupancy of ONO-2952 in rhesus monkey brain in pet study using ^{11}C-PBR28

Authors: *N. YAMADA[1], K. MITSUI[1], T. SASAMURA[1], S. KATSUMATA[2], S. NAKADE[1]
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Abstract: Translocator protein 18kDa (TSPO) has been proposed to have many physiological functions including regulation of neurosteroids biosynthesis. We have found that ONO-2952, a novel TSPO antagonist, prevented restraint stress-induced defecation at doses of more than 0.3 mg/kg (p.o.) in rats with approximately 50% or more TSPO occupancy in the brain. The aim of this study was to elucidate a correlation between plasma concentration and brain TSPO occupancy of ONO-2952 in conscious rhesus monkeys using ^{11}C-PBR28 as a PET ligand. PET measurements were performed 2 hours and 24 hours after single or multiple oral administration of ONO-2952 at several doses ranging from 0.3 mg/kg to 30 mg/kg. After the head of the test animal was fixed into a stereotactic apparatus, an emission scan (55 frames in 121 min in total) was started simultaneously with the start of intravenous bolus administration of ^{11}C-PBR28. Total distribution volume of ^{11}C-PBR28 was calculated for each region of interest (hippocampus, parietal lobe, frontal lobe, temporal lobe, occipital lobe, corpus striatum, thalamus and cerebellum) by the Logan Plot method. TSPO occupancy was calculated by comparing total distribution volume following ONO-2952 administration with that following vehicle administration. Before ONO-2952 study, a known TSPO antagonist PK11195 was confirmed to occupy TSPO in the monkey brain in PET study using ^{11}C-PBR28, providing that ^{11}C-PBR28 functioned as a PET ligand for TSPO. ONO-2952 was demonstrated to occupy TSPO in the monkey brain. The highest TSPO occupancy rate of ONO-2952 was 94%, suggesting few non-specific binding of ^{11}C-PBR28 in the monkey brain. There was no marked brain region-specificity in the TSPO occupancy of ONO-2952 at any doses. In the evaluation of the relationship between plasma concentration and brain TSPO occupancy of ONO-2952, it was shown that the TSPO occupancy was dependent on the plasma ONO-2952 concentration in individual animal, irrespective of the time from the final dosing. These results indicate that the TSPO occupancy of ONO-2952 in the monkey brain increases up to maximal rate in parallel with its plasma concentration, and ONO-2952 do not accumulate in excessive amounts in the brain. Moreover, these results suggest that ^{11}C-PBR28 is a useful PET ligand to determine the TSPO occupancy by ONO-2952 in the monkey brain and probably in the human brain.

Disclosures: **N. Yamada:** Employment; ONO Pharmaceutical Co., Ltd. **K. Mitsui:** Employment; ONO Pharmaceutical Co., Ltd. **T. Sasamura:** Employment; ONO Pharmaceutical Co., Ltd. **S. Katsumata:** Employment; ONO Pharma UK Ltd. **S. Nakade:** Employment; ONO Pharmaceutical Co., Ltd..

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.02/TT19

Topic: E.05. Stress and the Brain

Title: Effects of ONO-2952, a novel translocator protein 18kDa antagonist, on stress-induced rectal hyperalgesia and defecation in rats

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Abstract: Translocator protein 18kDa (TSPO) localized on the outer mitochondrial membrane regulates cholesterol transport from intracellular stores into mitochondria, a rate limiting step of neurosteroidgenesis. It is well known that neurosteroids act as allosteric modulators of excitatory and/or inhibitory neurotransmission and its amount is drastically changed by stress. ONO-2952 is a novel potent and selective TSPO antagonist and we have demonstrated that ONO-2952 inhibited restraint stress-induced pregnenolone production in hippocampus of rats. ONO-2952 thus may have a potential in the treatment of stress-related disorders like irritable bowel syndrome (IBS). The aim of this study was to investigate effects of ONO-2952 on stress-induced disturbance of bowel movement, abdominal pain/discomfort and anxiety-related behavior in rats. To determine effects of ONO-2952 on the bowel movement, weight of feces during 1 hour restraint stress, and relationship between plasma concentration and TSPO occupancy in the brain of ONO-2952 were examined in rats. ONO-2952 or a vehicle was orally administered 2 hours before restraint stress loading. In the experiments of abdominal nociception, ONO-2952 or vehicle was orally administered 1 hour before forelimb restraint stress loading followed by rectal balloon-distension applied at 5 minute interval for 1 hour from 5 minutes after 1 hour stress exposure. The nociceptive threshold was determined by abdominal electromyogram. To examine effects of ONO-2952 on stress-induced anxiety, rats were conditioned 15 times at 1 minute intervals by a 3 second warning beep followed by a 5 second 2 mA foot shock and flash of light. On the following day, rats were again placed in the apparatus 2 hours after oral administration of ONO-2952 or vehicle. Freezing time under the 30 minutes stress without exposure to foot shock was measured. The results showed that ONO-2952 prevented restraint stress-induced defecation at doses of more than 0.3 mg/kg in rats with approximately 50% or more TSPO occupancy in the brain. ONO-2952 also prevented the decrease of visceral nociceptive threshold induced by forelimb restraint stress at doses of 0.3 mg/kg or higher. Moreover, ONO-2952 prevented the freezing behavior induced by conditioned fear stress at doses of 1 mg/kg or higher with an efficacy similar to that of diazepam at 3 mg/kg. These results suggest that ONO-2952 may be useful for the treatment of IBS.

Disclosures: **K. Mitsui:** Employment; ONO Pharmaceutical Co., Ltd. **T. Sasamura:** Employment; ONO Pharmaceutical Co., Ltd. **S. Katsumata:** Employment; ONO Pharma UK Ltd. **T. Niwa:** Employment; ONO Pharmaceutical Co., Ltd. **Y. Kawahara:** Employment; ONO Pharmaceutical Co., Ltd. **N. Yamada:** Employment; ONO Pharmaceutical Co., Ltd. **S. Nakade:** Employment; ONO Pharmaceutical Co., Ltd..

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.03/TT20

Topic: E.05. Stress and the Brain

Support: NIMH IRP

Title: Glucocorticoids orchestrate divergent effects on mood through adult neurogenesis

Authors: *M. L. LEHMANN[1], R. A. BRACHMAN[2], M. HERKENHAM[3]
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Abstract: In mice, both social defeat stress and environmental enrichment (EE) stimulate glucocorticoid (GC) secretion but have opposing effects on hippocampal neurogenesis and mood. HPA-axis dysregulation and decreased neurogenesis are consequences of social defeat. Both outcomes are correlated with depression, but a causal role for either remains elusive. Additionally, enrichment has antidepressant actions that are neurogenesis-dependent, but the contribution of GCs to this effect is unexplored. Thus, for both social defeat and environmental enrichment, how glucocorticoids interact with neurogenesis to change affect remains unknown. Here we explore causal roles of glucocorticoids and neurogenesis in both the induction of depressive-like behavior and its amelioration by EE. By inhibiting neurogenesis and surgically clamping adrenal hormone secretions, we show that neurogenesis_through interactions with the HPA axis_is directly involved in precipitating the depressive phenotype following social defeat. Mice adrenalectomized prior to social defeat showed enhanced behavioral resiliency and increased survival of adult born hippocampal neurons compared to sham-operated mice. The protective effects of adrenalectomy were not conferred to mice with conditional ablation of hippocampal neurogenesis. Additionally, we show that GCs secreted during environmental enrichment promote neurogenesis and are required for the restoration of normal behavior after social defeat. These data demonstrate that glucocorticoid-dependent declines in neurogenesis drive affective disorders following social defeat and that glucocorticoids secreted during

enrichment promote neurogenesis and restore normal behavior after defeat. These data also provide new evidence for the direct involvement of neurogenesis in the etiology of depression, suggesting that treatments promoting neurogenesis will enhance stress resilience.

Disclosures: M.L. Lehmann: None. **R.A. Brachman:** None. **M. Herkenham:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

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Topic: E.05. Stress and the Brain

Support: NIDA Grant 1T32DA031111-01

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Grant from OSVC, Health Sciences, Univ Pittsburgh

Title: Effects of unpredictable chronic mild stress in adolescent rats on emotional behavior and hippocampal CREB

Authors: *E. K. ZIMMERMAN[1,2,3], N. PORCH[2,6], J. N. PARRISH[2,4], E. C. DONNY[3,4,5], A. F. SVED[2,4,5], E. THIELS[1,2,3]
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Abstract: Unpredictable chronic mild stress (UCMS) is a widely-used model for inducing depressive-like behavior in adult rodents. The ability of UCMS to impact emotional behavior varies across individuals. Work suggests that rats exhibiting low levels of locomotor activity (low responders; LR) during a 2-hr test in a novel open field (OFT) are more sensitive to UCMS than are high responders (HR). Adolescence is a period of heightened vulnerability to stress. We wanted to determine whether UCMS exposure during adolescence has a greater impact on emotional behavior than UCMS exposure during adulthood, and whether the LR/HR dimension is similarly predictive of UCMS effects in adolescents as it is in adults.
First, we tested the effect of a very mild UCMS protocol (vmUCMS) on adult male rats (starting age: >postnatal day 60; pd60). Rats (vmUCMS-exposed rats and controls) were weighed and received sucrose preference tests (SPT) weekly, and were tested in the OFT before and during

week 5 of vmUCMS. Results confirmed that the vmUCMS protocol was sub-optimal for producing significant changes in emotional behavior in adult rats, but that the LR/HR tendencies are persistent, including across the vmUCMS exposure period. We then exposed adolescent rats (starting age: pd28) to vmUCMS or control conditions and assessed the effect on body weight, coat state, and SPT weekly. OFT performance was assessed before and during week 5 of vmUCMS. Elevated plus maze (EPM) performance was examined during week 5, and the rats' brains were collected for measurement of glucocorticoid receptor (GR), CREB, and ERK protein levels in hippocampus and amygdala by Western blot analysis.
Different from the effect in adults, vmUCMS attenuated weight gain, worsened coat appearance, and decreased locomotor activity in the OFT in adolescent rats. Although overall performance on the EPM was unaffected, vmUCMS-exposed adolescent rats either totally avoided open arms or visited them very frequently, whereas control rats exhibited a normal distribution of open arm entries. SPT scores were near ceiling levels in both groups, possibly because of a general high sucrose preference by adolescent rats. Also different from adults, the LR/HR dimension was not stable in adolescent rats, and did not confer differential sensitivity to vmUCMS. Hippocampal GR levels were unaffected by vmUCMS; however, CREB levels tended to be reduced after vmUCMS in adolescence. Additional biochemical analyses are ongoing.
These findings suggest that adolescent rats are more susceptible to vmUCMS than are adult rats, but that susceptibility to vmUCMS among adolescent rats does not vary with differences in locomotor activity in a novel environment.

Disclosures: E.K. Zimmerman: None. **N. Porch:** None. **J.N. Parrish:** None. **E.C. Donny:** None. **A.F. Sved:** None. **E. Thiels:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.05/UU2

Topic: E.05. Stress and the Brain

Support: NIMH 090420

Title: Sex differences in the effects of acute social defeat in adolescence and in adulthood.

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Abstract: Social defeat is a potent stressor for both males and females and females are particularly sensitive to social stressors, especially during adolescence. However, little is known about the impact of social stress in the form of aggression in females either during adolescence or in adulthood. Here, we used the social defeat paradigm (resident-intruder paradigm) in male and female rats in order to investigate their behavior during defeat and their neural activity in response to defeat. Both adolescent and adult male and female rats were subjected to an acute social defeat by a resident lactating female rat. Results indicated that subpopulations emerged in both sexes in which roughly half of the adolescent and adult rats displayed the defeat posture whereas the other half resisted defeat. These differences occurred despite similarly rapid attacks by the lactating resident females. All rats exposed to the lactating female resident showed increases in HPA function as shown through elevated ACTH and corticosterone levels. Furthermore, sex differences in the patterns of neural activity emerged at both ages in the defeat compared to the non-defeat subpopulations. Specifically, males and females showed differences in neural activity within the Insular Cortex, the Paraventricular Nucleus of the Hypothalamus and the Paraventricular Nucleus of the Thalamus. In the prelimbic division of the prefrontal cortex, expression of cytokines, cyclooxygenase-2 and markers for microglia and astrocytes was examined in the female rats. Both defeated and non-defeated intruders showed elevated levels of c-fos, COX-2 and pro-inflammatory cytokines. The level of increase differed between females exhibiting defeat compared to the non defeated females and this effect seemed to be greater in adult compared to adolescent females. Thus, two contrasting patterns of behavior and neuronal activity occurred in response to social aggression in female and male rats and these patterns were different in adolescence compared to adulthood. Furthermore, these patterns of behavior during exposure to the lactating female are associated with variations in expression of pro- and anti-inflammatory cytokines in both adolescent and adult rats. Further study of these variations in responses to social aggression may provide insight into resilience or vulnerability to social stress-associated pathology.

Disclosures: S. Bhatnagar: None. **E. ver Hoeve:** None. **G. Kelly:** None. **S. Luz:** None. **G. Barr:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.06/UU3

Topic: E.05. Stress and the Brain

Support: NIH Grant MH41256 to BM

The Gary R. Helman Fellowship to JG

Help For Depression Research Foundation to RH

Title: Chronic restraint stress alters the gene expression profile of the hippocampus to a novel, heterotypic stressor

Authors: *J. D. GRAY, T. R. RUBIN, R. G. HUNTER, B. S. MCEWEN
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Abstract: Chronic and acute stressors have been linked to a variety of changes in hippocampal function. Both produce changes in gene expression that can endure beyond the discontinuation of stress, though the magnitude and direction of the response varies with stress intensity, duration and predictability. The present studies seek to characterize how changes in the hippocampus resulting from chronic restraint stress (CRS) differentially modulate gene expression in response to a novel, acute stressor. Male c57/bl6 mice were subjected to 1) a forced swim test (FST), 2) CRS for 21 days and then a FST, or 3) allowed to recover 21 days after CRS and subjected to FST. mRNA from the hippocampus of each animal was extracted and used to generate a cDNA library for hybridization to Illumina MouseRef8-v2 microarray. Changes in gene expression were compared against cDNA from the hippocampus of age-matched controls that were not subjected to any stress. Hybridization data was analyzed for statistical significance using a series of pairwise comparisons to identify genes differentially expressed across conditions. 1,369 genes were identified with significantly altered expression levels ($p < .05$) in mice subjected to FST, 4,448 genes were significantly changed in mice subjected to FST at the end of CRS, and 1,291 genes were significantly different in mice subjected to FST after recovery from CRS. Across comparisons, 104 genes reached significance in all FST conditions. Pathway analysis identified significant clusters of differentially expressed genes. Several pathways were significantly altered across conditions, principally that of Nf-kB. RT-PCR was performed to validate changes identified by the arrays in genes established as responsive to an acute stress. The immediate early gene, cFos, was found to be comparably increased on the array and by RT-PCR after FST, indicating the arrays are reliable indicators of changes in mRNA levels. The large number of genes changed in FST immediately after CRS, which minimally overlap with the naïve FST condition (only 9.2% of genes), suggests that CRS uniquely alters how cells in the hippocampus respond to a novel stressor. Mice allowed to recover from CRS and subjected to FST shared 14.0% of the significantly different genes with naïve FST mice, and 34.7% of genes with mice subjected to FST after CRS. This suggests that even after recovery, mice with a stress history have a more similar hippocampal response then naïve mice to a novel stressor. Ongoing analysis of the genes identified will provide new insight in the molecular mechanism underlying the stress response in the hippocampus.

Disclosures: J.D. Gray: None. **T.R. Rubin:** None. **R.G. Hunter:** None. **B.S. McEwen:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

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Topic: E.05. Stress and the Brain

Support: T32 DA016176 (JNB)

R01 AA013388 (MAP)

Title: Chronic corticosterone sensitizes the mesolimbic dopamine reward pathway to excitatory NMDA receptor activity

Authors: *J. N. BERRY, L. J. SHARRETT-FIELD, A. R. REYNOLDS, M. A. PRENDERGAST
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Abstract: Elevations in circulating corticosteroids during periods of stress may influence activity of the mesolimbic dopamine reward pathway, which plays a crucial role in drug dependence, and compromise neuronal viability, possibly by increasing glutamatergic *N*-methyl-D-aspartate (NMDA) receptor expression and/or function in a glucocorticoid receptor-dependent manner. The current study used organotypic co-cultures of the ventral tegmental area and nucleus accumbens designed to mimic the mesolimbic pathway *in vitro* and examined effects of chronic pre-exposure to corticosterone on NMDA receptor-mediated cell death. Co-cultures were pre-exposed to corticosterone (1 μM) or vehicle for 5 days prior to 24 hour co-exposure to NMDA (200 μM). Chronic pre-exposure to a non-toxic, but stress-relevant, concentration of corticosterone significantly enhanced subsequent NMDA-induced neuronal toxicity in the ventral tegmental area as indicated by increased propidium iodide uptake. The effect of corticosterone pre-exposure on subsequent NMDA-induced neurotoxicity in the ventral tegmental area was NMDA receptor dependent, as co-exposure to the selective NMDA receptor antagonist 2-amino-7-phosphonovaleric acid (APV) attenuated the observed neurotoxicity. In contrast, no significant effects were observed in the nucleus accumbens. These results suggest an enhancement of NMDA receptor systems or downstream effectors in areas of the mesolimbic reward pathway following chronic pre-exposure to corticosterone, which leads to enhanced neuronal vulnerability to future excitotoxic insults. This may be of critical importance as use of psychostimulants and other drugs of abuse may produce excitotoxic events in these areas, thus further compromising neuronal viability.

Disclosures: J.N. Berry: None. **L.J. Sharrett-Field:** None. **A.R. Reynolds:** None. **M.A. Prendergast:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.08/UU5

Topic: E.05. Stress and the Brain

Support: NIH Grant MH041256

Title: Chronic non-invasive disruption of the hypothalamic-pituitary-adrenal stress axis has effects on learning and memory.

Authors: C. D. WILSON[1], B. S. MCEWEN[1], *I. N. KARATSOREOS[2]
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Abstract: Stress is an inescapable reality of modern life. The physiological and behavioral responses that underlie the stress system are based in systems that evolved to protect an organism from threats to homeostasis, such as predation, agonistic encounters with conspecifics, or changes in the environment. In humans, disruption of the hypothalamic-pituitary-adrenal (HPA) axis (the neuroendocrine system central in the stress response) is associated with numerous neuropsychiatric conditions, as diverse as depression, anxiety, post-traumatic stress disorder, and is clearly observed during aging. As such, understanding how a disrupted HPA axis affects neural circuits underlying emotionality and cognition is of key importance.
The purpose of our study was to investigate the behavioral and neural effects of a disrupted HPA axis on spatial and emotional learning systems, both of which are affected by chronic stress. We have characterized a model of chronic reversible HPA disruption induced by non-invasively delivering adrenal stress hormone corticosterone (CORT) in the drinking water of male C57 mice for 28d. This treatment suppresses function of the HPA axis, including eliminating endogenous hormonal responses to stress. Under this model, CORT levels follow a diurnal rhythm, as the animals consume most of their fluids in early night. This results in peak plasma CORT value of around 100ng/ml a few hours after the start of dark phase as opposed to the naturally occurring peak of 60-70ng/ml at the start of the dark phase. As such, this unique treatment results in almost complete inhibition of the endogenous HPA axis, but rhythmic plasma CORT, a situation that is not possible with adrenalectomy and/or pellet or mini-pump experiments. It also avoids the inherent issues involved with daily CORT injections. We found that this treatment results in impaired learning and memory in the Morris Water Maze, a hippocampal based learning and memory task, but no apparent differences in a contextual fear conditioning, a task that also engages the amygdala. This suggests that our model of HPA

disruption may affect aspects of spatial cognition but not certain types of emotional learning. Further studies to explore specific neural changes in the hippocampus and amygdala after chronic HPA disruption are necessary to fully understand the mechanisms by which our model impairs behavioral function.

Disclosures: C.D. Wilson: None. **B.S. McEwen:** None. **I.N. Karatsoreos:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.09/UU6

Topic: E.05. Stress and the Brain

Support: R01 NS38632

Title: Increased EPO level, but decreased VEGF expression and microvascular density in chronic moderate hyperoxia in the mouse cerebral cortex

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Abstract: The partial pressure of oxygen in the brain parenchyma is tightly controlled, and normal brain function is delicately sensitive to continuous and controlled oxygen delivery. Chronic moderate hypoxia leads to angiogenesis, suggesting a modulatory role for oxygen in determining capillary density. The objective of this study was to determine physiologic and brain angiogenic adaptational changes during chronic moderate normobaric hyperoxia in mice. Four-month old C56BL/6J mice were kept in a normobaric chamber at 50% O_2 for up to 3 weeks. Normoxic littermates were kept in the same room outside the chamber. Freshly collected or fixed brain specimens were analyzed by RT-PCR, Western blot analysis and immunohistochemistry. Results show accumulation of hypoxia inducible factors 1 and 2 (HIF-1 and 2), and increased expression of erythropoietin (EPO), cyclooxygenase-2 (COX-2) and angiopoietin-2 (Ang-2). Conversely, vascular endothelial growth factor (VEGF), and VEGF receptor-2 (KDR/Flk-1), Peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1) and prolylhydroxylase-2 (PHD-2) expressions were decreased. VEGF mRNA level was diminished but there was no change in HIF-1 mRNA and von Hippel Lindau E3 ubiquitin ligase (VHL) protein expression. Microvascular density was significantly diminished by the end of the 3rd week of hyperoxia. Overall, our results are: 1) increased expression of the potent neuroprotective molecule, EPO; 2) diminished expression of the potent angiogenic factor, VEGF; and 3)

decreased microvascular density. We can, therefore, conclude that brain microvascular density can be controlled by HIF-independent mechanisms, and that brain capillary density is a continuously adjusted variable with tissue oxygen availability as one of the controlling modulators.

Disclosures: J.C. LaManna: Employment; Case Western Reserve University. **G.F. Benderro:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.10/UU7

Topic: E.05. Stress and the Brain

Support: NIMH Grant MH41256

Title: The glutamate dysfunction in the stressed tripartite synapse

Authors: *C. NASCA[1,2], T. R. RUBIN[1], J. D. GRAY[1], A. PITTALUGA[3], F. NICOLETTI[2,4], B. S. MCEWEN[1]
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Abstract: Vulnerability to stress is a cardinal feature of many psychiatric disorders. Growing evidence indicates that both stress and psychiatric disorders are associated with epigenetic modifications and atrophy and loss of neurons and glia, which contribute to the decreased size and function of limbic brain regions that are involved in the pathophysiology of mood disorders, such as the hippocampus and prefrontal cortex. We hypothesized that glutamatergic dysfunction associated with stress-related disorders could involve changes in the expression of the catalytic subunit (Xc-) of the glutamate cystine antiporter, which supplies extrasynaptic glutamate, thereby regulating the endogenous activation of extrasynaptic receptors, such as presynaptic mGlu2/3 receptors, and postsynaptic mGlu5 and NR2B-containing NMDA receptors. We found that acute exposure to restraint stress in mice caused a substantial reduction in the transcripts encoding for Xc-, mGlu2, mGlu3, and mGlu5 receptors, and the glial high affinity glutamate transporter, GLT-1, in the hippocampus with no changes in any of these transcripts in the prefrontal cortex. Interestingly, in mice exposed to chronic stress in the form of restraint stress or a chronic oral administration of corticosterone, we found reductions in Xc-, mGlu2, mGlu3, and mGlu5 receptors, and GLT-1 mRNA levels in the prefrontal cortex, but not in the hippocampus.

Moreover, these data seem to be correlated to an in progress gene array analysis. We conclude that stress causes adaptive changes in the expression of glutamate transporters and mGlu receptors that sequentially involves the hippocampus and the prefrontal cortex. Unraveling the nature of these changes might pave the way to novel therapeutic interventions for stress-related disorders.

Disclosures: C. Nasca: None. **T.R. Rubin:** None. **J.D. Gray:** None. **A. Pittaluga:** None. **F. Nicoletti:** None. **B.S. McEwen:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.11/UU8

Topic: E.05. Stress and the Brain

Support: NIH COBRE

VA Research

Title: Afferent corticotropin releasing factor (crf) projections to the nucleus accumbens.

Authors: K. E. TSCHETTER[1,3], *L. B. CALLAHAN[4,3], P. J. RONAN[5,2,3]
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Abstract: Stress is a major factor leading to both drug use and relapse. Common neurobiological substrates and pathways underlie actions of both drugs of abuse as well as stress. One such substrate is the neuropeptide corticotropin-releasing factor (CRF); a major coordinator of endocrine, autonomic and behavioral output to stress. In animal models, CRF has been directly implicated in mediating both stress-induced reinstatement of drug-seeking behavior as well as the anxiety associated with withdrawal. Dopaminergic projections from the midbrain ventral tegmental area (VTA) to the nucleus accumbens (NAc) and other forebrain structures are particularly significant components of the reward pathway. We have previously shown that dopamine cells of the VTA express CRF1 receptors and others have shown that the VTA receives afferent CRF fibers from cells of the PVN, CeA and BNST. Until now, the sources of afferent CRF pathways to NAc have been unknown. The purpose of this study was to determine these pathways and further characterize the expression of components of the CRF family (CRF, CRF_1 receptors, CRF_2 receptors, and corticotropin releasing factor binding protein; CRF-BP) in

these regions. Using a combination of retrograde tracer, immunohistochemistry and confocal microscopy techniques we found clear evidence for CRF innervation of NAc shell from CRF cells originating primarily from the CeA and PVN with a lesser contribution from cells of the BNST. Additionally, we provide many details of specific expression of CRF system components within the VTA-NAc pathway. This is the first study to provide clear evidence of direct CRF innervation of the NAc. This study adds weight to the notion that regions that the CeA, PVN and BNST should be considered part of an extended brain reward/reinforcement system.

Disclosures: K.E. Tschetter: None. **L.B. Callahan:** None. **P.J. Ronan:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

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Program#/Poster#:899.12/UU9

Topic: E.05. Stress and the Brain

Support: NIDA T-32-DA7290

RO1MH085298

RO1DA024680

Title: The role of stress-induced ghrelin elevations in hippocampal neurogenesis: implications for the regulation of mood and food reward behavior

Authors: *A. WALKER[1,2], S. OSBORNE-LAWRENCE[2], J.-C. CHUANG[2], J. M. ZIGMAN[2]
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Abstract: Many studies show a correlation between psychosocial stress, depression, and obesity, although mechanisms linking these disease states have yet to be fully elucidated. Mouse models to investigate these mechanisms include the chronic social defeat stress (CSDS) protocol, which induces both depressive-like behavior and enhanced reward-based, hedonic eating behavior. Of interest, CSDS and other forms of stress raise circulating levels of ghrelin, a stomach-derived orexigenic peptide hormone which possesses antidepressant-like actions and mediates reward-based behaviors of various types. Mice lacking the ability to respond to CSDS-induced ghrelin elevations (GHSR-null mice) display an increase in depressive-like behavior and a loss of food-reward behavior as compared to similarly CSDS-exposed wild-type littermates. The brain region(s) and mechanisms through which ghrelin may be acting to have these behavioral effects

remain mostly unknown. Since it is well-established that chronic stress reduces neurogenesis and cell survival in the hippocampal dentate gyrus (DG) of wild-type mice, that ghrelin receptors are expressed in the hippocampus, and that ghrelin has neuroproliferative and neuroprotective properties, we focused here on determining the role of altered hippocampal neurogenesis in the behavioral differences observed between GHSR-null and wild-type littermates upon stress. Using Western blot analyses, DG expression of pERK, pCREB, and BDNF - all signaling molecules involved in cell growth and survival -- was shown to be significantly lower in CSDS-exposed GHSR-null mice as compared to CSDS-exposed wild-type littermates. Using immunohistochemisty, the number of proliferating (Ki67-immunoreactive) cells in the DG of CSDS-exposed GHSR-null mice was shown to be significantly reduced as compared to CSDS-exposed wild-type littermates. In contrast, the number of apoptotic (caspase 3-immunoreactive) cells in the DG of CSDS-exposed GHSR-null mice was significantly elevated as compared to CSDS-exposed wild-type littermates. The overall reduction of neurogenesis in stressed GHSR-null mice correlated with the increase in depressive-like behavior as well as loss of food reward behavior observed in these mice. Therefore, these data suggest that ghrelin may modulate mood and food reward behavior upon stress through its regulation of neurogenesis in the DG.

Disclosures: A. Walker: None. **S. Osborne-Lawrence:** None. **J. Chuang:** None. **J.M. Zigman:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.13/UU10

Topic: E.05. Stress and the Brain

Support: CRP3_63 (L.R. 7/2007) R.A.S.

Title: Enhanced ethanol voluntary intake and decreased hippocampal neuronal excitability in c57bl/6j mice exposed to post-weaning social isolation

Authors: *E. SANNA[1,2], G. TALANI[1], V. LICHERI[1], F. CANNAS[1], S. LUTZU[1], T. FANUTZA[1], F. TRUDU[1], N. MASALA[1], G. BIGGIO[1,2]
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Abstract: Exposure early in life to stressful experiences is considered a critical factor for an increased vulnerability, during adulthood, to development of psychiatric disorders, including abuse of psychotropic substances such as alcohol. Rearing rodents in isolation (SI), starting at the

time of weaning and lasting several weeks, is a valuable model of chronic stress by which to examine the neurobiological and neuroendocrine mechanisms involved in the interaction between early stress and alcohol abuse. Our previous work has shown that post-weaning SI in C57BL/6J mice is associated with a decreased plasma and brain levels of neuroactive steroids, and a parallel up-regulation of extrasynaptic GABA-A receptors in dentate gyrus granule cells (DGGCs). These changes are accompanied by an enhanced voluntary intake of ethanol in the free-choice drinking paradigm compared to group-housed (GH) animals. Furthermore, we also demonstrated that SI significantly reduced long-term synaptic plasticity in CA1 pyramidal cells and ethanol was more effective in inhibiting LTP in SI than GH mice. More recently, current-clamp analysis of DGGCs revealed a decrease in resting membrane potential, membrane input resistance, and firing of action potentials in SI compared to GH mice, suggesting that SI results in a reduced level of neuronal excitability in DGGCs. Since daily administration of progesterone during the 6-weeks of isolation period reverts the effects caused by SI on tonic current and neuronal excitability in DGGCs, it likely that the changes induced by SI may be triggered, at least in part, by the decrease of plasma and brain levels of neuroactive steroid. This effect, in turn, may drive the up-regulation of extrasynaptic GABA-A receptors in DGGCs, and consequently the reduced excitability of the whole hippocampal excitatory circuitry.
This work was funded by a grant #CRP3_63 (L.R. 7/2007) from Regione Autonoma della Sardegna.

Disclosures: E. Sanna: None. **G. Talani:** None. **V. Licheri:** None. **F. Cannas:** None. **S. Lutzu:** None. **T. Fanutza:** None. **F. Trudu:** None. **N. Masala:** None. **G. Biggio:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

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Title: The influences of reproductive status and acute stress on the levels of phosphorylated delta opioid receptor immunoreactivity in rat hippocampus

Authors: *S. R. BURSTEIN[1], T. J. WILLIAMS[1,2], D. A. LANE[1], M. G. KNUDSEN[1], V. M. PICKEL[1], B. S. MCEWEN[3], E. M. WATERS[3], T. A. MILNER[1,3]
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Abstract: Ovarian hormones and stress influence the hippocampal opioid system, which plays an important role in learning. Acute immobilization stress (AIS) differentially alters the level of phosphorylated mu opioid receptors in the hippocampus of males and females, which may contribute to reported sex differences in the hippocampally-dependent behavior of stressed animals. As ovarian hormones can alter the trafficking of delta opioid receptors (DORs) in addition to mu opioid receptors, we assessed the interactions of gonadal hormones and AIS on the activation of DORs in the hippocampus of male and female rats using a well-characterized antibody to the phosphorylated DOR at the SER363 carboxy-terminal residue (pDOR; Cell Signaling). First, the distribution of pDOR-immunoreactivity (ir) was determined using light and electron microscopy. Light microscopy showed pDOR-ir predominantly located in apical dendrites in the CA1-3 layers, especially in the CA2/CA3a region. pDOR-ir also was seen in interneuron somata and dendrites in stratum oriens of CA1-3 and the hilus of the dentate gyrus. At the subcellular level, pDOR-ir was primarily located in postsynaptic profiles in association with dendritic spines and endomembranes within dendrites. Rarely, pDOR-ir was found in axons and terminals. The cellular distribution of the pDOR is consistent with that of DORs (Williams et al., 2011 PMC3045654) and strategically places the pDOR for modulating neurons known to be involved in stress responses. Second, pDOR-ir level was characterized using quantitative light microscopic densitometry. To investigate opiate activation of DORs, the level of pDOR-ir was examined in male rats one hour after a single morphine injection (20mg/kg, I.P.). We found a significant increase in pDOR-ir level in the CA2/CA3a region of male rats exposed to morphine in comparison to controls, confirming morphine activation of DORs. Next, we determined if pDOR-ir level was altered by hormone status and/or in response to AIS. In unstressed rats, the level of pDOR-ir in stratum radiatum of CA2/CA3a was increased in estrus (elevated estrogen and progesterone) females compared to proestrus and diestrus females and males. However, immediately following 30 minutes of AIS, no significant differences in pDOR-ir level were seen across estrous cycle phase or sex. These findings suggest that levels of activated DORs vary with estrous cycle phase and that acute stress may dampen the differential effects on DOR activation in females.

Disclosures: S.R. Burstein: None. **T.J. Williams:** None. **D.A. Lane:** None. **M.G. Knudsen:** None. **V.M. Pickel:** None. **B.S. McEwen:** None. **E.M. Waters:** None. **T.A. Milner:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.15/UU12

Topic: E.05. Stress and the Brain

Support: P01 Grant AA17056

Title: Effects of ethanol on nucleus accumbens monoamine levels in socially isolated and group housed rats

Authors: *A. KARKHANIS, J. LOCKE, J. L. WEINER, S. R. JONES
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Abstract: Exposure to adverse experiences such as social isolation for an extended period of time during childhood results in chronic stress; which often results in psychiatric problems such as anxiety and affective disorders, and increased probability of drug and alcohol abuse problems. Chronic stress alters the normal development of monoamine systems, affecting adult behavior. Behavioral differences in isolation and group reared rats suggest that there are alterations in the limbic system. Isolation rearing affects appetitive behavior during ethanol (EtOH) self-administration; isolated rats have a greater preference for EtOH than group reared rats. However, to our knowledge no studies have examined the effects of acute EtOH on monoamine levels in group and isolation reared rats. Previous studies have shown that acute EtOH results in increased extracellular dopamine (DA) levels in the nucleus accumbens (NAc), and intraperitoneal (i.p.) administration of amphetamine results in significantly larger DA increases in isolated than in group housed rats. Thus, we hypothesized that increase in levels of DA would be greater in isolated rats compared to group reared rats following EtOH administration.
In the current study, male Long Evans rats were either group reared (groups of 4) or isolation reared beginning on postnatal day 28 for 6 weeks, followed by an assessment of anxiety-like behavior. Following behavioral testing, in vivo microdialysis was conducted to measure NAc monoamine levels in freely moving group and isolation reared rats. Rats were challenged with acute (i.p.) administration of EtOH (1 g/kg). Brain samples were collected every 20 min and extracellular levels of DA, norepinephrine (NE), and serotonin (5-HT) were quantified using High Performance Liquid Chromatography.
Isolation reared rats were observed to have increased levels of DA at baseline compared to group

reared rats. Interestingly, although DA levels in both groups increased, the increase in isolation reared rats was significantly smaller than in group reared rats. Moreover, the DA response to EtOH was temporally delayed in isolation reared rats. Levels of NE were lower in isolated rats; EtOH resulted in an increase in NE levels in both groups, albeit a temporal delay was observed in isolation reared rats. No difference was observed in levels of 5-HT at baseline. Preliminary data show extracellular levels of 5-HT decreased in both groups following EtOH challenge. Thus, we show that DA, NE, and 5-HT in the NAc differentially respond to acute EtOH in isolated and group reared rats. Such changes may be involved in the documented increases in anxiety-like and drinking behaviors in isolated rats.

Disclosures: A. Karkhanis: None. **J. Locke:** None. **J.L. Weiner:** None. **S.R. Jones:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.16/UU13

Topic: E.05. Stress and the Brain

Support: School of Life Sciences, ASU

Title: Effects of varying timelines of restraint stress on neuroplasticity.

Authors: J. J. HANNA[1,2], K. SAINZ[2], C. D. CONRAD[1], *D. P. BALUCH[2]
[1]Psychology, [2]Sch. of Life Sci., Arizona State Univ., Tempe, AZ

Abstract: Stress influences neurogenesis in the hippocampus and modifies the structure/volumes of the hippocampus and medial prefrontal cortex (mPFC), regions involved in executive function and memory. It has been suggested that that chronic stress and these resulting morphological changes may contribute to neurological disorders, such as depression and post-traumatic stress disorder. Several chronic stress studies reveal that the morphological alterations, such as dendritic retraction in the CA3 region of the hippocampus, occur over weeks (i.e., 21 days and not 10 days). To date, no study has documented a thorough timeline of various metrics of neuronal plasticity as they might emerge early in the stress experience in both the hippocampus and mPFC. In this study, we examined the effects of restraint stress at various durations on neurogenesis and dendritic arbor morphology in mice within the hippocampus and mPFC. Wild type C57BL/6J mice were placed in Plexiglas restraints for various time periods over a duration of 2-21 days (1 hour for 2 days, 0.5 hour for 5 days, 1 hour for 5 days, and 5 hours for 21 days). Non-stressed controls were either left alone with food and water or yoked to a respective restraint stress group with their food and water removed. Metrics of neuroplasticity in the

hippocampal CA3 region and in the mPFC (prelimbic and cingulate) at level II-III, included measuring neurogenesis, identified using BrdU, functional activation of the immediate early gene product, FOS, using immuno-fluorescence, and changes in dendritic complexity of pyramidal apical dendrites as determined from Golgi stained sections. The results are expected to show that the hippocampus and mPFC would express different timelines for neuroplastic changes and that stress-induced changes in neurogenesis and FOS would precede dendritic remodeling.

Disclosures: J.J. Hanna: None. **K. Sainz:** None. **C.D. Conrad:** None. **D.P. Baluch:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

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Program#/Poster#:899.17/UU14

Topic: E.05. Stress and the Brain

Support: NIH NCRR 5P20RR016463-12

NIH 8P20GM103423-12

Title: Triggering the epigenome to combat stress: Modulation of depressive symptoms in adult rats by prenatal choline supplementation

Authors: *K. ERICKSON, D. WISE, M. ROSENGREN, M. J. GLENN
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Abstract: Availability of the nutrient choline in early development has lasting effects on neural function and behavior. Prenatal choline supplementation in rats exerts marked neuroprotective effects against severe disorders such as fetal alcohol syndrome and epilepsy; it also prevents age-related declines in plasticity and cognitive function. Choline levels may exert neuroprotective effects by increasing adult neural plasticity and neurotrophic expression, possibly via epigenetic effects on gene regulation. Interestingly, a failure in neural plasticity and diminished neurotrophic expression are believed to be important contributors to the pathology associated with depression. Recent work in our lab (Glenn et al., 2012) compellingly showed that choline supplementation early in life has an antidepressant effect in adult female rats. The aim of the present study was to extend these findings to both male and female rats, as depression in humans affects males and females differently, and to examine both prenatal choline supplementation and deficiency, as prenatal choline deficiency in rats may lead to vulnerability to pathology such as depression. This study also investigated the effects of prenatal choline availability and chronic

stress on the epigenome as a possible mechanism for antidepressant properties of choline supplementation. From gestation day 10 until birth, pregnant Sprague Dawley rat dams were fed either deficient (0 g/kg choline), standard (1.1g/kg choline) or supplemented (5 g/kg choline) diets. Offspring, who served as subjects in the study, were cross-fostered and weaned on postnatal day 25 and fed a standard diet for the remainder of the study. In adolescence, the open field test was used to gauge activity and anxiety based on diet condition. As adults (approximately 120 days of age), half of the rats from each diet condition underwent 14 days of chronic variable stress (CVS), a widely used method for inducing depression-like symptoms in rats; the other half of each group were handled normally and not stressed. Following CVS, rats were assessed for emotional reactivity: the open field test, the elevated plus maze and the forced swim test. Rats were sacrificed and brains extracted to look for neurogenesis and epigenetic changes. The open field and elevated plus maze revealed that choline deficient males and females were more anxious and vulnerable to stress. Choline supplemented males also displayed increased anxiety, although choline supplemented females did not. A histone acetylation assay revealed that stressed, supplemented females had increased histone acetylation, a feature associated with antidepressants.

Disclosures: K. Erickson: None. **D. Wise:** None. **M. Rosengren:** None. **M.J. Glenn:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.18/UU15

Topic: E.05. Stress and the Brain

Title: Distribution of corticosteroid binding globulin and glucocorticoid receptors in rat hippocampus

Authors: *J. D. CALDWELL[1], E. SIVUKHINA[2], G. F. JIRIKOWSKI[2]
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Abstract: Adrenal steroid hormones are known to be involved in central stress responses. They are thought to act through nuclear glucocorticoid receptors (GR), which have been described in various brain regions, including the hippocampus. Recently we observed the intrinsic expression of corticosteroid binding globulin (CBG) in various brain regions. Here we examined the immunocytochemical co-localization of CBG and GR in the rat hippocampus. While nuclear GR staining was found in most of the pyramidal cells in the CA 1 region and in the dentate gyrus, the CA 2 and the CA 3 regions were almost devoid of GR. Intense cytoplasmic immunofluorescence

for CBG was found in the CA 2 and CA 3 regions while CBG appeared in the CA 1 region predominantly in non-pyramidal cells. Co-localization of both antigens was most pronounced in the subiculum and in the dentate gyrus. We conclude that glucocorticoids act on the limbic system through both nuclear receptors and non-genomic effects mediated by CBG and its putative membrane receptor.

Disclosures: J.D. Caldwell: None. **E. Sivukhina:** None. **G.F. Jirikowski:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:899.19/UU16

Topic: E.05. Stress and the Brain

Support: Canadian Institutes of Health Research Grant 38069

Title: Glucocorticoid receptors in the prefrontal cortex mediate stress-evoked dopamine release via descending glutamatergic input to the ventral tegmental area

Authors: *K. A. BUTTS, A. G. PHILLIPS
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Abstract: Enhanced dopamine release in the medial prefrontal cortex (mPFC) is a well-documented response to acute stress. We have previously shown that glucocorticoid receptors in the mPFC regulate stress-evoked dopamine release (Butts et al., 2011) but the underlying mechanism(s) is unknown. Dopamine neurons in the ventral tegmental area (VTA) receive excitatory input from and send reciprocal projections to the mPFC (Sesack and Carr, 2002). We hypothesized that blockade of prefrontal glucocorticoid receptors could reduce activity of descending glutamatergic input to the VTA, thereby attenuating stress-evoked dopamine release in the mPFC. We also examined the possible role of glucocorticoid-induced synthesis and release of endocannabinoids acting presynaptically via cannabinoid CB_1 receptors to inhibit GABA release onto prefrontal pyramidal cells (Hill et al., 2011), thus enhancing descending glutamatergic input to the VTA during stress. *In vivo* microdialysis was performed in freely-moving rats subjected to an acute tail-pinch stress. A glucocorticoid receptor antagonist or a cannabinoid CB_1 receptor antagonist was delivered by reverse-dialysis into the mPFC, while glutamate and dopamine was continuously sampled from the VTA or mPFC. Glutamate and dopamine in microdialysates were separated by high-pressure liquid chromatography and quantified by electrochemical detection. We demonstrate that acute tail-pinch stress leads to a significant increase in glutamate release in the VTA but not in the mPFC. Glucocorticoid

receptor blockade in the mPFC significantly attenuated the stress-evoked glutamate release in the VTA. In contrast, administration of the cannabinoid CB_1 receptor antagonist into the mPFC failed to attenuate stress-evoked dopamine release in the mPFC. Taken together, our data suggest that glucocorticoids act locally within the mPFC to modulate mesocortical dopamine release by potentiation of glutamatergic drive onto dopamine neurons in the VTA.

Disclosures: K.A. Butts: None. **A.G. Phillips:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

Location: Hall F-J

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Topic: E.05. Stress and the Brain

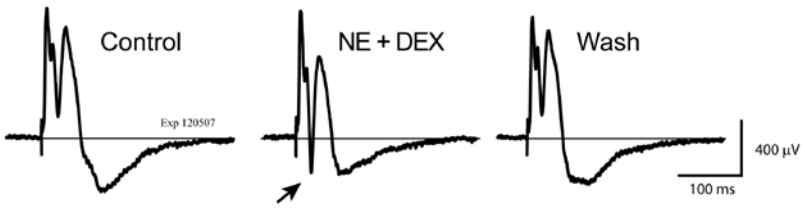
Support: NSF Grant UBM0634588

Title: Cooperativity of corticosteriods and norepineprhine in non-genomic modulation of corticocortical transmission

Authors: A. K. PETKO, E. WHITE, *D. M. SENSEMAN
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Abstract: In a variety of mammalian and non-mammalian species, increased levels of corticosteroids trigger overt changes in behavior (e.g. increased locomotion) over time scales too short to involve *de novo* protein synthesis. Recently, several electrophysiological studies of rat brain slices have led to a rapid increase in our understanding of how such non-genomic effects of corticosteroids modulate neurotransmitter release . What remains to be determined, however, is how these modulatory effects lead to alterations in the organization and activities of neuronal ensembles that underlie overt behavioral changes. The isolated, *in vitro*, turtle telencephalon provides an especially amenable preparation in which to study non-genomic corticosteroid actions on neuronal assemblies. Since the preparation is basically a flat sheet with its excitatory and inhibitory interconnections largely intact, both whole-cell recording of individual cortical, hippocampal, and septal neurons can be combined with simultaneous monitoring of population activity using voltage-sensitive dye imaging (VSDI). As a first step to developing this promising preparation, we monitored the local field potential (LFP) evoked in the turtle's homologue of hippocampal area CA3 (i.e. dorsomedial cortex) in response to brief electrical stimulation of the turtle's homologue of the dentate gyrus (medial cortex). The waveforms of these LFP's were complex with multiple positive and negative peaks indicating activation of several polysynaptic pathways (see fig below). We found that in most preparations, the LFP waveform in the

homologue of CA3 could be reversibly altered (arrow in fig) by bath application of 20 microM norepinephrine (NE) and 10 microM dexamethasone (DEX). (Comparatively high levels of DEX mostly likely reflect its difficulty in crossing the semi-intact blood brain barrier). Significantly, similar effects were not observed when either drug was applied individual, but only when both drugs were applied in combination.



Disclosures: A.K. Petko: None. **E. White:** None. **D.M. Senseman:** None.

Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

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Support: VA Career Development Award to K.M.S.

VA Merit Awards to K.E.S. and C.E.A.

MH15442

MH073725

MH086383

MH81177

MH68582

Title: Prenatal stress in rats alters hippocampal nicotinic acetylcholine receptor levels in adult offspring

Authors: *M. B. BURKE[1,2], K. M. SCHULZ[2,4], K. M. ANDRUD[2,4], J. N. PEARSON[3], A. KREISLER[5], C. E. ADAMS[2,4], S. LEONARD[2,4], K. E. STEVENS[2,4]

[1]Univ. of Colorado Denver, Denver, CO; [2]Dept. of Psychiatry, [3]Neurosci. Program, Univ. of Colorado Anschutz Med. Campus, Aurora, CO; [4]VAMC, Denver, CO; [5]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Prenatal stress in humans is associated with psychiatric problems in offspring such as anxiety, depression, and schizophrenia. These same illnesses are also associated with neuronal nicotinic receptor abnormalities. Despite the known associations between prenatal stress exposure and offspring mental illness, and between mental illness and nicotinic receptor abnormalities, it is not known whether prenatal stress exposure impacts neuronal nicotinic receptors. Thus, we tested the hypothesis that maternal stress alters the development of hippocampal alpha4 beta2 and alpha7 nicotinic acetylcholine receptor levels in offspring. Female Sprague-Dawley rats experienced unpredictable variable stressors 2-3 times daily during the last week of gestation. At weaning (21d) the offspring were assigned to same-sex prenatally stressed or nonstressed groups. In adulthood, offspring brains were collected and processed for quantitative autoradiography using an 125I-epibatidine (alpha4 beta2 nicotinic receptor-selective), and 125I-alpha-bungarotoxin (alpha7 nicotinic receptor selective) ligands. We found that prenatal stress significantly increases epibatidine binding in the offspring's alveus, CA1 lacunosum moleculare, CA3 laconusum moleculare, and the dentate gyrus molecular layer, suggesting that prenatal stress significantly increases hippocampal alpha4 beta2 receptor levels. No effects of stress or interactions between sex and stress were observed for epibatidine binding. We are also currently quantifying hippocampal alpha-bungarotoxin binding to determine whether prenatal stress exposure differentially impacts alpha7 and alpha4 beta2 nicotinic receptor subtypes, or whether the effects of prenatal stress exposure are limited to the alpha4 beta2 nicotinic receptor subtype. Taken together, our results suggest that nicotinic receptors may be an important link between prenatal stress exposure and risk for neuropsychiatric illness.

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Poster

899. Stress-Modulated Pathways: Cortex, Hippocampus, and Striatum

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Program#/Poster#:899.22/UU19

Topic: F.02. Animal Cognition and Behavior

Support: COECYTJAL

FOMIX UDG

Title: Chronic exposure to environmental noise stress in early life produces long-term improvement in the rat spatial learning

Authors: *O. HUET[1], Y. RUVALCABA-DELGADILLO[1], R. GONZÁLEZ-CASTAÑEDA[1], G. CHIPRES-TINAJER[1], P. HERNÁNDEZ-CARRILLO[1], G. YAÑEZ-DELGADILLO[1], J. GARCÍA-ESTRADA[3], S. LUQUIN[1], F. JÁUREGUI-HUERTA[2]
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Abstract: There is a growing concern that the excess environmental noise experienced by infants may disrupt their growth and development, putting them at risk for cognitive and neurological alterations. In the present experiment, we used the Morris Water Maze to assess short and long-term spatial learning changes in juvenile rats exposed to environmental noise. Recently weaned male Wistar rats were subjected to chronic environmental noise (urban sounds adapted to the rat audiogram) during the 15 days after weaning (from 21-36 posnatal days). Then, Morris Water Maze was used to evaluate the rat spatial learning performance both, at the end of the noise exposure (36 posnatal day) and two months later when the rat was adult. In addition, body weight measures were taked periodically. Our results, shows that the environmental noise exposed rats exhibit a retardation in their body weight gain. However, Morris Water Maze assessment revealed no impairment in spatial learning abilities but a remarkable improvement in those who were exposed to environmental noise. Exposed rats performed better both, at the end of the exposition when they where youngs, and two months later when they were adults. Such results suggest that our noise model works as a moderate stressor which indeed stimulate spatial learning abilities. Such effect persisted long after the noise protocol was concluded.

Disclosures: O. Huet: None. **Y. Ruvalcaba-Delgadillo:** None. **R. González-Castañeda:** None. **G. Chipres-Tinajer:** None. **P. Hernández-Carrillo:** None. **G. Yañez-Delgadillo:** None. **J. García-Estrada:** None. **S. Luquin:** None. **F. Jáuregui-Huerta:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Research Council of Norway grant 199728 SALMOWA

Title: Omission of expected reward sensitizes the brain dopaminergic system of classically conditioned Atlantic salmon

Authors: M. A. VINDAS[1], E. HOGLUND[2], O. FOLKEDAL[3], I. B. JOHANSEN[4], B. O. BRAASTAD[1], L. H. STIEN[3], T. S. KRISTIANSEN[3], *O. OVERLI[5]
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Abstract: For several reasons, such as easy maintenance, rapid generation times, and increasingly mapped genomes, teleost fishes are emerging as an alternative to small mammals in biomedical, neural, and behavioral research. Behavioral, genetic, and physiological screening of high numbers of individuals across treatments and generations is one particularly attractive feature of fish model systems. Both animal welfare considerations and fundamental scientific questions regarding the evolution of learning and memory have directed particular attention towards possible cognitive and emotional processes in fishes. Here we show that the omission of expected reward (OER) leads to increased aggression towards conspecifics in classically conditioned Atlantic salmon (*Salmo salar*). Furthermore, in response to an acute stressor, OER fish displayed increased dopaminergic (DA) neurotransmission compared to controls. There was also a general downregulation of dopamine receptor D1 gene expression in the telencephalon of OER groups, which suggests a coping mechanism in response to unbalanced DA metabolism. These results indicate that animals subjected to unpredictable reward conditions develop a senzitation of the DA signalling system, manifest as a potentiated response to novel, stressful stimuli. Similarities between fish and mammals in this response to unpredictability illustrates a role for teleost fish as models to understand the development of different types of DA dysfunction.

Disclosures: M.A. Vindas: None. **E. Hoglund:** None. **O. Folkedal:** None. **I.B. Johansen:** None. **B.O. Braastad:** None. **L.H. Stien:** None. **T.S. Kristiansen:** None. **O. Overli:** None.

Poster

900. Stress-Modulated Pathways: Neuromodulatory Systems

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:900.01/VV1

Topic: E.05. Stress and the Brain

Support: Michael Smith Foundation for Health Research Postdoctoral Fellowship

Title: Serotonergic mechanisms underlying sex differences in habituation to repeated restraint stress

Authors: *N. GOEL, Q. ZHENG, L. QIU, V. VIAU
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Abstract: Maladaptive stress coping is associated with increased risk of affective disorders such as depression and anxiety. These disorders are more prevalent in women than in men and involve dysfunction of both the serotonin (5-hydroxytryptamine, 5-HT) system and the hypothalamic-pituitary-adrenal (HPA) stress axis. We hypothesized that the ability of the HPA axis to adapt to repeated stress differs in males and females and that habituation of stress hormone secretion is associated with changes in serotonergic markers. To investigate sex differences in stress habituation, we exposed adult male and female rats to 10 daily episodes of 3 hour restraint stress. ACTH responses in males declined significantly by the 5th day of restraint compared to the first day. At day 10, ACTH levels were further decreased by a small degree. In contrast, ACTH responses in females did not show a significant decline, even by the 10th day of repeated stress. Similarly, restraint-induced corticosterone levels in males were attenuated by day 5, while females did not display a significant reduction on day 5 or 10. These data indicate that the capacity to habituate to repeated stress is impaired in females. A potential mediator of HPA axis function and changes in mood is the 5-HT 1A receptor, which has been implicated in previous studies of chronic stress and depression. We examined 1A binding in several forebrain limbic regions and the dorsal raphe and found no stress- or sex-dependent changes on day 5. We hypothesize that sex differences in 1A binding capacity and mRNA will emerge by day 10 and will correlate with the ability to habituate to stress. These studies provide us with a framework for determining whether the sex difference in neuroendocrine habituation may be explained by differences in the recruitment of 5-HT circuits and function of the 1A receptor. These mechanisms may improve our understanding of the bases for individual and sex-related differences in affective disorders.

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Poster

900. Stress-Modulated Pathways: Neuromodulatory Systems

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Program#/Poster#:900.02/VV2

Topic: E.05. Stress and the Brain

Support: NIH grant MH069860

Title: Regulation of HPA axis and cardiovascular responses to chronic stress exposure: Role of hindbrain catecholaminergic neurons

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Abstract: Chronic stress exposure is accompanied by hypothalamic-pituitary-adrenal (HPA) axis hyperactivity and cardiovascular dyshomeostasis. Chronic stress can sensitize the subsequent activation of HPA axis in response to a novel acute stressor. Brainstem norepinephrine (NE) A2 neurons in the nucleus of the solitary tract (NTS) play role in integration of endocrine (HPA axis) and autonomic (cardiovascular system) stress responses. A2 neurons provide the primary NE input to the parvocellular paraventricular nucleus (PVN), which expresses corticotropin-releasing hormone, which is required for ACTH and corticosterone (CORT) secretion. Enhanced NE function contributes to sensitization of HPA axis responses following chronic stress. We hypothesized that A2 cells mediate sensitization to novel stressor following unpredictable stress exposure. To study the role of A2 neurons in the HPA axis and cardiovascular responses to acute restraint stress (RS) and chronic variable stress (CVS), four groups of animals were used. Animals were injected with vehicle or 6-OHDA (10 mg/ml, 2µl). Over half of the animals receiving vehicle or 6-OHDA injection were implanted with radiotelemetry transmitters to afford continuous recording of heart rate, blood pressure and activity. All experimental groups were exposed to acute RS, followed by 14 days of CVS and a novel stressor (NS) (elevated plus maze) on the 15th day. 6-OHDA animals had significantly lower plasma CORT levels 30 min after exposure to acute RS relative to vehicle injected animals. However, we did not find any significant differences in CORT secretion following NS. During RS exposure 6-OHDA animals showed significantly decreased heart rate relative to vehicle injected animals. During CVS (on the 7th day) vehicle animals showed increased low frequency (LF, reflects sympathetic activity) and high frequency (HF, reflects parasympathetic activity) components of heart rate variability and elevated LF/HF ratio (assessed on the 14th day of the CVS). In contrast, no elevations in LF activity were observed in 6-OHDA rats; however, HF activity was diminished (assessed on the 14th day of CVS). After NS exposure, vehicle animals had increased LF/HF ratio and 6-OHDA animals decreased HF. Importantly, while A2 do not appear to be involved in CVS-mediated HPA axis sensitization, they are required for CVS-induced changes in sympathetic drive, and may contribute to deleterious effects of long-term stress on cardiovascular function.

Disclosures: J. Bundzikova: None. **S. Ghosal:** None. **B.A. Packard:** None. **J.P. Herman:** None.

Poster

900. Stress-Modulated Pathways: Neuromodulatory Systems

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Topic: E.05. Stress and the Brain

Support: HHMI individual grant (SM)

UROP individual grant (AJM)

NIMH grant R01MH086539 (CAL)

Title: Chronic glucocorticoid intake abolishes the diurnal pattern of slc6a4 and htr1a mRNA expression in anxiety-related serotonergic systems

Authors: *S. MANI, N. C. DONNER, A. J. MATTI, A. J. WACKER, C. A. LOWRY
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Abstract: Research has demonstrated that the diurnal expression pattern of *tph2*, the gene encoding the rate-limiting enzyme during serotonin (5-hydroxytryptamine, 5-HT) synthesis, depends on the circadian glucocorticoid (GC) rhythm. Research from our lab has furthermore established that chronic GC exposure causes an anxiety- and depression-like phenotype associated with an abolished diurnal variation of *tph2* expression due to increased *tph2* expression during the inactive phase (light phase in rodents). We here hypothesized that *slc6a4*, the gene encoding the serotonin transporter (SERT), and *htr1a*, the gene encoding the autoinhibitory 5-HT_{1A} receptor, are also expressed in a diurnal pattern that is altered by chronic GC exposure. To test this hypothesis, we subjected adrenal-intact adult, male rats to 40, 100, or 400 μg/ml corticosterone (CORT) or vehicle (0.45% 2-hydroxypropyl-β-cyclodextrin) via the drinking water for 21 days, and assessed *slc6a4* and *htr1a* mRNA expression within the serotonergic dorsal raphe nucleus (DR), the main source of brain 5-HT, using *in situ* hybridization histochemistry. We found that CORT-treatment specifically affected *slc6a4* and *htr1a* mRNA expression within specific DR subregions. In vehicle-treated rats, the anxiety-related dorsal DR (DRD) displayed a diurnal variation in *slc6a4* expression that was abolished in CORT-treated rats by suppressing *slc6a4* expression dose-dependently during the rats' active dark phase. CORT treatment also decreased dark-phase *slc6a4* expression in the caudal part of the ventral DR (cDRV), an area that provides a dense innervation of the sensorimotor cortex, among other brain regions, and is consistently affected in chronic anxiety models that involve vulnerability to panic-like responses. Expression of *htr1a* occurred in a diurnal pattern within the anxiety-related caudal DR (DRC), a pattern that was, again, abolished by chronic CORT treatment. No other subdivisions displayed a diurnal expression pattern of either gene, suggesting an indirect effect of chronic CORT treatment on gene expression, possibly via alteration of afferent signaling from the extended amygdala complex. The observed changes in *tph2, slc6a4*

and *htr1a* expression may result in an overactive serotonergic system (more 5-HT production, less 5-HT reuptake, and disrupted diurnal 5-HT_{1A}-mediated autoinhibition) particularly within those DR subdivisions previously associated with control of anxiety-related behavior. The effects of CORT-induced changes in *slc6a4* and *htr1a* expression on serotonergic activity in forebrain structures remains to be determined.

Disclosures: S. Mani: None. **N.C. Donner:** None. **A.J. Matti:** None. **A.J. Wacker:** None. **C.A. Lowry:** None.

Poster

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Topic: E.05. Stress and the Brain

Support: Emerald Foundation, Inc.

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Sobel and Keller Fund

Title: Stress, adrenergic function and post-traumatic stress disorder

Authors: *D. WONG[1], K. M. MYERS[2], R. CLAYCOMB[1], W. A. CARLEZON, Jr.[2]
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Abstract: Stress and epinephrine (EPI) contribute to both physiological and behavioral illnesses. As a stress hormone, epinephrine (EPI) plays a pivotal role in enabling organisms to restore homeostasis and overcome stress. We have shown that EPI itself is stress-regulated through its biosynthesis by phenylethanolamine N-methyltransferase (PNMT). In the adrenal of rats subjected to single or repeated immobilization (IMMO) or rat adrenal medulla-derived PC12 cells exposed to moderate hypoxic stress (5% oxygen), marked elevation of PNMT mRNA and protein is induced, preceded by a rise in the PNMT gene transcriptional activators, Egr-1, Sp1 and HIF1alpha mRNA and protein. However, little is known about mechanisms by which EPI contributes to illness. Post-traumatic stress disorder (PTSD) is a behavioral illness that continues to escalate, particularly in war veterans returning from Iraq and Afghanistan, children and teenagers. PTSD patients show memory extinction deficits and elevated EPI, with relief of symptoms provided by adrenergic antagonists. We have recently developed a rat PTSD model

using immobilization (IMMO) stress and have demonstrated that stress-induced elevation of PNMT and EPI alters adrenergic drive through central learning and memory circuitry. Long-term adaptive/maladaptive changes are apparent, with maladaptive changes having the potential to evoke illness. Rats trained in fear extinction exhibit deficits in extinction memory consolidation and/or retrieval in the rat fear-potentiated startle (FPS) paradigm when subjected to 2 hr sessions of daily IMMO for at least one week. Adrenal adrenergic indices are concomitantly elevated, including Egr-1, HIF1alpha and PNMT mRNA and protein. In contrast, behavior deficits and changes in adrenergic indices do not occur in rats trained in fear extinction and exposed to a single IMMO. Present studies are examining how IMMO duration and adrenergic antagonists affect these observed behavioral and adrenergic changes in this rat PTSD model.

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Poster

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Topic: E.05. Stress and the Brain

Support: NIMH grant R01MH086539 (CAL)

HHMI individual grant (SM)

Title: Chronic glucocorticoid intake increases basal- and stress-induced tryptophan hydroxylase activity in anxiety-, sleep/wake-, and memory-related serotonergic systems

Authors: *N. C. DONNER, P. H. SIEBLER, S. MANI, C. A. LOWRY
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Abstract: Major depressive disorder is linked to both disruption of hypothalamic-pituitary-adrenal (HPA) axis function and dysfunction of brain serotonin (5-hydroxytryptamine, 5-HT) systems. However, causal relationships leading to dysregulation of these systems are still unclear. To test the hypothesis that chronic glucocorticoid (GC) exposure, without external stressors *per se*, alters both basal and stress-induced activity of tryptophan hydroxylase (TPH), the rate-limiting enzyme for 5-HT synthesis, we treated adrenal-intact adult, male rats with 100 μg/ml corticosterone (CORT) or vehicle (0.45% 2-hydroxypropyl-β-cyclodextrin) via the drinking water for 21 days. We monitored weight gain and diurnal food and water consumption, and assessed the rats' emotionality in the elevated plus-maze (EPM) and forced swim tests (FST)

after two weeks. On day 21, all rats were injected with 100 mg/kg NSD-1015, an inhibitor of aromatic amino acid decarboxylase, to block the conversion of 5-hydroxytryptophan (5-HTP) to 5-HT. Following the injection, rats were either exposed to acoustic startle (AS) for 30 min or remained in home cages until rapid decapitation. *Post mortem,* we assessed *ex vivo* adrenal functionality in a tissue culture assay, and measured 5-HTP accumulation, a readout of TPH activity, in the median raphe nucleus (MnR) and dorsal raphe nucleus (DR) subregions, and in anxiety-related forebrain target areas, using high-pressure-liquid chromatography. Chronic CORT intake caused reduced weight gain, anxiety-like behavior on the EPM, increased immobility in the FST, an increased AS response, and adrenal insufficiency. The diurnal pattern and the amount of food and water intake remained unchanged. Most importantly, chronic CORT elevated both basal and AS-induced TPH activity in the dorsal (DRD) and ventral DR (DRV), as well as basal TPH activity in the MnR. In the caudal DR (DRC), the infralimbic cortex (IL), the dorsal *cornu ammonis* region of the hippocampus (dCA1) and the caudal pontine reticular formation (PnC), AS elevated TPH activity only in CORT-, but not in vehicle-treated rats, indicating a permissive effect of chronic CORT. The increase of TPH activity in the PnC was strongly correlated with the magnitude of the AS response. Our results are novel, and suggest that GCs exert their chronic actions by creating either a basal hyperactivity or a hypersensitivity to an acute stressor in those serotonergic circuitries that modulate anxiety-related behavior (DRC, DRD, DRV, IL) as well as sleep/wake function (MnR, PnC) and learning and memory (dCA1).

Disclosures: N.C. Donner: None. **P.H. Siebler:** None. **S. Mani:** None. **C.A. Lowry:** None.

Poster

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RR000163 for the operation of ONPRC

Title: The effect of short-term stress on serotonin gene expression in high and low resilient macaques

Authors: C. L. BETHEA[1,2,3,4], K. PHU[1], A. P. REDDY[1], *H. M. RIVERA[1], J. L. CAMERON[5]
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Abstract: Female cynomolgus monkeys exhibit different degrees of reproductive dysfunction with moderate metabolic and psychosocial stress. When stressed with a paradigm of relocation and diet for 60 days, or 2 menstrual cycles, highly stress resilient monkeys continue to ovulate during both stress cycles (HSR); medium stress resilient monkeys ovulate once (MSR) and stress sensitive monkeys do not ovulate for 60 days or more (SS). This study examines serotonin-related gene expression in monkeys with different sensitivity to stress and exposed to 5 days of moderate stress. Monkeys were first characterized as HSR, MSR or SS. After resumption of menstrual cycles, each monkey was re-stressed for 5 days in the early follicular phase. The expression of 3 genes pivotal to serotonin neural function was assessed in the 3 groups of monkeys (n=4-5/group). Tryptophan hydroxylase 2 (TPH2), the serotonin reuptake transporter (SERT), and the 5HT1A autoreceptor were determined at 7 morphological levels of the dorsal raphe nucleus with in situ hybridization (ISH) using digoxygenin-incorporated riboprobes. Positive pixel area and cell number were measured with ImageJ. All data were analyzed with ANOVA. After 5 days of stress, there was no significant difference in TPH2, SERT or 5HT1A gene expression between groups. This pattern of expression differed markedly from the pattern observed in the absence of stress in previous studies. Therefore, the pattern of expression was examined in HSR and SS groups in the absence and presence of stress. In the absence of stress, TPH2, SERT and 5HT1A gene expression was 54%, 128% and 36% higher in the HSR group than the SS group, respectively. In the presence of stress, TPH2 and SERT expression were less than or equal in the HSR and SS groups. However, 5HT1A remained 36% higher in the HSR group than the SS group. In addition, the ratio of HSR/SS gene expression for TPH2, SERT and 5HT1A equaled, respectively, 1.54, 2.28, and 1.36 in the absence of stress and 0.72, 0.98 and 1.36 in the presence of stress. We speculate that the SS group had an ‘acute stress response’ with an immediate increase in TPH2 and serotonin production, which correlates with an increase in SERT expression, while the HSR group may not have responded profoundly. However, 5HT1A expression may have been slower to change, which is also observed after antidepressant treatment. In summary, SS animals perceived stress and serotonin neurons responded quickly to 5 days of moderate metabolic and psychosocial stress. How the serotonin system functions after a longer period of stress that is sufficient to suppress ovulation is the subject of ongoing experiments.

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Poster

900. Stress-Modulated Pathways: Neuromodulatory Systems

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Topic: E.05. Stress and the Brain

Support: Natural Science Foundation of China (no. 30870862)

Creative Research Groups of The National Natural Science Foundation of China (81121003)

Title: Neuropeptide y reverses chronic stress-induced baroreflex hypersensitivity in rats

Authors: *J. AI, F. XIE, L. SUN, X. SU, Y. WANG, J. LIU, R. ZHANG, N. WANG, T. BAN
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Abstract: Chronic stress, as a risk factor for cardiovascular diseases, has been reported to result in elevated plasma neuropeptide Y (NPY) and be highly associated with abnormal cardiac autonomic function. This study aimed to explore the effect of NPY on the chronic stress-induced abnormal baroreceptor reflex sensitivity (BRS). Seven types of recognized stressors were used to develop chronic stress rat model. Subcutaneously implanting ALZET mini-osmotic pumps containing NPY were used to evaluate the action of NPY on the stressed male rats. We found that chronic stress showed no influence on baseline systolic blood pressure (SBP) and heart rate (HR), whereas NPY (85 μg for 30 days) could elevate baseline SBP and induce bradycardia in rats intervened by various stimuli. NPY pretreatment could preserve chronic stress-induced decreases in left ventricular systolic pressure (LVSP) and the maximum rate of change in left ventricular pressure in the isovolumic contraction period ($+dp/dt_{max}$) but has shown no effect on left ventricular end diastolic pressure (LVEDP) and the maximum rate of change in left ventricular pressure in the isovolumic relaxation period ($-dp/dt_{max}$). Notably, chronic stress led to baroreflex oversensitivity indicated by the elevated ratio of ΔHR/Δmean arterial blood pressure (MABP) in rats followed by vasoconstrictor (phenylephrine, PE) or vasodilator (sodium nitroprusside, SNP) administration, which was almost completely reversed by NPY pretreatment. The expressions of substance P (SP) and gamma aminobutyric acid A receptor ($GABA_AR$) in nucleus tractus solitarius were increased in chronic stress rats, which were counteracted by NPY pretreatment. We conclude that chronic stress-induced baroreflex hypersensitivity could be blocked by NPY pretreatment. Furthermore, the altered expressions of neurotransmitters and receptors in the brainstem might contribute to this process. Conflict of interest: The authors declare no competing financial interest. This study was supported in part by the project of Natural Science Foundation of China (no. 30870862), the Funds for Creative Research Groups of The National Natural Science Foundation of China (81121003).

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Poster

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Topic: E.05. Stress and the Brain

Support: DA09082

MH40008

Title: Targeting of the dorsal raphe nucleus by amygdalar afferents

Authors: *E. J. VAN BOCKSTAELE[1], T. A. RETSON[1], R. J. VALENTINO[2]
[1]Neurosci., Thomas Jefferson Univ., PHILADELPHIA, PA; [2]Dept. of Anesthesiol. and Critical Care Med., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: According to the National Institute of Mental Health, nearly 10% of the American adult population is affected by a mood disorder in a given year, with the costs of mental healthcare reaching billions of dollars. One of the major forms of treatment in the management of affective disorders is administration of selective serotonin reuptake inhibitors. The dorsal raphe nucleus (DRN) is enriched in serotonergic neurons that provide the primary source of serotonin to the forebrain and is a region profoundly impacted by stress. To better understand the regulation of the DRN by the amygdalar stress-related peptides, corticotropin releasing factor (CRF) and dynorphin (DYN), we examined projections from the central nucleus of the amygdala (CeA) to the DRN-5HT system using retrograde tract-tracing from the DRN, and immunocytochemical identification of CRF and DYN in the CeA. Fluorescent latex microspheres (Retrobeads, Lumafluor) were injected into the DRN of adult male rats and were directed at its ventromedial quadrant at the anterior-posterior coordinate of Bregma -7.7mm (Paxinos and Watson, 1986). Rats were perfused one week after injection, and brains were sectioned for analysis. Retrogradely labeled neurons were identified within the CeA confirming previous studies. Specifically, when injections were placed in the ventromedial division of the DRN (n=3), analysis revealed that 595±32 neurons were present in the rostral aspect of the CeA, 843±87 were present in the mid-region of the CeA and 252±32 in the caudal region of the nucleus. Approximately 28% of DRN-projecting neurons in the CeA exhibited CRF, while 35% contained DYN. Considering the importance of the amygdala in stress sensitivity and associating salience with emotional cues in the environment, understanding the influences of this limbic

region on the DRN may help fill a gap in our knowledge regarding neural circuits impacting 5HT systems, and may offer additional insight into treatment targets for affective disorders.

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Poster

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Topic: E.05. Stress and the Brain

Support: JSPS KAKENHI 22592158

Title: Restraint stress affects Galectin-1 level in serum and organs of adult rats

Authors: *T. KADOYA[1], M. IWAMOTO[2], S. KOIZUMI[2], H. HORIE[2], E. KOSHINO[1], T. YAMAMOTO[3], K. SASAGURI[2]
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Abstract:
Stress is inherent in modern society, and it has the potential to disturb the normal functions of many systems of the human body, including the nervous system, the endocrine system, and the immune system. Therefore, the investigation of the markers that show stress at an early stage will be useful and important for diagnostic and therapeutic purposes.
Galectin-1 (Gal-1), an animal lectin with an affinity for β-galactoside, is widely expressed in various normal and pathological tissues and shows a variety of biological activities, such as immune-cell homeostasis, tumorigenesis, nerve regeneration after injury, and the inflammatory response. However, the effect of various types of stress on the Gal-1 level is unclear. In this study, we employed a restraint stress model using male adult rats and investigated the effect on Gal-1 level in serum and several organs by western blot analysis and ELISA. The *in vivo* experiments demonstrated that Gal-1 quickly responds to the stress, the restraint stress markedly increases the serum Gal-1 level. The Gal-1 increase in the serum was inhibited by pre-treatment with a neurotoxin 6- hydroxydopamine, which destroys the noradrenergic nerve terminals. On the other hand, a bilateral adrenalectomy had no effect on the Gal-1 increase. These results suggest that the stress-induced increase of Gal-1 in serum is regulated by the sympathetic nervous system under stress conditions. Furthermore, the Gal-1 levels increased in thymus and spleen at 30min and 60 min after the restraint stress. Gal-1 may therefore play an important role in the reaction to the stress and be a good novel candidate as a stress marker.

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Poster

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Topic: E.05. Stress and the Brain

Support: R01MH 077152

Title: Endocannabinoid signaling as an intrinsic component of the circuits mediating adaptive responses to repeated stress exposures

Authors: *R. J. NEWSOM, C. V. MASINI, T. J. NYHUIS, H. E. DAY, S. CAMPEAU
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Abstract: Evidence implicates the endocannabinoid (eCB) system as a negative modulator of neural and endocrine responses to acute stressors. Recently, eCBs were also shown to influence the development of habituated hypothalamo-pituitary-adrenal (HPA) axis responses to repeated homotypic stress, in which repeated exposures to the same stressor reliably decreases subsequent activation of the HPA axis. The present studies were initiated to distinguish a potential role of eCB signaling in the acquisition as compared to the expression of habituated HPA axis responses. Adult male Sprague Dawley rats (Harlan) were exposed to daily, 30 minute sessions of loud white noise (95 dB) for 8 days. Rats received either the cannabinoid receptor 1 (CB1) antagonist AM251 (2mg/kg or 0.5mg/kg, i.p.) or vehicle 30-min before the loud noise sessions on the first 7 days, but not on the 8th day of stress exposure. Blood samples were taken after loud noise on days 1, 4, 7, and 8. The rats treated with 2 mg/kg AM251 had higher levels of plasma corticosterone (CORT) on days 1,4, and 7, and a slower rate of habituation than rats receiving vehicle or 0.5 mg/kg injections of AM251. However, on day 8 of noise (drug-free), 2 mg/kg AM251-treated rats had near complete attenuation of HPA axis response, as observed in rats receiving vehicle or 0.5 mg/kg AM251 injections during the initial 7 loud noise exposures. This suggests that disruption of CB1 receptor signaling does not disrupt the plasticity associated with acquisition of habituation to repeated homotypic stress. In a second study, rats were exposed to daily loud noise stress for 7 days to establish HPA axis habituation without drug treatments. Twenty-four hrs later, rats were injected with 1 mg/kg AM251 or vehicle, 30 min prior to a final 30-min loud noise exposure. Vehicle-treated rats displayed reliable habituation of HPA axis response, but CB1 antagonism disrupted the expression of this habituated response, by restoring

plasma CORT to levels observed during the initial (day 1) loud noise exposure. Rats were given an additional mild stress exposure (novel environment) 24-hours later, along with a cohort of rats not previously habituated to noise stress. 1 mg/kg AM251 treatment prior to novel environment resulted in a significantly larger HPA axis response, but only in rats recently habituated to repeated loud noises. These results point to eCB signaling as an intrinsic component of the circuits expressing adaptive responses to repeated stress, without itself mediating the plasticity associated with repeated stress exposures.

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Poster

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Topic: E.05. Stress and the Brain

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NSF Grant IOB 0448060

Title: Gender differences in serotonergic stress response in stalk-eyed flies(Teleopsis dalmanni)

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Abstract: The impact of acute stress on brain function, including perturbations of the monoaminergic systems, have important implications for behavioral coping responses. Stress responses often vary among individuals and between genders in phylogenetically diverse organisms. In the stalk-eyed fly (*Teleopsis dalmanni*), a sexually dimorphic species, we initially tested if brain 5-HT concentrations differed between males and females. In a second experiment, we tested the effects of restraint and a combination of restraint and heat stress on brain 5-HT in both sexes. Restraint was accomplished by placing the flies in a 1.5 mL tube for 30 min. Heat stress was induced by emerging the tubes in a water bath set at 36 ^{o}C for 30 min. Brain 5-HT from each individual fly was measured using HPLC with electrochemical detection. We found that 5-HT levels did not differ between control, non-stressed, male and female flies (mean $\pm$ SEM: 7.22±1.73, 7.65±1.56 respectively). In addition, exposure to restraint did not significantly affect 5-HT levels for flies of either sex when compared to untreated controls. However, whole

brain 5-HT levels in males increased 89.6% in response to the combined heat and restraint stress ($P < .027$). In contrast, females exhibited a trend for a decrease in 5-HT in response to the combined stress ($P<0.053$). Additionally, stress-induced changes in 5-HT levels differed when males were compared to females ($p<.003$). These results suggest that the central control of stress responsiveness is sexually dimorphic in the stalk-eyed fly. Supported by NSF grants IOS 0921874 (KJR) and IOB 0448060 (JGS).

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Poster

901. Inflammation and Glia in Intake and Metabolism

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Support: New Zealand Lottery Health Grant 215912

New Zealand National Research Centre for Growth and Development MSc scholarship to TRS

Title: Maternal obesity and IL-6 lead to aberrant developmental gene expression and deregulated axon growth in the fetal arcuate nucleus

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Abstract: Maternal obesity during pregnancy is a well-accepted predetermining factor for offspring risk of obesity. Several observations have pointed to a causative role for the pro-inflammatory cytokine interleukin 6 (IL-6), but how and where it acts on the developing fetus is unclear. We reasoned that elevated IL-6 might alter the formation of neural connections made by body weight control neurons in the fetal arcuate nucleus of the hypothalamus (ARC), the primary site for neural control of body weight. Such an effect may thereby program offspring for deregulated body weight control later in life.
In initial experiments, explants of fetal ARC treated with IL-6 in vitro showed a significantly reduced ability to extend neurites, indicating that IL6 exposure may have a detrimental effect on the ability of the fetal ARC to make neural connections. To assess whether the expression of genes involved with neurite growth regulation may be modulated by IL-6, the expression of

several genes was quantitated in ARC tissue treated with IL-6 in vitro. The expression of Deleted in Colorectal Cancer (DCC), a receptor for the axon growth and guidance molecule Netrin-1, and Gli1, a signaling molecule in the canonical Sonic hedgehog pathway, were found to be significantly increased by IL-6 treatment of ARC tissue in vitro. Strikingly, both genes were also found to be increased in the ARC of fetuses developing in obese, but not normal weight, mothers.
Together, these data suggest that one mechanism by which maternal body weight may program offspring for elevated body weight is through an IL-6-dependent disruption of gene expression that regulates the normal formation of body weight control circuits in the fetal ARC.

Disclosures: C. Jasoni: None. **T.R. Sanders:** None.

Poster

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Support: CNPq

FAPESP

IBRO

Title: Reduction of insulin secretion and desensitization to insulin-brain effects may contribute to hypophagia induced by prolonged LPS treatment.

Authors: *R. C. RORATO, B. C. BORGES, E. T. UCHÔA, P. B. MARANGON, J. ANTUNES-RODRIGUES, L. L. K. ELIAS
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Abstract: It is well-known that insulin and leptin induce hypophagia after intracerebroventricular (icv) injection. The plasma levels of both hormones are increased after acute lipopolysaccharide (LPS) administration. On the other hand, prolonged LPS injection induces desensitization of leptin secretion and leptin-induced hypophagia. In the present study we aimed to investigate if prolonged LPS treatment also desensitizes insulin secretion and their central hypophagic effects. For this purpose rats were assigned to three different groups of LPS i.p. treatment at 16:00: saline once daily for 6 days (saline), saline once daily for 5 days and an injection of LPS at the 6th day (1LPS) and LPS once daily for 6 days (6LPS). On the day of the

experiment, food were removed after treatments and two (18:00) and four (20:00) hours after, the rats were decapitated for plasma collection. We also collected the brain of rats decapitated 2 hours after injections for western blot analyses of mediobasal hypothalamus. A second set of rats were previously icv cannulated and on the day of the experiment, at 15:30, they received insulin (12uM/5uL) or vehicle (saline) and their food consumptions and body weight were determined 2 and 14 hours after, respectively. We observed in 1LPS group, but not 6LPS group, an increased plasma insulin levels 4h after treatments. In addition, 1LPS, but not repeated LPS rats, showed higher expression of IRS-1 in the mediobasal hypothalamus. As expected, we observed in vehicle-treated group a decrease of food intake and body weight in 1LPS-treated rats and no changes in rats that received 6 doses of endotoxin. On the other hand, insulin reduced food intake and body weight gain in saline treated group, but there were no changes in food intake and body weight in 1LPS or 6LPS group. The present data suggest that desensitization of hypophagia after long term LPS treatment may be related to reduction of insulin secretion and decreased insulin signaling. IRS-1 could play a role in the development of desensitization of LPS responses and insulin hyporesponsiveness under prolonged endotoxin exposure.

Disclosures: R.C. Rorato: None. **B.C. Borges:** None. **E.T. Uchôa:** None. **P.B. Marangon:** None. **J. Antunes-Rodrigues:** None. **L.L.K. Elias:** None.

Poster

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DK56373

NS60664

Title: Astrocytes as glucosensors in the solitary nucleus

Authors: D. H. MCDOUGAL, *R. C. ROGERS, G. E. HERMANN
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Abstract: The regulation of serum glucose is critical for brain function. The autonomic response to hypoglycemia is characterized by a collection of responses referred to as counter-regulation (CR) which ultimately raises glucose to euglycemic levels. Brainstem astrocytes may be

important chemodetectors for autonomic control contributing to the initiation of the CR. These experiments were conducted to see if brainstem astrocytes actively signal during conditions of low glucose availability. Further, we examined whether one manifestation of CR, hypoglycemia-induced “cephalic phase” increases in gastric motility, were sensitive to inactivation of brainstem astrocytes. In vitro, confocal live cell imaging of acute brainstem slices from adult Long Evans rats was used to quantify cellular activation of astrocytes and neurons in the nucleus of the solitary tract (NST) during low glucose or glucoprivic [2DG] challenges. Changes in intracellular calcium levels reflected the activation of pre-labeled astrocytes and neurons. Slices were exposed to 10min interval of low glucose or glucoprivic challenge [0.5mM glucose, 1.0mM glucose or 5mM 2-deoxyglucose (2-DG)]. These conditions reversibly evoked significant increases in intracellular calcium in both NST astrocytes and neurons. Activated astrocytes had significantly larger responses and shorter latencies to respond relative to the NST neurons. Additionally, the astrocytic activation induced by low extracellular glucose was maintained even in the presence of 1μM TTX, eliminating the possibility of a neuronal origin of the astrocytic response. A separate experiment suggests that astrocyte detection of glucoprivation is essential to at least one aspect of CR. 2DG applied to the floor of the fourth ventricle [17umole] elicits a significant increase in gastric motility. Fluorocitrate, [astrocyte metabolic inhibitor; 5nM] applied to the fourth ventricle has no effect on basal gastric motility but blocks the 2DG driven increase in gastric motility. These data suggest that NST astrocytes are intrinsically glucosensitive and are capable of actively signaling in response to low glucose availability. This active signaling may play a pivotal role in driving autonomic mechanisms associated with CR.

Disclosures: D.H. McDougal: None. **R.C. Rogers:** None. **G.E. Hermann:** None.

Poster

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Title: Role of astrocytes and ketone bodies in neuronal fatty acid sensing and feeding.

Authors: *A. A. DUNN-MEYNELL[1], B. E. LEVIN[1,2], C. LE FOLL[2]
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Abstract: Select metabolic sensing neurons in the brain alter their activity when ambient levels of metabolic substrates such as glucose and fatty acids (FA) change. Select neurons that “sense” long chain fatty acids reside in the ventromedial hypothalamus (VMH). Astrocytes are potential mediators of the responsiveness of these neurons since astrocytes are the major source of long chain fatty acid oxidation and the only known source of ketone body production in the brain. To assess the role of ketone bodies in VMH neuronal FA sensing, fura-2 calcium imaging was used as a surrogate marker for neuronal activity in dissociated VMH neurons from 3-4 wk old rats. Glucose levels were changed from 2.5 to 0.5 to 2.5 mM glucose to identify glucosensing neurons and then 15nM oleic acid (OA) was added to identify FA sensing neurons. When then exposed to β-hydroxybutyrate (βOHB; 10nM to 100μM), neuronal responses were dependent upon the glucose concentration; the overall proportion of βOHB excited neurons was 66% higher in 2.5mM (48%) than in 0.5mM glucose (29%). In addition, 40% of the OA-excited neurons were further excited by βOHB, while 50-70% of OA inhibited neurons were excited by increasing concentrations of βOHB. To assess the potential role of astrocyte-derived ketone body production on feeding, VMH microdialysis for βOHB was performed on male Sprague-Dawley rats after they were trained over a 3 wk period to eat all their daily calories in a 3h period as 4.5% fat chow (n=10) or 60% fat (HFD; n=10)). On the day of testing, after their initial 3h diet feeding periods, they were then allowed to eat 4.5% chow ad libitum to assess the satiating effect of their respective diet preloads. Jugular venous blood and VMH microdialysis samples were taken every 30 min over an 8h period and food intake was monitored for 24h in a BioDAQ Food Intake Monitor (Research Diets). Rats in both diet groups ate comparable calories during the 3h preload but subsequent chow was 40% and 22% lower over the next 3h ($P<0.05$) and total 24h test period in rats previously preloaded with HFD vs. chow ($P<0.05$) . During the first 1h of the 3h preload, the VMH/ blood ketone ratio increased by 400% during HFD with a variable rise or return of the ratio to pre-feeding levels over the next 8h. Chow feeding caused no increase in VMH/blood ketones. These results suggest that ketone bodies have a primarily excitatory effect on VMH FA sensing neurons, even in the presence of FA, and that astrocytes utilize excess dietary FA to produce these ketones. Thus, HFD-induced increase in VMH ketone levels may alter neuronal FA sensing and play a role in the reduced food intake that follows a HFD preload.

Disclosures: A.A. Dunn-Meynell: None. **B.E. Levin:** None. **C. Le Foll:** None.

Poster

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NIH Grant DK53181

Title: Role of FAT/CD36 in neuronal fatty acid sensing and energy homeostasis regulation

Authors: C. LE FOLL[1,2], A. A. DUNN-MEYNELL[1], S. MOUSATOV[3], C. MAGNAN[4], *B. E. LEVIN[1,2]
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Abstract: The hypothalamus contains "metabolic sensing neurons" which play an integral role in the regulation of glucose and energy homeostasis and the development of obesity and diabetes. Many of these neurons utilize both glucose and long chain fatty acids (LCFA) to alter their activity as a means of monitoring the metabolic status of the body. Using pharmacologic agents, we previously found that 50% of these neurons responded to the LCFA, oleic acid (OA), by utilizing the fatty acid (FA) translocator/receptor, FAT/CD36. To further elucidate the role of CD36 in neuronal FA sensing, adeno-associated viral (AAV) vectors expressing CD36 or luciferase shRNAs as well as mCherry fluorescent marker were injected into the ventromedial hypothalamus (VMH) of P5 Sprague-Dawley rats. At P21-P25, dissociated VMH neurons were obtained from these rats and subjected to fura-2 calcium imaging as a surrogate marker for changes in neuronal activity. Glucose levels were first changed from 2.5 to 0.5 to 2.5 mM to identify glucosensing neurons. They were then exposed to 15 nM OA to identify FA sensing neurons. Neuronal glucosensing was not affected by the CD36 AAV shRNA injections. However, whereas 16% of control (Ctrl) glucose inhibited neurons were also inhibited by OA, none were inhibited in CD36 AAV shRNA rats ($P<0.05$ vs. Ctrl). Also, whereas 47% of Ctrl glucose excited neurons were also excited by OA, only 25% of CD36 AAV shRNA neurons were excited by OA ($P<0.05$ vs. Ctrl). Next, to assess the role of CD36-mediated neuronal FA sensing in the regulation of energy homeostasis, CD36 AAV shRNA or Ctrl AAV were injected into the VMH of P21 male Sprague-Dawley rats. Rats were fed 4.5% fat chow (Ch) for 6 wk, followed by 9 wk on 45% high fat diet (HFD). After 6 wk on chow, the CD36 AAV shRNA rats ate less than Ctrl rats at 1h (-36%, $P<0.05$), 5h (-34%, $P<0.05$) and 24h (-22%, $P<0.05$) when refed after a 24 h fast. Chow-fed CD36 AAV shRNA rats also had elevated fasting plasma glucose (+11%), insulin (+72%) and leptin (+171%) levels vs. Ctrl rats ($P<0.05$). After 9 wk on HFD, CD36 AAV shRNA rats showed no difference in food intake or body weight gain but displayed heavier inguinal fat pad weights (+68%, $P<0.05$) and had higher leptin levels (+54%, $P<0.05$) as compared to Ctrl rats. They also had a markedly abnormal oral glucose tolerance test with 98% higher glucose ($P<0.05$) and 244% higher insulin ($P<0.05$) areas-under-the-curve levels vs. Ctrl rats. These alterations were associated with a 69% decrease of CD36 mRNA expression in the arcuate nucleus of CD36 AAV shRNA rats ($P<0.05$). These results demonstrate

that CD36 is a critical factor in both VMH neuronal FA sensing and the regulation of energy and glucose homeostasis.

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Poster

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Support: French Grant of the Agence Nationale de la Recherche (ANR-11-BSV1-007-01-CONNEXSENSING)

From Grant of Région Bourgogne, PARI-FABER 2010-2012

Title: Involvement of the astroglial networks in the hypothalamic glucose-sensing: Role of Connexin 43

Authors: *C. ALLARD[1], L. CARNEIRO[1], S. GRALL[1], B. CLINE[1], X. FIORAMONTI[1], C. CHRETIEN[1], F. BABA-AISSA[1], C. GIAUME[2], L. PENICAUD[1], C. LELOUP[1]
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Abstract: Introduction
The hypothalamus plays a pivotal role in regulating glucose homeostasis. Hypothalamic detection of increased blood glucose levels, by specific hypothalamic glucose sensitive neurons, triggers physiological responses such as increased insulin secretion and decreased hepatic glucose production. Astrocytes are emerging as important candidates involved in brain glucose-sensing. They create an intricate organized network formed by numerous gap-junctions (GJ) comprised of connexins, allowing for the transfer of glucose from the bloodstream to neurons. The main connexin expressed in astrocytes is connexin 43 (Cx43). We hypothesize that the Cx43-dependant astroglial networks play a critical role in hypothalamic glucose-sensing.
Materials and Methods
Expression of Cx43 was first characterized by immunochemistry in the rat ventro-median hypothalamus (VMH) and then studied using different metabolic conditions (hyperglycemia or fasting). VMH Cx43 was inhibited, in vivo, by stereotaxic injection of siRNA against Cx43 in rats. Hypothalamic glucose-sensing was evaluated by monitoring pancreatic insulin secretion in

response to an intracarotid injection of a glucose bolus towards the brain.
Results
Cx43 was highly expressed in the VMH at astrocytic end-feet enwraping blood vessels. Cx43 expression increased after 3h-hyperglycemia and decreased after fasting. Inhibition of Cx43 (about 30%) led to a decrease of food intake, 72h after the injection, without modifying weigth, blood glucose and insulin levels. Intracarotid glucose injection-induced insulin secretion was significantly decreased in siCx43 rats.
Conclusion
These results show that Cx43 presents a specific expression pattern in the hypothalamus. Moreover, this Cx43 expression varies upon the metabolic status. The central response to glucose (insulin secretion) was lost by inhibition of hypothalamic Cx43. We conclude that Cx43-dependant astroglial networks are critical for hypothalamic glucose sensitivity.

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Poster

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant HL083810

Title: The effect of obesity on microglial activation, inflammation and the renin-angiotensin system in specific brain nuclei

Authors: *A. D. DE KLOET[1], J. A. SMITH[2], D. NGUYEN[2], E. G. KRAUSE[2], C. SUMNERS[1]
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[2]Pharmacodynamics, Univ. of Florida, Col. of Pharm., Gainesville, FL

Abstract: Although energy balance is tightly regulated, the incidence of obesity continues to rise. Obesity is associated with increased prevalence of hypertension and cardiovascular disease. As a consequence, it is imperative that effective treatment strategies for obesity and its co-morbidities are uncovered and this can only be achieved by attaining a thorough understanding of the factors that regulate the homeostatic systems involved. Among these factors are proinflammatory cytokines and the components of the renin-angiotensin system (RAS), which are elevated during both obesity and hypertension.

Here, we hypothesize that an important mechanistic link between high-fat diet (HFD)-induced metabolic and cardiovascular dysregulation is an increase in RAS activity and inflammation within brain regions regulating energy balance and blood pressure. Consistent with this, previous studies indicate that consumption of HFD enhances microglial activation within the arcuate nucleus (ARC; a metabolic control center). Moreover, we have found that leptin, an endocrine factor that becomes elevated during obesity, increases the synthesis of proinflammatory cytokines (tumor necrosis factor-α, interleukin-1β, interleukin-6) in hypothalamic microglial cultures. Nonetheless, the effect of HFD feeding and other obesity-related factors on microglial activation, inflammation and RAS activity in cardiovascular and other metabolic control centers is largely unknown.
In the present studies, adult male C57BL/6 mice were given either a HFD or a low-fat diet (LFD) for 3 days or 8 weeks and the effect of HFD-feeding on microglial activation, inflammation and the RAS within metabolic and cardiovascular control centers was examined via immunohistochemistry and RT-PCR. Eight weeks of HFD feeding resulted in increased body mass and adipose mass. Consumption of HFD led to an increase in the number and activation (based on morphological changes) of microglia within the ARC, the subfornical organ (SFO), and the parvocellular region of the paraventricular nucleus of the hypothalamus (PVN), but not within the magnocellular region of the PVN. Collectively, these data are consistent with the hypothesis that HFD feeding initiates an inflammatory cascade within specific brain regions that may then contribute to disturbed energy and cardiovascular homeostasis.

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Poster

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Title: Generation of transgenic mice with vimentin-driven reporter fluorescence in the ependymocytes

Authors: *S. MINABE[1], C. DEURA[1], T. GOTO[1,2], M. SANBO[2], K. TOMITA[2], M. HIRABAYASHI[2], Y. UENOYAMA[1], K.-I. MAEDA[1], H. TSUKAMURA[1]
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Abstract: Ependymocytes, lining the ventricular surface of the central nervous system, have widely been known to play functional roles, such as energy supports for neighboring neurons. Our previous studies showed that the ependymocytes in the fourth ventricle (4V) or central canal (CC) express key molecules for glucose sensing, such as glucokinase and glucose transporter 2. Thus, we proposed that ependymocytes in the lower brainstem were equipped with a glucose-sensing mechanism, thereby controlling feeding behavior and reproductive functions. To study the intracellular mechanism by which ependymocytes sense the levels of glucose or other energetic molecules, we generated a new transgenic mouse model to visualize ependymocytes with fluorescence proteins. Vimentin, a marker of ependymocytes, was chosen to drive the ependymocytes-specific gene expression. Two types of mouse vimentin promoters (*pVim*) were chosen: full-length *pVim* (3.2 kb) and short *pVim* (0.2 kb). Five transgenic founder mice were generated by microinjection of each DNA construct into mouse oocytes. The transgenic mice with full-length *pVim* successfully expressed reporter fluorescence in ependymocytes of the lateral ventricle, third ventricle, 4V and CC. On the other hands, the short *pVim*, which was reported as essential region for basic promoter function *in vitro*, did not drive reporter fluorescence expression in any ependymocytes. These results indicate that upstream region (3.0 kb) of short *pVim* is essential for the ependymocytes-specific gene expression *in vivo*. The current transgenic mice will be useful for the analysis on functions of ependymocytes, such as intracellular energy-sensing mechanism.

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Poster

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Support: European Research Council Grant StG 260820

Swedish Research Council Grant 63P-21677

Title: Distinct routes for interleukin-1 and lipopolysaccharide-induced anorexia

Authors: *A. NILSSON, L. ELANDER, A. BLOMQVIST, D. ENGBLOM
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Abstract: Inflammation-induced anorexia seems to be mediated both by PGE_2-dependent and independent pathways. Thus, the loss of appetite elicited by interleukin (IL)-1β is attenuated in mice with deletion of microsomal prostaglandin E synthase-1 (mPGES-1), the terminal inducible PGE_2 isomerase, whereas such deletion has no effect on the anorexia elicited by bacterial wall lipopolysaccharide (LPS). Furthermore, LPS-induced anorexia has been reported be dependent on melanocortin receptors, known to be critical for energy homeostasis.
In a series of experiments we have further examined in C57Bl/6 mice the components of the signaling pathways engaged by these two immune stimuli. Pretreatment with the unselective cyclooxygenase (Cox) inhibitor indomethacin or the Cox-2 inhibitor parecoxib, given intraperitoneally (i.p), but not the Cox-1 inhibitor SC 560, attenuated the anorexic response to LPS. Also when administered intracerebroventricularly, indomethacin reduced the LPS-induced hypophagia. Deletion of the MC4-receptor had no effect on the LPS-induced anorexia.
The IL-1β-induced anorexia was attenuated in mice with selective deletion of the EP_4-receptor in neural cells, but unaffected in mice lacking EP_1-, EP_2- or EP_3 receptors.
Taken together with previous observations, these findings show that LPS-induced anorexia is dependent on Cox-2, but not on mPGES-1, and that the signaling pathway is independent of MC4-receptors. The IL-1β-induced loss of appetite seems at least partly to be dependent on PGE_2 and its binding to EP_4-receptors. These results further support the notion that distinct prostanoids may elicit the anorexic response to LPS and IL-1β, respectively.

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Poster

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Support: Georgia State University Brains & Behavior Program Seed Grant

Georgia State University Neuroscience Institute

Title: Neonatal inflammatory pain increases food intake and body weight in adulthood and impairs hippocampal-dependent memory

Authors: *Y. OGAWA[1], N. C. VICTORIA[1], A. Z. MURPHY[2], M. B. PARENT[3]
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Abstract: Each year in the United States, over 500,000 infants are born prematurely. Preterm infants spend on average >2 weeks in the neonatal intensive care unit, where they undergo an average of 14 painful and inflammatory procedures each day, including repeated heel lances, endotracheal intubation, and respiratory and gastric suctioning. The hippocampus is a brain region important for learning and memory and is exquisitely vulnerable to the effects of stress. We have shown previously that neonatal injury decreases hippocampal glucocorticoid receptor expression and impairs hippocampal-dependent memory in adult rats. Given extensive evidence implicating the hippocampus in food intake, we hypothesized that neonatal injury also disrupts food intake in adulthood and that these effects are caused, at least in part, by hippocampal-dependent memory deficits. To test this, Sprague-Dawley rats were given an intraplantar injection of the inflammatory agent carrageenan (1%; edema 24-72 hrs) on the day of birth (Postnatal Day; PN0). Control rats were handled in an identical manner. In adulthood (PN232), food intake behavior and memory in the spatial water maze were measured. Our preliminary data indicate that a single injury on PN0 increased weight gain and decreased the satiety ratio, a measure of the interval between two meals. Interestingly, this effect was only observed in rats that had poor memory scores in the water maze task. These data are the first to demonstrate neonatal inflammatory pain disrupts energy intake and body weight in adulthood and that this effect is related to neonatal inflammatory pain-induced attenuation of hippocampal-dependent memory function.

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Poster

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JSPS Grant 23580402

Title: Neuronal tract tracing of a pathway relaying energetic signals originating in ependymocytes of the lower brainstem

Authors: *C. DEURA, S. MINABE, Y. UENOYAMA, K.-I. MAEDA, H. TSUKAMURA
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Abstract: Energy deficiency causes the suppression of reproductive functions and induction of feeding behaviors. In fact, food deprivation induced suppression of gonadal function in several mammalian species. We have previously suggested that the ependymocytes of the lower brainstem have a possible role in glucose sensing to regulate reproductive functions and food intake. It has also been demonstrated that the malnutrition-induced suppression of gonadotropin secretion is mediated by noradrenergic inputs to the hypothalamic paraventricular nucleus (PVN) from noradrenergic neurons of A2 region in the solitary tract nucleus (NTS). The aim of the present study is to test whether malnutrition-induced suppression of reproduction and feeding is mediated by neural pathway originating in ependymocytes projecting to the PVN through A2 noradrenergic neurons. To this end, wheat-germ agglutinin (WGA), a transsynaptic tracer, was injected into the fourth ventricle (4V) in adult Wistar-Imamichi male rats. These animals were perfused with 4% paraformaldehyde 48 h after WGA injection. Immunohistochemistry was performed to detect WGA in ependymocytes and noradrenergic neurons with specific antibodies for WGA, an ependymal marker vimentin and a noradrenergic neuronal marker dopamine β-hydoroxylase. WGA immunoreactivities were found in vimentin-immunopositive ependymocytes of the 4V and the central canal (CC), suggesting that ependymocytes have taken up WGA from the ventricular lumen. WGA signals were also detected in DBH-immunopositive neurons in the A2 region as well as magno- and parvocellular neurons in the PVN. These results indicate that brainstem ependymocytes connect to PVN through A2 noradrenergic neurons and suggest that the energetic signals were transduced from the ependymocytes to the hypothalamus via A2 noradrenergic pathway.

Disclosures: C. Deura: None. **S. Minabe:** None. **Y. Uenoyama:** None. **K. Maeda:** None. **H. Tsukamura:** None.

Poster

901. Inflammation and Glia in Intake and Metabolism

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:901.12/WW3

Topic: E.07. Food Intake and Energy Balance

Support: FAPESP

CNPq

Title: Intraperitoneal LPS injection leads to hypothalamic AMPK dephosphorylation

Authors: D. C. V. VITORINO[1], *G. F. ANHE[2], R. M. MOURA[1], L. A. V. VELLOSO[1], E. A. R. R. ROMAN[1], C. E. N. NUÑEZ[1], D. S. R. RAZOLLI[1], G. A. S. SANTOS[1], M. A. T. TORSONI[1]
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Abstract: Aim:
AMP-activated protein kinase (AMPK) has long been known by its role in regulating the energetic metabolism in several tissues. Currently, researches have focused the role of AMPK on inflammatory mechanisms, raising the possibility that the kinase plays a crucial role in events triggered by acute endotoxemia. The presence of pathogenic organisms can lead to an increase in energy expenditure and satiety through toll like receptor-4 activation and inflammatory cytokine expression. Thus, AMPK modulation following the TLR-4 activation could be an important event related to several metabolic diseases as obesity and type 2 diabetes. Therefore, the aim of this study is to evaluate the effects on hypothalamic AMPK pathway following acute injection of LPS.
Methods:
Swiss male mice were submitted to stereotaxic surgery to implant of guide cannula into the lateral ventricle. A specific inhibitor of TLR-4 was administered via intracerebroventricular (ICV) and the endotoxin lypopolyssaccharide (LPS) was administered via intraperitoneal (IP) (1 mg/Kg). Four hours later, the animals were killed by decapitation and the hypothalamus was quickly removed and stored at -80ºC. All blood samples were centrifuged and the plasma stored. The plasmatic cytokines were determined by the ELISA procedure and the protein analysis by western blot. Glucose oxidase method and immunofluorescence assay were also performed.
Results:
The LPS treatment significantly reduced AMPK phosphorylation in the hypothalamus with concomitant increase in the plasmatic levels of TNF-α and IL1β, which was not prevented by the use of the TLR4 inhibitor. Blood glucose in LPS-treated animals was increased by 3 fold either in presence or absence of the icv inhibitor, when compared to control group ($p<0.05$). Besides, the inhibitor use per se did not alter de blood glucose compared to control group.
Conclusions:
AMPK dysregulation has been linked with obesity and metabolic syndrome. Hypothalamic AMPK plays important role in the satiety signal and energy expenditure, two events that can be damaged by model of sepsis and obesity. The present results have showed that ip systemic injection with LPS decreased the AMPK activation in fasted mice, which was followed by an increase in the circulating proinflammatory cytokines, suggesting therefore that the LPS-induced hypothalamic AMPK activity might be influenced by endogenous production of cytokines.
Support: FAPESP

Disclosures: D.C.V. Vitorino: None. **G.F. Anhe:** None. **R.M. Moura:** None. **L.A.V. Velloso:** None. **E.A.R.R. Roman:** None. **C.E.N. Nuñez:** None. **D.S.R. Razolli:** None. **G.A.S. Santos:** None. **M.A.T. Torsoni:** None.

Poster

902. Sleep: Systems Architecture

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:902.01/WW4

Topic: E.08. Biological Rhythms and Sleep

Support: IAPA DF

Title: Assessment of the subjective sleep quality in an elderly mexican population using the pittsburgh sleep quality index and its association with depressive symptoms

Authors: *M. M. MELENDEZ[1,2], A. GALLEGOS[3], G. VALDEZ GONZALES[3], M. LOPEZ BRAMBILA[3], E. AGUILAR BUSTOS[3], R. CAMACHO SOLIS[3]
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Abstract: Mexican population is getting older. In 2010 people with more than 65 years old represents 6.2 % of the total population and in 2020 is expected that it will grow to 8.5 %, in 2030 it could reach 12.5 %. Indeed, life expectancy among Mexican people has increased to 75.4 years old (73.1 years in men and 77.8 years in women). This data show the challenge we will confront and the urgent need of programs to attend this new elderly population. Among these needs are included economic security and health care. Many individuals will face the risk of having at least one chronic disease. Moreover, emotional health is also very important, such as symptoms of depression, may significantly reduce the likelihood that elderly patients will receive preventive health services. Also, depressive symptoms are correlated with sleep quality changes and previous research has shown that sleep quality deteriorates with aging. Therefore, the aim of this study was to determine the prevalence of subjective sleep quality in an elderly Mexican population using the Pittsburgh Sleep Quality Index and a depressive symptoms test. The sample was taken among the men and woman with more than 65 year old living in Mexico City.We analyzed sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleeping medication and daytime dysfunction in this population. In addition, a Pearson correlation was made
with the depressive symptoms. The results show that we need to be prepared todeal with increasing disturbances in sleep quality and emotional disorders among elderly people, not only

in Mexico, but around the world. This view requires intervention at community and family levels, because none of this issues are isolated but in interaction with others.

Disclosures: M.M. Melendez: None. **A. Gallegos:** None. **G. Valdez gonzales:** None. **M. Lopez brambila:** None. **E. Aguilar bustos:** None. **R. Camacho solis:** None.

Poster

902. Sleep: Systems Architecture

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:902.02/WW5

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant NS065451

Title: Markov modeling of sleep-wake dynamics for applications in neurotherapy

Authors: F. YAGHOUBY[1], T. ZHANG[2], M. STRIZ[2], K. D. DONOHUE[3], B. F. O'HARA[2], *S. SUNDERAM[1]
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Abstract: Understanding the consequences of genetics, degenerative neural disorders, or acute neural injury on behavior involves extensive screening via sleep-wake and cognitive analysis of animal models. However, convenient metrics that track sleep-wake dynamics over time_beyond simplistic measures such as the percent time spent in each state (Wake, REM, non-REM) or mean bout duration_are lacking. Here, the utility of hidden Markov models (HMMs) estimated from invasive (EEG, EMG) and noninvasive (motion) physiological measurements in mice for tracking progressive changes in sleep-wake dynamics is investigated.
With IACUC approval, mice were surgically implanted with EEG/EMG preamplifiers and placed in cages equipped with piezoelectric motion sensors on the floor. The “piezo” signal discriminates sleep from wakefulness based on muscle activity, and senses thoracic movements associated with breathing, which is less regular in REM than in non-REM sleep. Piezo features that convey energy and breathing regularity can potentially be used to discriminate these stages of sleep. An HMM was used to sequence EEG/EMG data in 4s windows into REM, non-REM, and Wake states; at its heart is a 3x3 matrix representing the probabilities of transitions between the three states independent of state history. HMMs were estimated separately from EEG/EMG and piezo features every four hours. The accuracy of piezo HMM estimates was validated against HMMs estimated from EEG/EMG--the gold standard for sleep scoring--using a confusion matrix analysis. Trends in HMM properties were inspected to track progressive

changes in behavior such as circadian variation and recovery from surgery.
HMMs were able to track sleep-wake state with reasonable accuracy from continuous EEG/EMG measurements. HMMs estimated from piezo features alone were similar to HMMs based on EEG/EMG, except that REM sleep may be overestimated. The trace Tn of the HMM's transition matrix was used to estimate the probability that any state will persist uninterrupted. After surgery, Tn followed a decreasing trend without distinctive features for a few days; but a week later, Tn showed pronounced circadian rhythmicity indicative of normal sleep and behavior. This study suggests that HMMs estimated from physiological measurements could provide quantitative behavioral correlates of neural disorders as well as recovery from brain injury. We intend to further model the effects of interventions such as sensory or electrical stimulation on mouse sleep regulation and dynamics, with the end goal being a novel framework for dynamic programming of neurotherapy.

Disclosures: F. Yaghouby: None. **T. Zhang:** None. **M. Striz:** None. **K.D. Donohue:** Ownership Interest; Signal Solutions, L.L.C. **B.F. O'Hara:** Ownership Interest; Signal Solutions, L.L.C.. **S. Sunderam:** None.

Poster

902. Sleep: Systems Architecture

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:902.03/WW6

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant R21AA017718

NIH Grant R01DA020677

Title: Improved phenotyping and QTL analysis of sleep and wake traits in mice using a non-invasive, high-throughput piezo system

Authors: T. ZHANG[1], M. STRIZ[1], D. GOLDOWITZ[4], K. D. DONOHUE[2], E. J. CHESLER[5], K. M. HAMRE[6], *B. F. O'HARA[3,1]
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Abstract: Sleep and wake are influenced by genetic and environmental factors, but little is known about the specific genes involved in variation of sleep:wake parameters. Traditional sleep

phenotyping has been limited by the invasive and labor-intensive nature of EEG/EMG methods, which creates a bottleneck for high-throughput genetic studies, such as quantitative trait locus (QTL) analysis. This study utilized a noninvasive piezoelectric sleep-wake activity monitor for high-throughput screening. The system has demonstrated a 90-95% accuracy compared to EEG/EMG and human observation, and has already proven successful in screening over two thousand mice for sleep and wake traits, including BXD recombinant inbred mice and a large population of pre-collaborative cross (CC) mice during multiple generations of inbreeding. The present study employs the BXD piezo data to further evaluate sleep:wake parameters to find QTLs associated with the temporal dynamics of sleep, environmental influences, and other variables.

Over 450 female & male mice from 54 lines of expanded BXDs were individually housed in a 12h:12h light:dark cycle. Mice were recorded in the piezo system for 4-5 days to monitor their sleep-wake behavior. For data analysis, hourly sleep:wake parameters, including percent sleep and median bout length, were extracted from the raw data. The first day recordings were classified as an acclimation day and analyzed separately. For the following 3 days, 24 hours were divided into 3 hour segments and 6 hour segments according to the Zeitgeber Time. Percent sleep one hour ahead and after lights on/off were treated as specific traits. Interval mapping was performed using WebQTL to identify general QTLs that influence the sleep:wake parameters within a specific time period. This analysis confirmed several previously reported QTLs and identified several novel loci associated with sleep and wake. In addition, it suggested that QTLs associated with total sleep time overlap QTLs associated with bout length, which is expected and strengthens the results, but was not clear in the previous studies. Additionally, using the recordings of the first day, which were omitted in previous analyses, several QTLs were found that contribute to strain differences in sleep in novel environments.

To enhance and expand this phenotyping system, work has been done to develop a 3-state classifier (REM, nonREM, wake) (See adjacent poster “Markov modeling of sleep-wake···”) in addition to other classifiers that distinguish quiet wake, grooming and locomotor activities. Using these new algorithms, more traits and QTLs, and the dynamic interaction of these traits and loci, can be examined.

Disclosures: T. Zhang: None. **M. Striz:** Employment; Signal Solutions LLC. **D. Goldowitz:** None. **K.D. Donohue:** Ownership Interest; Signal Solutions LLC. **E.J. Chesler:** None. **K.M. Hamre:** None. **B.F. O'Hara:** Ownership Interest; Signal Solutions LLC.

Poster

902. Sleep: Systems Architecture

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:902.04/WW7

Topic: E.08. Biological Rhythms and Sleep

Support: CHIR

NIH

NSERC

FRSQ

Title: Sleep-wake cycle in laboratory and domestic cats

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Abstract: Previous studies conducted on cats in isolated small size chambers demonstrated a very fragmented pattern of sleep. It was unclear to what extent these experimental conditions influenced the exact pattern of sleep-wake alterations in these species.
We examined activity patterns of 4 cats kept in the animal facility and 4 cats in the domestic environment. Laboratory cats were housed in colonies under a standardized environment in which employees had defined access to the room. Domestic cats lived together with their owners. Activities of all animals were recorded with an accelerometer. In two laboratory cats, continuous accelerometer, video and electrophysiological (wireless) recordings were carried out for 1 month.
In laboratory cats an increased activity was observed: (1) about 2 hours before lights-on, (2) at the time of entries of personnel in the experimental room and (3) at the onset of dark period. The increased activity lasted 2-3 hours. Occasional periods of activity were found at other times. The overall measured inactivity level was 60%. Electrographic data indicate that laboratory cats slept 50-55% of time. Therefore, activity measures predicted sleep with 87% accuracy. Domestic cats were usually active between 4 and 9 am and 5 and 8 pm although occasional periods of activity could occur at other times. Similar to laboratory cats, the inactivity periods in domestic cats occupied 65% of the 24-hour period of time.
We conclude that domestic and laboratory cats have very similar profile of activity-inactivity. Within the 24-hour period, cats have 2 periods of increased activity: before lights-on and after lights-off; each period lasting 2-4 hours. Laboratory cats sleep 50-55% of the time suggesting that domestic cats sleep 55-60% of the time. During the long sleep period, the cats often have brief periods of waking. Parallel recordings of accelerometer and electrographic activities show that the accelerometer alone is a useful tool for analyzing the sleep-wake cycle of cats.

Disclosures: L.B. Grand: None. **S. Chauvette:** None. **I. Timofeev:** None.

Poster

902. Sleep: Systems Architecture

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:902.05/WW8

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant NS14610

The Medical Research Service of the Dept of Veterans Affairs

Title: Lesions of the putamen elicit motor disorders in sleep

Authors: *Y.-Y. LAI, J. M. SIEGEL
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Abstract: Muscle activity across the sleep-wake cycle is state-dependent; normally high in wake, low and regular in slow wave sleep (SWS), and atonic in rapid eye movement (REM) sleep. Over the past decades, studies of brain control of motor activity have been focused on wake and REM sleep. However, modulation of motor activity during SWS remains unclear, despite the fact that abnormal motor activity in SWS has been reported in the majority of sleep-related motor disorders. More than 10% of the population has periodic leg movements (PLM) in sleep. Our previous study showed that cats with lesions of the substantia nigra pars reticulata (SNR) generate PLM in sleep. The SNR receives GABAergic projections from the putamen via direct and/or indirect pathways. Abnormalities of the putamen have been reported in human restless legs syndrome patients, who have PLM in wake and in SWS. In this study, we show that neurotoxic lesions of the putamen also increase motor activity in SWS in the rat. Eight young adult Sprague-Dawley rats were used, 5 for controls and 3 for the putamen lesion group. Under isoflurane anesthesia, electrodes were implanted in the skull to record EEG. Flexible wires were implanted in the hindlimb and neck musculatures to record muscle activity. Experimental rats were given 50 nl NMDA (0.5 M) injections into the putamen, bilaterally (2 rats) or unilaterally (1 rat). Sleep recordings were performed after the animal recovered from the surgery. No signs of tremor, rigidity, or bradykinesia in wake were observed in the lesioned animals. However, motor hyperactivity in SWS was observed in all the lesioned rats. The average PLM index (total number of PLM/total time of SWS/24 hour) of lesioned rats was 37.3 ± 10.3, while the PLM index of the normal rats was 1.8 ± 1.7. The sleep pattern was not changed in the lesioned rats (minute/24 hour; wake, control rats: 619.4 ± 59.7, lesioned rats: 603 ± 99.1; SWS, control rats: 714.1 ± 43.5, lesioned rats: 730.8 ± 102.2; REM sleep; control rats: 106.5 ± 5.7, lesioned rats: 106.1 ± 6.4). No differences were seen in the sleep pattern or PLM activity between rats which received bilateral putamen lesions and the rat that received a unilateral lesion. We hypothesize that, like the SNR, the putamen also plays an important role in the control of motor activity in sleep.

Disclosures: Y. Lai: None. **J.M. Siegel:** None.

Poster

902. Sleep: Systems Architecture

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:902.06/WW9

Topic: E.08. Biological Rhythms and Sleep

Support: KAKENHI 23591273 and 23591275

Title: Sleep stage changes connectivity in human cortices: A cortico-cortical evoked potential study

Authors: *K. USAMI[1], R. MATSUMOTO[1], T. HITOMI[2], K. KOBAYASHI[1], A. SHIMOTAKE[1], T. KUNIEDA[3], N. MIKUNI[5], S. MIYAMOTO[3], H. FUKUYAMA[4], R. TAKAHASHI[1], A. IKEDA[1]
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Abstract: **Introduction:** How the connectivity of brain network changes with sleep stage remains to be elucidated for better understanding of normal information processing or propagation of epileptic discharges during sleep. By directly stimulating a part of the cortices during sleep and by recording cortico-cortical evoked potentials (CCEPs) that emanate from the adjacent and remote cortices, we could trace cortico-cortical connections *in vivo*. The parameters of CCEPs [generally consisting of the 1st sharp (N1) and the 2nd slow (N2) negative components] should reflect the degree of connectivity or interaction between stimulus and recording sites. Making use of its excellent spatio-temporal resolution, we attempted to investigate how sleep modulates cortico-cortical connections in human brain. We hypothesized that 1) CCEPs change during sleep and 2) the degree of
modulation of CCEPs differs between the primary sensorimotor cortex (Pr) and the non-primary association cortex (NPr).
Methods: Subjects were 8 patients with intractable partial epilepsy who underwent invasive presurgical evaluation with chronic implantation of subdural electrodes (IRB No. 443). Repetitive single-pulse electrical stimulation (1 Hz) was applied to cortical surface away from the epileptic focus, and CCEPs were recorded by averaging electrocorticogram time locked to the stimuli, sequentially at different sleep stages (W: awake, L: stage I-II, S: stage III-IV, and R: REM) that were judged from the simultaneous scalp EEG/EOG recording. The modulation of connectivity was assessed with the size and latency of N1/N2 component of the

adjacent or remote CCEPs that were spatio-temporally isolated from their neighborhood.
Results: A total of 11 pairs of electrodes were stimulated (1-2 stimulus sites per patient), and adjacent or remote CCEPs were identified at 28 electrodes among patients. Sleep stage significantly changed CCEP parameters (N1 and N2 size, N1 latency). Significant change was observed between W vs. L and/or S. We then assessed the degree of
CCEP modulation in reference to the stimulus-recording sites pair [Pr-Pr (n=5 pairs) vs. Npr-Npr (n=20)]. Analysis of N1 size change demonstrated an inverse relationship between Pr and NPr cortices; namely, as compared with stage W, N1 at stage L became larger in NPr but smaller in Pr. The behavior of CCEPs at stage R was similar to that at stage W regardless of cortical architecture.
Conclusion: Cortico-cortical connectivity changes once patients fall asleep. The degree of connectivity change most likely depends on the sleep stage [awake&REM vs. non-REM (L&S)] and cortical architecture (primary vs. non-primary cortex).

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Poster

902. Sleep: Systems Architecture

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:902.07/WW10

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant 41771-0002

Title: Transcranial electrical stimulation accelerates sleep homeostasis in humans

Authors: D. REATO[1], F. GASCA[2,3], A. DATTA[1], M. BIKSON[1], L. MARSHALL[3,4], *L. C. PARRA[1]
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Abstract: Sleep pressure is reflected in the power of slow-wave activity: it is high after extended wakefulness and gradually decays in the course of the night. Transcranial stimulation with slow-

oscillating currents can entrain electro-encephalographic slow-wave oscillations (SWO) and transiently increase their power. Here we show in humans that 25 minutes of stimulation attenuates the decay of SWO in the remainder of the night. A computational model of up/down-state transitions underlying these SWO predicted the entrainment to a specific phase of the oscillating stimulation. The model also shows a preponderance for increased firing rates during stimulation. This increased firing rate could accelerate homeostatic synaptic downscaling resulting in reduced SWO in the hours of nocturnal sleep after stimulation. To test these predictions we analyzed human EEG data recorded after transcranial electrical stimulation in sleep and found attenuated decay of power and spatial coherence of SWO. Combining the results from a Finite-Element Model (FEM) of electrical stimulation, that simulates anatomically accurate currents distributions that account for folding of human cortex and the computational model of up/down-state transitions, we were able to predict the magnitude and spatial distribution of the effects of electrical stimulation in the human EEG experiments. These combined experimental and modeling results show how electric stimulation can accelerate synaptic homeostasis, and thereby influence a putative processes of sleep regulation. The ability to accelerate the homeostatic function of sleep may have important practical implications.

Disclosures: D. Reato: None. **F. Gasca:** None. **A. Datta:** None. **M. Bikson:** None. **L. Marshall:** None. **L.C. Parra:** None.

Poster

902. Sleep: Systems Architecture

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:902.08/WW11

Topic: E.08. Biological Rhythms and Sleep

Support: Philips Healthcare

Title: Enhancing slow waves using acoustic stimuli

Authors: *B. A. RIEDNER, M. BELLESI, B. K. HULSE, G. SANTOSTASI, F. FERRARELLI, C. CIRELLI, G. TONONI
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Abstract: Increasing evidence suggests that electroencephalographic (EEG) sleep slow waves may perform a key role in sleep’s restorative function, perhaps mediating critical cellular benefits of sleep on the brain. If so, the ability to potentiate slow waves during sleep might mitigate the detrimental effects of sleep loss and could be particularly impactful for a chronically sleep-deprived society. Previous work from our laboratory has identified acoustic stimulation as

a promising non-pharmacological means for increasing slow waves (Riedner et al., 2010). Here, we present data further demonstrating that tones can enhance slow waves when administered during sleep. Six subjects (5 males, ages 21 - 28) were recorded with a six-channel EEG montage that included chin electromyography and two electro-oculographic channels. Blocks of 15 brief tones (2000 Hz, 50 ms duration) were played using headphones or speakers (ON blocks, 1 sec inter-tone interval) during deeper stages of NREM sleep preceded and followed by 15 seconds without stimulation (OFF blocks). ON blocks were then contrasted with the adjacent OFF blocks in order to make valid comparisons given the moment to moment changes in sleep depth. Stimulation blocks were delivered automatically using a software algorithm designed to detect sleep based on the ongoing EEG and importantly to adjust tone intensity based on sleep depth. Subjects received an average of 148 stimulation blocks (range 103 - 196) during N2 and N3 sleep. All subjects showed an increase in slow wave band-limited power (0.5 - 4 Hz) during ON compared to OFF blocks, ranging from 6 - 22%. Power in other frequency ranges were not statistically different from each other, suggesting that tones were not causing arousals or affecting other sleep EEG rhythms (i.e. spindles). The increase in slow waves resulting from the stimulation was apparent regardless of sleep cycle or whether the stimulation block occurred in N2 or N3 sleep. These results confirm the viability of using automatically administered acoustic stimulation to non-pharmacologically increase slow waves. Future work will examine the reliability of this effect and investigate alternative stimulation protocols, as well as determine whether stimulation enhanced sleep produces restorative benefits.

Disclosures: **B.A. Riedner:** Research Grant; Philips Healthcare. **M. Bellesi:** Research Grant; Philips Healthcare. **B.K. Hulse:** Research Grant; Philips Healthcare. **G. Santostasi:** Research Grant; Philips Healthcare. **F. Ferrarelli:** None. **C. Cirelli:** None. **G. Tononi:** Research Grant; Philips Healthcare. Other; Philips Healthcare.

Poster

902. Sleep: Systems Architecture

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:902.09/WW12

Topic: E.08. Biological Rhythms and Sleep

Support: National Research Foundation of Korea

Title: Functional mouse brain mapping and its application to sleep study

Authors: *J. CHOI[1], E. HWANG[2], B. KIM[2], S. LEE[2], S. LEE[2], S. HAN[2], Y. KIM[3], R. STRECKER[4]

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Abstract: In 2010, a new methodology called high resolution electroencephalography (HR-EEG) for mouse brain was introduced in Journal of Neurophysiology (Choi JH, Koch KP, Poppendieck W, Lee M, Shin HS (2010) High resolution electroencephalography in freely moving mice. J Neurophysiol 104: 1825-1834; Lee M, Kim D, Shin H-S, Sung H-G, Choi JH (2011) High-density EEG Recordings of the Freely Moving Mice using Polyimide-based Microelectrode. J Vis Exp: e2562). By combining this new technique with conventional local field potential recording in thalamus, we revisited the sleep wave including sleep spindle, K-complex, and slow-wave sleep oscillation in the viewpoint of the generation, propagation, and termination of these waves. We analyze the spatio-temporal patterns of the sleep waves and performed causality study between sleep waves in cortex and thalamus. We also analyzed the structures of sleep wave under the condition of acute sleep deprivation as well as chronic sleep deprivation.

Disclosures: J. Choi: None. **E. Hwang:** None. **B. Kim:** None. **S. Lee:** None. **S. Lee:** None. **S. Han:** None. **Y. Kim:** None. **R. Strecker:** None.

Poster

902. Sleep: Systems Architecture

Location: Hall F-J

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Program#/Poster#:902.10/WW13

Topic: E.08. Biological Rhythms and Sleep

Support: HHMI

NIH Grant P01 AG017628

Title: Monitoring neuronal activity with a new calcium indicator in D. melanogaster

Authors: L. BAI[1], S. DOSHI[2], W. JOINER[3], *A. SEHGAL[1]
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Abstract: The fruit fly Drosophila melanogaster has been an extremely useful model for understanding genetic and molecular mechanisms underlying circadian control of rest:activity and other rhythmic behaviors. A related effort is directed towards understanding the molecular

mechanisms of sleep-wake regulation. Most studies classify sleep-like states in the fly based solely upon behavioral locomotion patterns as, thus far, it is not possible to record electroencephalograms (EEGs) in the fly. Monitoring brain activity in vivo should help greatly to define sleep/wake states at the brain/neuronal level in the fly, just as EEGs do for mammals. Genetically encoded calcium indicators (GECIs) are becoming crucial tools for monitoring neuronal activity in vivo. However, mainstream GECIs are engineered with fluorescent proteins and thus require laser excitation, which could potentially have direct effects on sleep-wake and/or affect circadian control of behavior. To overcome this caveat, we designed a new GECI that reports calcium transients with bioluminescence changes. We are able to monitor bioluminescence from whole dissected brains of flies that express this GECI either pan-neuronally or in various brain regions. In ongoing experiments we are restricting expression of the GECI to either wake- or sleep-promoting neurons and attempting to correlate neural activity with behavioral activity. This new GECI may provide a valuable tool for characterizing neuronal circuits underlying different behavioral paradigms.

Disclosures: L. Bai: None. **S. Doshi:** None. **W. Joiner:** None. **A. Sehgal:** None.

Poster

902. Sleep: Systems Architecture

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:902.11/WW14

Topic: E.08. Biological Rhythms and Sleep

Support: Swiss National Foundation Grant No. 139778

Wisconsin Distinguished Rath Graduate Fellowship

NIH Grant MH064498

NIH Mental Health Grant 5P20MH077967

Title: Slow waves and sleep consciousness

Authors: *F. SICLARI, J. J. LAROCQUE, B. R. POSTLE, G. TONONI
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Abstract: *Aims and background*: Dreaming is a particular form of consciousness that occurs during sleep. Although mental activity is most frequently and vividly recalled when awakening from REM sleep, it is also reported in up to 80% of Non-Rem (NREM) sleep awakenings. Slow waves, the hallmark of NREM sleep, reflect a slow oscillation of cortical neurons between up

and down states. It has been suggested that the brain's capacity to integrate information and generate conscious experiences is reduced whenever the brain is in such a bistable state. We aimed at determining whether slow waves are a negative predictor of sleep consciousness and whether machine-learning techniques can reliably distinguish between periods with and without mental activity.
Methods: Two healthy participants each underwent six overnight high density-EEG recordings with 256 electrodes. They were awakened by a computerized sound at 15-20 minute intervals and questioned for the presence or absence of sleep consciousness. Recall without content ("I was experiencing something but do not remember what") was considered separately. A time frequency transform of the EEG signal in the two minutes preceding the awakening was performed to obtain power in the 1 to 4.5 Hz frequency band. Multivariate pattern analysis (L2-regularized logistic regression) was performed for the EEG signal within the delta band in the 10 seconds prior to waking.
Results: 253 questionings were performed (3% in wake, 30% in stage NREM1, 47% in NREM2, 26% in NREM3 and 26% in REM sleep). Recall with content was reported in 76 (41%) of the 185 awakenings obtained in stages N2 and N3. Awakenings yielding sleep consciousness reports were associated with significantly lower frontal delta power over all electrodes compared to awakenings without consciousness ($p<0.001$). Pattern classification predicted the presence of sleep consciousness with above chance accuracy.
Conclusion: Our results suggest that the brain's capacity to generate
conscious experience during sleep is reduced in the presence of slow waves. Initial analyses indicate that machine-learning techniques can predict the presence of sleep consciousness.

Disclosures: F. Siclari: None. **J.J. LaRocque:** None. **B.R. Postle:** None. **G. Tononi:** None.

Poster

902. Sleep: Systems Architecture

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Topic: E.08. Biological Rhythms and Sleep

Support: Wisconsin Distinguished Rath Graduate Fellowship (J.J.L.)

Swiss National Foundation Grant number 139778 (F.S.)

NIH MH064498 (B.R.P.)

NIH 5P20MH077967 (G.T.)

Title: Sleep spindles and sleep consciousness

Authors: *J. J. LAROCQUE, F. SICLARI, B. R. POSTLE, G. TONONI
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Abstract: Introduction: Sleep is associated with a reduction in the level of consciousness. However, reports of dreaming, or conscious experience during sleep, can be elicited upon waking from any stage of sleep. Here, we explored the influence that sleep spindles - 12-15 Hz oscillations arising from connections between thalamus and cortex - may exert on conscious experience during sleep. Reasoning that the capacity of the thalamocortical system to integrate information and generate conscious experience is compromised by the highly synchronized activity of sleep spindles, we predicted that sleep spindles would be associated with reduced reports of sleep consciousness.
Methods: We collected high-density electroencephalographic (EEG) data from two subjects for six nights each. Subjects were awakened with a tone every 15-20 minutes, and sleep consciousness was assessed with a questionnaire. To the primary question, “What was going through your mind immediately prior to the tone?” subjects could respond positively (with a report of the content of their sleep experiences) or negatively (denial of any sleep experiences). Ambiguous responses, in which sleep consciousness was reported without any recall of content, were considered separately. A time-frequency transform was applied to the EEG data preceding each waking, and analyses were performed on the resultant spectrogram at each electrode. We also calculated additional measures of spindle activity, including number of spindles, duration of spindles (integrating across all spindles present in the two minutes preceding waking), and time since last spindle.
Results: In stage 2 and 3 non-REM sleep, power in the spindle range (12-15 Hz) was significantly lower during epochs preceding positive reports of sleep consciousness compared with those preceding negative reports. This effect was strongest at bilateral frontal and central parietal electrodes. Additionally, for epochs preceding reports of sleep consciousness, spindle power was negatively correlated with the subjective estimate of the length of the conscious experience. Initial analyses also suggest that in right frontal electrodes, fewer spindles, shorter spindle duration, and longer time since last spindle all were associated with longer duration of conscious experience during sleep.
Conclusion: During non-REM sleep, 12-15 Hz spindles are negatively associated with incidence of sleep consciousness. Importantly, sleep spindles also correlate with quantitative features of sleep consciousness, such as subjective length of the experience. This provides strong support for the influence of spindle activity on sleep consciousness.

Disclosures: J.J. Larocque: None. **F. Siclari:** None. **B.R. Postle:** None. **G. Tononi:** None.

Poster

902. Sleep: Systems Architecture

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Program#/Poster#:902.13/WW16

Topic: E.08. Biological Rhythms and Sleep

Title: Basal ganglia functional connectivity in human NREM sleep

Authors: *C. GOH[1], R. KANAI[1], V. WALSH[1], O. JOSEPHS[2]
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Abstract: An emerging literature implicates the basal ganglia (BG) in the control of sleep-wake (S-W) behavior. Dopaminergic modulation of the BG and BG-dependent sites may exert sleep stage-specific influence on cortical activity levels. We measured whole-brain activity by functional MRI during human non-rapid eye movement (NREM) sleep, and show that functional connectivity (fc) between BG sites (striatum (caudate nucleus, putamen), globus pallidus (GP)) and the cortex differs across NREM sleep stages. Compared to wake, in both light and deep sleep the GP shows greater fc with the insular cortex, but less fc with primary motor and anterior temporal areas, while the striatum shows more coupling with primary motor and lateral occipital areas, and the middle temporal gyrus. In contrast to the GP, the striatum has greater fc to the insular cortex during light sleep, relative to wake. Additionally, the GP is more coupled to frontal regions during deep sleep. A possible explanation for increased coupling to the motor cortex during sleep is possibility is that increased functional connectivity reflects a progressive loss of inhibitory cortical influence by the basal ganglia. Furthermore, the greater fc between GP and frontal regions found during deep sleep is discussed in the context of the GP's putative role in the initiation of slow oscillations.

Disclosures: C. Goh: None. **R. Kanai:** None. **V. Walsh:** None. **O. Josephs:** None.

Poster

902. Sleep: Systems Architecture

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Topic: E.08. Biological Rhythms and Sleep

Support: CIHR

JS McDonnell Foundation

Title: Anatomical connectivity supports functional community structure in macaque cortex

Authors: *K. SHEN[1], G. BEZGIN[1], R. M. HUTCHISON[2,3], J. S. GATI[3], R. S. MENON[2,3], S. EVERLING[2,3], A. R. MCINTOSH[1,4]
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Abstract: Computational and empirical neuroimaging studies have suggested that the anatomical connections between brain regions largely constrain their functional interactions. Given that the large-scale organization of functional networks is determined by the temporal relationships between brain regions, the structural limitations may extend to the global characteristics of functional networks. Here, we explored the extent to which the functional network community structure is determined by the underlying anatomical architecture. We directly compared macaque functional connectivity (FC) assessed using spontaneous blood-oxygen-level-dependent functional magnetic resonance imaging (BOLD-fMRI) to anatomical connectivity derived from macaque axonal tract tracing studies. Consistent with previous reports in both humans and monkeys, we observed strong FC both within and across hemispheres. FC increased with increasing strength of anatomical connection and FC was also present between regions that had no direct anatomical connection. We observed high similarity between each region's FC and its anatomical connectivity. Notably, partitioning of the functional network was supported by dense intra-modular bidirectional anatomical connections that were not present when considering the non-partitioned network. Taken together, our data directly demonstrate that the observed FC in resting-state fMRI is dictated by the underlying neuroanatomical architecture. Importantly, we show how this architecture contributes to the global organizational principle of functional specialization.

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Poster

902. Sleep: Systems Architecture

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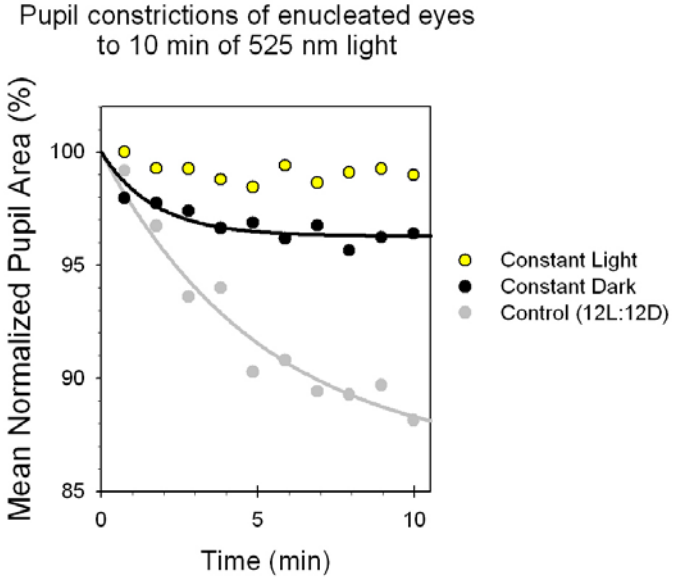
Program#/Poster#:902.15/WW18

Topic: E.08. Biological Rhythms and Sleep

Title: Local clock in the iris of the turtle?

Authors: G. S. TROUTMAN, K. NIOTIS, *J. R. DEARWORTH, Jr.
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Abstract: Studies done in our laboratory suggest that the degree of constriction by the pupil in response to light in turtles can be associated with their circadian rhythm. After turtles are subjected to the condition of constant darkness, their pupillary light reflex is diminished. However, the response of the pupil without feedback from the brain (the photointrinsic iris response) and how it can be affected by different photoconditions is unknown. This study explored whether there is a connection between the visual processing done for circadian rhythms and the photointrinsic iris response. This was done by subjecting turtles over long periods (≥10 days) to three different photoconditions (12 hours of light with 12 hours of dark = 12L:12D, constant light, and constant dark) and comparing their photointrinsic response from their enucleated irises. Turtles under control conditions (12L:12D) had a photointrinsic iris response that reduced the pupil size by ~14%. In contrast, the response in turtles in constant dark was less at ~4%, and the response was entirely eliminated in turtles exposed to constant light. These results suggest that the photoreceptive mechanism responsible for driving the photointrinsic iris response is influenced by prior photoconditions to which the turtles are subjected possibly regulated by a circadian clock.



Disclosures: G.S. Troutman: None. **K. Niotis:** None. **J.R. Dearworth:** None.

Poster

902. Sleep: Systems Architecture

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH Director's Pioneer Award to G.T.

Human Frontier Science Program Organization (HFSPO) long-term fellowship to Y.N.

Title: Auditory responses and stimulus-specific adaptation are largely preserved across NREM and REM sleep in rat primary auditory cortex

Authors: *Y. NIR[1], V. V. VYAZOVSKIY[1,3], C. CIRELLI[1], M. I. BANKS[2], G. TONONI[1]
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Abstract: Sleep entails a disconnection from the external environment, but to what extent signal propagation along ascending sensory pathways is preserved across vigilance states remains unclear. It has been suggested that the thalamus may not relay effectively sensory inputs to the cortex in sleep, but recent evidence challenges this view.
In this study, neuronal and LFP responses to sounds in rat primary auditory cortex were compared between wakefulness and sleep. Adult WKY rats were implanted with microwire arrays (~50 kΩ) in right A1 (n=6). After a week of recovery, sleep stabilization, and habituation to stimulation, acoustic stimuli were presented (free-field) in a sound-proof, acoustically-calibrated environment as rats spontaneously switched between vigilance states. In the first experiment, 24 sounds were presented pseudo-randomly with inter-stimulus intervals (ISIs) of 1250±250ms at 3 intensities (30,55,80 dB SPL). Stimuli included short (100ms) and long (600ms) tones, clicks and click-trains (duration = 500ms), more complex stimuli including environmental sounds and rat vocalizations (duration = 250-1000ms), as well as FM sweeps (0.5-2kHz, duration = 100ms) and 'chirp AM' tones [10kHz, f_{mod} = 20-200Hz, duration = 600 ms). In a second 'oddball' experiment, low (8kHz) and high frequency (45kHz) tones were presented frequently (90%) or rarely (10%) in a mixed and counter-balanced manner (ISIs = 750±100ms, 55 dB SPL). Local field potentials (LFPs), and single unit activity (SUA) were recorded continuously along with epidural EEG, EMG and video, and spike sorting was performed offline. Vigilance states were scored in 4s epochs on the basis of EEG, EMG, and behavior.
We recorded from a total of 520 units in A1 and quantitatively compared SUA across vigilance states. Trials categorized as wake, NREM or REM sleep showed robust differences in baseline LFP power spectra and neuronal discharges (e.g. firing rate in NREM was 80.5% of that in wakefulness, $p < 10^{-43}$). On average, both the selectivity and the magnitude of evoked SUA were comparable across wakefulness, NREM and REM sleep (differences < 8.1% between all states), and this was confirmed separately for onset, offset, and sustained responses. In the oddball paradigm, robust stimulus-specific adaptation (SSA) was observed following the onset of repetitive tones. SSA was similar in magnitude across vigilance states, suggesting that basic

deviance detection persists in sleep. These results support the notion that responses to external stimuli are preserved in primary sensory cortices across sleep. A functional disconnection may prevent activity to effectively drive high-order cortical regions.

Disclosures: Y. Nir: None. **V.V. Vyazovskiy:** None. **C. Cirelli:** None. **M.I. Banks:** None. **G. Tononi:** None.

Poster

902. Sleep: Systems Architecture

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Topic: E.08. Biological Rhythms and Sleep

Support: SRPBS “Brain Machine Interface Development”, MEXT

Title: Embodied global network of consciousness

Authors: *T. YANAGAWA, N. HASEGAWA, N. FUJII
BSI, RIKEN, Wako Saitama, Japan

Abstract: How does the brain generate consciousness? A hypothesis suggests that there might be sustained neural interaction in ongoing activity, which supports stable continuity of consciousness and a drop of the network interaction will cause elimination of consciousness (Alkire et al. 2008). Based on the hypothesis, previous studies in functional imaging and electrophysiology indicated importance of the global network interaction in the brain to maintain the stable conscious state. Specifically the interactions between frontal and parietal regions and between thalamus and those regions were reported to be reduced during loss of consciousness (Boveroux et al. 2010, Ku et al. 2011). However, neural interactions in entire cortical network in awake/anesthetic-unconscious state have not been well investigated with high spatio-temporo-spectral resolution by using electrophysiological technique.
Here we estimated neural interactions by calculating spectral granger causality from electrocorticographic data recorded from entire lateral cortex in four macaques during awake and anesthetic conditions. Ketamine and Medetomidine were used for anesthetic agent. We revealed sustained neural interactions in alpha and beta band that was stable over days, but was lost in anesthetic condition. The neural interactions were found between/within a block of premotor-sensorimotor-parietal (PSMP) cortices, prefrontal and temporal cortex.
Previous studies showed that beta-band oscillation in PSMP reflects sensorimotor integration (Baker 2007). So we hypothesized that the sustained neural interaction in PSMP in beta-band during resting state may produce unified and continuous bodily sensation that is often called

body schema. To test the hypothesis we applied local anesthesia in forearm which disrupted the monkey's body schema on the forearm and found modulation of the neural interaction in the PSMP.
The results suggest that the PSMP integrates multimodal somatosensory-motor information and generates body schema that anchors cognitive processes to physical body resulting the stable embodied conscious state.

Disclosures: T. Yanagawa: None. **N. Hasegawa:** None. **N. Fujii:** None.

Poster

902. Sleep: Systems Architecture

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Topic: E.08. Biological Rhythms and Sleep

Support: The Japan Society for the Promotion of Science

Japan Science and Technology Agency

Title: Measuring the level of consciousness based on the amount of integrated information computed from electrocorticogram (ECoG) recordings in monkeys before and after anesthesia

Authors: *M. OIZUMI[1,2,3], T. YANAGAWA[2], S.-I. AMARI[2], N. TSUCHIYA[2,4,5], N. FUJII[2]
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Abstract: Assessing the subjective conscious state of an organism based on the activity of the brain is an important issue in the clinical settings, yet it is one of the most challenging problems in neuroscience. The brain-based measure of the level of consciousness is most wanted in assessing the state of those who cannot speak (e.g., under anesthesia, in the vegetative state, etc) as has been suggested by recent studies that identified disadvantages in the conventional behavioral measures in assessing consciousness. Here we provide the results of our first step towards the goal of developing an empirical "consciousness meter" based on the real neuronal data.
One promising theory that allows us to quantify the level of consciousness is the information integration theory of consciousness proposed by Giulio Tononi. According to the theory, the level of consciousness corresponds to how much information is differentiated and

simultaneously integrated in the brain. In the theory, various forms of loss of consciousness are commonly explained as the drastic loss of the ability for the brain to integrate information. The measure proposed by the original theory, however, is intractable to compute in large systems. To cope with such intractability, some alternative measures that approximated the original one have been proposed. However, we found that these measures have some caveats, one of which manifests when neural activities are strongly correlated.
Here, we propose a yet another measure based on the concept of 'decoding', while keeping its applicability to real neural data. With this new measure, we quantified the amount of integrated information before, during and after anesthesia using the electrocorticogram (ECoG) recordings in 4 monkeys over 12 sessions. The ECoG data were simultaneously recorded from the surface of various cortical areas with 128 electrodes while the monkey subjects underwent a transition from an awake state to anaesthetized state. To separately evaluate the contribution of different frequency bands (delta, theta, alpha, beta, and gamma), we first applied the linear band-pass filter to the data and then computed the integrated information in each frequency band. We found that the integrated information computed in beta-band (12-30 Hz), but not in the other bands, decreased in a large amount after the injection of anesthetic drugs consistently across subjects and recording sessions. Our results suggest that our new measure of the integrated information based on the beta-band activity across the entire brain may be a promising candidate for the future development of the "consciousness meter".

Disclosures: M. Oizumi: None. **T. Yanagawa:** None. **S. Amari:** None. **N. Tsuchiya:** None. **N. Fujii:** None.

Poster

903. Energy Metabolism

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Program#/Poster#:903.01/XX2

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01 DK056132

Title: Altered glucokinase expression and function in identified GABA neurons in the nucleus tractus solitarius in mice with type 1 diabetes

Authors: S. MAIMAITI, K. C. HALMOS, P. GYARMATI, *B. N. SMITH
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Abstract: Type 1 diabetes is characterized uncontrolled hyperglycemia due to loss of insulin secretion from pancreatic beta cells. Effects on brainstem circuits of prolonged hyperglycemia

and hypoinsulemia have been documented, and GABA neurons in the nucleus tractus solitarius (NTS) express glucokinase and respond to changes in glucose concentration. Synaptic control of central vagal circuits is tightly regulated by glucose and displays plasticity in diabetic animals. Identified GABA neurons from mice that express GFP in a subset of inhibitory neurons (GIN mice; FVB-Tg(GadGFP)4570Swn/J) were targeted for whole-cell patch-clamp recordings. We tested the hypothesis that modulation of glucokinase activity would alter synaptic transmission in identified NTS GABA neurons and that this effect would be reduced in diabetic mice after 3-6 consecutive days of hyperglycemia. GIN mice (3-8 wk old) were fasted for 6 hrs before being injected with streptozotocin (STZ; 200mg/kg; i.p.) to induce hyperglycemia or vehicle (control). Blood glucose levels were monitored until the day of experimentation. In recordings from GABA neurons in control mice, application of the glucokinase-inhibitor glucosamine (GA; 5 μM) reversibly suppressed sEPSC frequency by 40 +/- 6% ($p<0.01$; $n=14$) within 5 min of application, with no effect on amplitude. In neurons from STZ-treated, diabetic mice, GA induced no effect on sEPSC frequency or amplitude ($p>0.05$; $n=6$). Pre-incubation of slices for 30 min with insulin (1 μM) failed to restore the effect of GA on sEPSC frequency in diabetic mice ($n=4$), suggesting the loss of effect was not due to deficiency of insulin-dependent protein trafficking. Quantitative PCR indicated a significant decrease in GCK expression in trimmed slices containing the dorsal vagal complex from STZ-treated mice versus controls ($p<0.05$). Results indicate that synaptic input to NTS GABA neurons is sensitive to glucokinase modulation and that this modulation is suppressed following several days of hyperglycemia, which coincided with glucokinase downregulation. Such modulation is consistent with the hypothesis that GABAergic neurons in the NTS are sensitive to glucose modulatory mechanisms that may contribute to autonomic dysregulation in metabolic disorders.

Disclosures: S. Maimaiti: None. **K.C. Halmos:** None. **P. Gyarmati:** None. **B.N. Smith:** None.

Poster

903. Energy Metabolism

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Program#/Poster#:903.02/XX3

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Canadian Institutes of Health Research (CIHR)

Canadian Foundation for Innovation (CFI)

Ontario Research Fund (ORF)

University of Western Ontario

Title: Striatal vacht ko mice are hyperphagic and lean

Authors: *M. S. GUZMAN LENIS[1], X. DE JAEGER[1], M. G. CARON[2], M. A. M. PRADO[1], R. GROS[1], V. F. PRADO[1]
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Abstract: The balance dopamine (DA)/ acetylcholine (ACh) in dorsal striatum has a role in movement disorders such as PD. This balance is also important for motivational disorders. In the striatum (STR), cholinergic neurons seem to counteract the effects of DA. This has been also observed during feeding and satiety. DA in NAcc is thought to increases appetite, whereas an increase in ACh is thought to be related to the onset of satiety. In this regard, it has been reported that rats with cholinotoxic lesions in STR show lower body weight and increased food consumption. Also, ACh levels in the NAcc normally increase during a meal when feeding slows down and may peak when feeding stops. ACh also seems to modulate STR opioid mRNA expression. The STR is among the brain regions with the highest levels of opioid peptides and receptors. In the STR the opiod peptides dynorphin and enkephalin help activating food-directed behaviour regulated by the hypothalamic energy-sensing system. In our laboratory we have developed a novel mouse line where the vesicular ACh transporter (VAChT) has been specifically eliminated in the STR, resulting in the elimination of ACh release. In our effort to characterize this mouse line, we found that striatal VAChT KOs were leaner when compared to their littermate controls suggesting that ACh release in striatum might play a role in feeding behaviour. Using indirect calorimetry we found metabolic differences between young and adult striatal VAChT KO mice. Young animals are hyperactive and have increased respiratory exchange ratio (RER) during the light cycle and lower RER in the dark cycle. On the other hand, adult mice are hyperphagic and have increased RER, both during the light and dark cycle. Both group of animals show no changes in heat production and body temperature. They also have higher oxygen consumption and, interestingly, they sleep less. Young striatal VAChT KO mice show a better tolerance for glucose in the glucose tolerance test while adult mice show no difference when compared against controls. We also analyzed mRNA expression levels of preprodynorphin and preproenkephalin in the STR and VAChT expression levels in the hypothalamus and found no differences. Paradoxically, hypothalamic mRNA levels of MCH, Orexin, POMC and AgRP in adult striatal VAChT KO mice were not changed, while young mice showed a tendency for lower levels of POMC and increase levels of Orexin. Our data indicate that cholinergic tone in the STR participates in modulation of food intake. The study of the metabolic changes in our mouse line might offer new therapeutic perspectives for the treatment of obesity.

Disclosures: M.S. Guzman Lenis: None. **X. De Jaeger:** None. **M.G. Caron:** None. **M.A.M. Prado:** None. **R. Gros:** None. **V.F. Prado:** None.

Poster

903. Energy Metabolism

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Program#/Poster#:903.03/XX4

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIDDK DK45024 to R. G.-J

Title: Acute exposure of the mediobasal hypothalamus to β-amyloid peptide induces hepatic insulin resistance

Authors: *I. ARRIETA-CRUZ[1], R. GUTIÉRREZ-JUÁREZ[2]
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Abstract: Alzheimer's disease (AD) is the most common form of dementia and increasing age is the best known risk factor for the development of this disease. β-amyloid peptide (Aβ) is the main component of amyloid plaques, an established hallmark of AD, which are responsible in part for the progressive deterioration of cognitive and behavioral functions characteristic of the disease. Ample evidence exists suggesting that hyperglycemia and insulin resistance, two hallmarks of type 2 diabetes mellitus (DM2), are factors that contribute to accelerating the appearance of AD. In fact, insulin signaling appears to be involved in the modulation of Aβ production and degradation in transgenic mice models of AD. The mediobasal hypothalamus (MBH) has emerged as a neuronal center directly involved in the control of whole body insulin action, glucose homeostasis and energy balance. Although a number of in vivo neurotoxic actions of Aβ have been described, there is evidence to date of a no direct effect of Aβ on insulin action. Here we examined the hypothesis that Aβ exerts an acute neurotoxic effect in the MBH resulting in hepatic insulin resistance and impairment of glucose metabolism. We performed central (MBH) infusions of Aβ in normal young Sprague-Dawley male rats during basal pancreatic insulin clamps to assess whole insulin action and glucose kinetics by tracer dilution methodology. Our results indicate that during pancreatic clamps the acute infusion of Aβ but not scrambled peptide in the MBH decreased hepatic insulin sensitivity manifested by a failure of insulin to efficiently suppress hepatic glucose production (inhibition of basal glucose production 9.8 ± 2.7% for Aβ group vs. 28 ± 4.6% scrambled peptide; and net glucose production 11.2 ± 0.6 vs 9 ± 0.3 mg/kg/min, respectively). No changes in glucose disposal were observed. Changes in the phosphorylation of AKT, p70S6 kinase and S6 ribosomal protein were observed in both the MBH and livers of the treated animals. Importantly, the insulin-resistance induced by central Aβ occurred without changes in circulating insulin or glucose levels thus revealing a direct action of Aβ in the impairment of insulin action via the MBH. The ability of Aβ to acutely impair hepatic

glucose metabolism through its action in the hypothalamus is a previously unrecognized neurotoxic effect of Aβ.

Disclosures: I. Arrieta-Cruz: None. **R. Gutiérrez-Juárez:** None.

Poster

903. Energy Metabolism

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: FAPESP 2009/50809-5; 2010/51683-2

Title: Knock-down of hypothalamic stearoyl-coa desaturase-2 reduces body mass of diet-induced obese mice

Authors: R. F. MOURA[1], L. F. R. NASCIMENTO[1], L. M. IGNÁCIO-SOUZA[1], G. F. P. SOUZA[1], J. MORARI[1], B. BOMBASSARO[1], *L. A. VELLOSO[2]
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Abstract: Stearoyl-CoA desaturase is encoded by the scd gene, and is responsible for the conversion of saturated to monounsaturated fatty acids. The evaluation of SCD knockout mice unveiled a tissue-specific role for this gene in obesity. However, the specific role of hypothalamic SCD is yet to be addressed. Thus, the present study aims at investigating the role of hypothalamic SCD (SCD2) and its role in obesity. For that, five-week old Swiss male mice were fed on a high-fat diet during six weeks. After the four initial weeks, the animals were selected for its obesity predisposition phenotype as: obesity resistant (RS), or obesity prone (PR). Both groups were treated either with 4nmol SCD2 antisense (ASO) or vehicle (VE), once a day for two weeks. Body mass was evaluated once a week and spontaneous activity, O2 consumption and CO2 production were determined after ten days. The hypothalamus was extracted and the expressions of orexigenic/anorexigenic neurotransmitters were analyzed by real time PCR. After two weeks of ASO treatment, PRASO and RSASO groups had 11% and 5% reductions of body mass as compared to respective controls (p<0.05 for both groups). During the light period, ASO treatment promoted 38% decrease (p<0.05) in the RS groups activity, but no significant change in PR groups. However, during the dark period, ASO treatment promoted 25% and 44% increases of activity for RSASO and PRASO, respectively (p<0.05 for both groups). O2 consumption was not affected by the treatment, but CO2 production was 10% higher in either PRASO and RSASO groups as compared to controls. This change in CO2 production resulted in 11% and 13% increase in respiratory quotient for PRASO and RSASO, respectively, as

compared to controls. The orexigenic AGRP and the anorexigenics POMC and CART genes presented no differences regarding the treatment or phenotype. In conclusion, hypothalamic SCD2 plays a role on obesity, its inhibition promotes reduction of body mass mainly through increased nocturnal activity and increased CO2 production. Thus, SCD2 is a novel candidate target for the treatment of obesity.

Disclosures: R.F. Moura: None. **L.F.R. Nascimento:** None. **L.M. Ignácio-Souza:** None. **G.F.P. Souza:** None. **J. Morari:** None. **B. Bombassaro:** None. **L.A. Velloso:** None.

Poster

903. Energy Metabolism

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:903.05/XX6

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: DK085122

Title: Estrogen is neuroprotective of hindbrain A2 noradrenergic metabolic sensory neurons during insulin-induced hypoglycemia.

Authors: *P. TAMRAKAR, B. A. IBRAHIM, K. P. BRISKI
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Abstract: Energy deprivation due to insulin-induced hypoglycemia (IIH) poses a risk of CNS dysfunction and injury. Nerve cell metabolic status is monitored in select brain sites, including the hindbrain dorsal vagal complex, where the cellular energy sensor, adenosine 5'-monophosphate-activated protein kinase (AMPK), is localized to A2 noradrenergic neurons. Estrogen is reportedly neuroprotective against various bio-energetic insults, including stroke, but its actions on CNS neurons during IIH remain unclear. We utilized a novel combinatory approach, involving laser-catapult microdissection and high-sensitivity Western blotting, to examine premise that estradiol enhances A2 energy metabolism during IIH, thereby regulating A2 metabolo-sensory functionality. Quick-immunolabeled A2 cells were harvested 2 hr after neutral protamine Hagedorn insulin (INS) or vehicle (V) injection of ovariectomized female implanted with estradiol benzoate (E) or oil (O) for Western blot analysis of rate-limiting glycolytic, tricarboxylic acid cycle, and AMPK-malonyl CoA pathway enzymes. E enhanced basal A2 phosphofructokinase (PFKL) and isocitrate dehydrogenase (IDH2), but decreased acetyl Co-A carboxylase (ACC), calcium/ calmodulin kinase (CaMMKβ), and fatty acid synthase (FAS) expression. These enzyme profiles, as well as PP2A, were elevated, whereas phosphoAMPK levels were decreased in E vs. O A2 neurons during IIH. These data support the

view that estradiol improves nerve cell energy status during eu- and hypoglycemia through control of substrate fuel metabolism. Ongoing studies seek to determine if estrogenic regulation of pAMPK is achieved by effects on ATP production and/or direct control of upstream kinase and phosphorylase activity. The current research extends current knowledge of neurochemical, hormonal, and nutrient stimuli that govern CNS AMPK function to include estrogen. The results also establish a platform for investigation of estrogenic control of neuronal fatty acid biosynthesis during energy balance versus deficiency.

Disclosures: P. Tamrakar: None. **B.A. Ibrahim:** None. **K.P. Briski:** None.

Poster

903. Energy Metabolism

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Program#/Poster#:903.06/XX7

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIDA-IRP (NIH)

Title: Rapid, area-specific fluctuations in extracellular brain glucose levels induced by natural arousing stimuli and intravenous cocaine in freely moving rats: Critical role of neural activity.

Authors: *E. A. KIYATKIN, M. LENOIR
Behavioral Neurosci Br., NIDA-IRP, NIH, DHHS, BALTIMORE, MD

Abstract: Glucose, a primary energetic substrate for neural activity, is continuously influenced by two opposite forces, which tend to either decrease its extracellular levels due to enhanced utilization or increase its levels due to entry from peripheral circulation via enhanced cerebral blood flow. In this study, enzyme-based glucose sensors coupled with high-speed amperometry were used in freely moving rats to evaluate rapid fluctuations in extracellular levels of glucose induced by a brief audio stimulus, tail-pinch (TP), social interaction with another rat (SI), and intravenous cocaine (1 mg/kg). Our measurements were conducted in two basal ganglia structures, nucleus accumbens shell (NAcc) and substantia nigra pars reticulata (SNr), which drastically differ in neuronal activity and responsiveness to afferent challenges. In the NAcc, where most cells are silent or sporadically active and show powerful excitations following salient stimulation, all stimuli induced a rapid (latency 2-6 s) increase (30-100 microM or 6-20% over baseline) in glucose levels; the increase differed in its magnitude and duration for each stimulus. In the SNr, where most cells have high rates of basal impulse activity and show transient inhibitions following salient stimulation, TP, SI and cocaine transiently decreased glucose levels (-20-40 microM or 5-10% below baseline); the decrease has longer latencies (~40 s) and

followed by a rebound-like glucose increase. To confirm the role of neuronal activity in mediating the initial glucose response, electrochemical currents were monitored following local microinjections of either glutamate (GLU) or procaine (PRO). While intra-NAcc injection of GLU transiently increased glucose levels in this structure, intra-SNr PRO injection resulted in rapid, transient decreases in SNr glucose levels. Therefore, extracellular glucose levels in the brain could change very rapidly following physiological stimulation, the response is structure-specific, and the pattern of neuronal activity appears to be a critical factor affecting the direction and magnitude of physiological fluctuations in extracellular glucose levels.
Supported by the Intramural Research Program of NIH, NIDA.

Disclosures: E.A. Kiyatkin: None. **M. Lenoir:** None.

Poster

903. Energy Metabolism

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: CONACyT Grant 83113 to MH

Title: Comparative study of the chronic effect of chorogenic, caffeic acid, and caffeine on glucemy, triglyceridemia, cholesterolemia, and, glucose and Insulin resistance in healty and diabetic rats

Authors: *M. HUERTA-VIERA, Z. URZÚA, C. RAMOS-FLORES, X. TRUJILLO
Ctr. Univ. Inv Biomed (CUIB), Univ. De Colima, CUIB, Colima, Mexico

Abstract: Previous studies indicate that the long-term consumption of roasted coffee regularly (with Caffeine, Chlorogenic and Caffeic acid), and decaffeinated coffee (without caffeine) reduces the risk of diabetes mellitus type 2 (DM2) in people taking both types of coffee (Van Dam and Hu, 2005; Van Dam et al, 2006). To determine whether chronic administration of Caffeine, Chlorogenic acid, and Caffeic acid decreases the levels of Glucose, Triglycerides and blood cholesterol and improve Glucose tolerance in rats with experimental diabetes. Using 5 groups of 11 male Wistar rats weighing 250-350 g type 2 diabetes was generated by high-fat diet for 60 days and then applied three doses of Streptozotocin (30 mg/Kg IP) administered on day 60, 67 and 74. One group was the control and other groups were given Caffeine (93 mg/Kg/day), Chlorogenic acid (31 mg/Kg/day), and Caffeic acid (1 mg/Kg/day), other group, were administered with the three substances together, respectively; for 4 months. Each month were tested for Insulin tolerance (IT) and Glucose tolerance (GT). These were also measured every 72

h blood Glucose, Triglycerides (TG) and Cholesterol, every 7 days for 4 months. Statistical analysis was performed with repeated measurements ANOVA Post Hoc test like Scheffe, when the distribution was normal, otherwise the test was with the Kruskal Wallis by using SPSS v15. We found significant differences in the group with Caffeine in Cholesterol, Triglycerides and in the weight. In the group with Chlorogenic acid alone, there was difference in the variable TG. For the group with the administration of the three substances together, significant differences were found in Cholesterol, Triglycerides, and in weight. Similarly with fasting glucose, GT difference remained significant in this group. However, Caffeic acid does not alter the study variables. Finally, our Results suggest that Caffeine reduces the levels of Cholesterol, Triglycerides and weight; Chlorogenic acid decreases the TG levels; Caffeic acid has no effect on the studied variables, but the three substances were together administrated significative differences were found in Cholesterol, TG, weight, fasting glucose and GT. Partially financed by CONACyT-Mexico 83113 to MH. ZUG, and CRF are CONACyT fellowships.

Disclosures: **M. Huerta-Viera:** Research Grant; CONACyT-Mexico 83113-MH. **Z. Urzúa:** None. **C. Ramos-Flores:** None. **X. Trujillo:** None.

Poster

903. Energy Metabolism

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01-DK092587

NIH Grant P30-DK072476

NIH Grant P20-RR021945

Title: Stimulation of dorsomedial hypothalamic leptin receptor (LepRb)-expressing neurons induces thermogenesis and energy expenditure in LepRbcre mice.

Authors: *K. P. REZAI-ZADEH, A. LAQUE, K. BUI, T.-A. NGYEN, S. GETTYS, C. MORRISON, H. MUNZBERG
Pennington Biomed. Res. Ctr., Baton Rouge, LA

Abstract: Obesity is a significant problem in the U.S. and current non-invasive treatments like dieting and physical exercise remain largely ineffective. Modification of energy expenditure via the induction of non-shivering thermogenesis (NST) in brown adipose tissue (BAT) is currently

reemerging as a potential obesity therapy. The dorsomedial hypothalamus (DMH) is thought to be a key site for the regulation of BAT NST. We recently showed that DMH neurons express leptin receptors (LepRb) and innervate premotor neurons that control BAT thermogenesis. Here, we show that systemic leptin administration also stimulates neuronal activity in DMH LepRb-expressing neurons, suggesting that neuronal activity in these cells may directly translate into increased energy expenditure. Furthermore, we found that intra-DMH leptin injection corrects hypothermia in leptin deficient *ob/ob* mice and causes significant weight loss, despite having no impact on food intake. We further examined if stimulation of neuronal activity in DMH LepRb-expressing neurons regulates energy expenditure and body weight in mice. By injecting a viral vector [AAV-hSyn-DIO-hM3D(G_q)-mCherry – made available by Dr. Bryan Roth, UNC] into the DMH of $LepRb^{cre}$ mice we were able to express cre-inducible designer receptors exclusively activated by designer drugs (DREADD) and thereby pharmacologically control the activity of DMH LepRb neurons. After implantation of subcutaneous temperature transmitters and recovery, animals were adapted to metabolic chambers and injected with saline or the designer drug clozapine-N-oxide (CNO) for 3 days each (twice daily). CNO caused a robust increase in energy expenditure accompanied by a significant decrease in body weight in CNO-responsive mice (CNO-induced energy expenditure) compared to CNO non-responders (no change in energy expenditure). Histological analysis revealed that CNO-responders showed DREADD expression in the DMH, while non-responders did not. Altogether, our data demonstrate that induction of neuronal activity in DMH LepRb-expressing neurons translates into increased energy expenditure leading to decreased body weight.

Disclosures: K.P. Rezai-Zadeh: None. **A. Laque:** None. **K. Bui:** None. **T. Ngyen:** None. **S. Gettys:** None. **C. Morrison:** None. **H. Munzberg:** None.

Poster

903. Energy Metabolism

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Program#/Poster#:903.09/XX10

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Lundbeck foundation Grant Nr R77-A6808

Title: Ablation of Glutamate Dehydrogenase expression in cultured mouse astrocytes alters both oxidative glutamate and glucose metabolism

Authors: *M. H. STRIDH[1], D. M. SKYTT[1], K. PAJECKA[1], M. KARACA[2], F. FRIGERIO[2], P. MAECHLER[2], A. SCHOUSBOE[1], H. S. WAAGEPETERSEN[1]

[1]Dept. Of Drug Design and Pharmacol., Univ. of Copenhagen, Copenhagen, Denmark; [2]Dept. of Cell Physiol. and Metabolism, Geneva Univ. Med. Ctr., Geneva, Switzerland

Abstract: As an integral part of the tri-partite synapse, the astrocytes provide optimal conditions for neuronal function and signalling. Astrocytes are equipped with high affinity transporters to ensure an efficient clearance of glutamate from the synapses and they ensure transmitter recycling via the glutamate/glutamine cycle. However, glutamate is also a valuable energy substrate and astrocytes are capable of fully oxidizing glutamate in the TCA-cycle. The mitochondrial enzyme glutamate dehydrogenase (GDH) catalyzes the oxidative deamination of glutamate to α-ketoglutarate and may therefore play an interesting role in linking glutamate homeostasis to energy metabolism. To evaluate the role of GDH in astrocyte energy metabolism, cultured astrocytes were prepared from CNS-*Glud1*$^{-/-}$ mice. Energy and glutamate metabolism were investigated in cultured astrocytes using [U-^{14}C]glutamate or [U-^{14}C]glucose and monitoring of $^{14}CO_2$ production. The metabolism was further mapped by incubating astrocytes with [U-^{13}C]glucose or [U-^{13}C]glutamate followed by analysis of labeling in amino acids and TCA-cycle metabolites using mass spectrometry.
Incubation with [U-^{13}C]glucose generated an significant increase in uniformly labeled alanine in *Glud1*$^{-/-}$ astrocytes compared to wt. Since alanine is in equilibrium with pyruvate, this indicates increased glycolysis in *Glud1*$^{-/-}$ astrocytes. In contrast to the augmentation in glycolytic activity, the CO_2 production from [U-^{14}C]glucose was decreased in astrocytes from *Glud1*$^{-/-}$ mice indicating an impaired TCA-cycle activity. The ability to use glutamate as an alternative energy substrate is clearly compromised in *Glud1*$^{-/-}$ astrocytes. Upon glucose deprivation, wt astrocytes are able to increase the $^{14}CO_2$ production from [U-^{14}C]glutamate, whereas this increase is absent in *Glud1*$^{-/-}$ astrocytes. Online monitoring of ATP production revealed that *Glud1*$^{-/-}$ astrocytes were unable to respond with increased ATP production upon stimulation with 1mM glutamate, in contrast to WT cells. It was also evident from these results that the *Glud1*$^{-/-}$ astrocytes had a lower baseline level of ATP indicating that they might be energetically challenged by the short period of glucose deprivation preceeding the ATP measurements. This finding further supports the notion that lack of GDH function alters glucose metabolism.
In conclusion, ablation of GDH expression in astrocytes causes the astrocytes to shift toward a more glycolytic metabolism

and there is evidence of an impaired TCA cycle function. The capability of astrocytes to use glutamate oxidation as an alternative energy source during glucose deprivation is dependent on GDH function.

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Poster

903. Energy Metabolism

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Program#/Poster#:903.10/XX11

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH DK083410

Title: Synphilin-1 regulates ampk and atp

Authors: *D. YANG[1], T. LI[2], J. LIU[2], X. LI[3], G. ZHU[4], T. MORAN[5], W. SMITH[2]
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Abstract: We recently generated a human synphilin-1 transgenic mouse model that displays some key features of obesity including increased food intake, body weight and body fat. Synphilin-1 is a cytoplasmic protein enriched in brain with unclear function. To further study the roles of synphilin-1 in energy balance control, we generated a stable pool of GT1-7 cells expressing human synphilin-1. We found that synphilin-1 increased AMP-activated kinase (AMPK, a cellular energy sensor) phosphorylation. Moreover, synphilin-1 interacted with AMPK in cultured cells and in homogenates of human brain tissues. Treatment of compound C, an AMPK inhibitor reduced synphilin-1 interacted with AMPK. Synphilin-1 also bound with the major energy currency, adenosine triphosphate (ATP) and increased cellular ATP levels. The two ATP binding regions and five motifs in synphilin-1 were mapped with truncated constructs and site mutagenesis. Genetic alterations abolishing ATP binding sites significantly reduced synphilin-1-induced AMPK activation and normalized cellular ATP levels. These data suggest that synphlin-1 is an ATP binding protein and regulates AMPK activity via ATP binding. These studies may provide novel insights into synphilin-1's biological functions and potential mechanism for affecting energy balance.

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Poster

903. Energy Metabolism

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Program#/Poster#:903.11/XX12

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Humboldt postdoctoral fellowship

Title: An energy budget for the recovery of spreading depolarisation - Multimodal measurement and kinetic modelling.

Authors: D. FEUERSTEIN[1], H. BACKES[1], M. GRAMER[1], M. TAKAGAKI[1], *A. J. STRONG[2,1], R. GRAF[1]
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Abstract: Spreading depolarisation (SD) is a slowly propagating wave of tissue depolarisation that could be closely linked to secondary deterioration of brain injury patients. It is hypothesised that progressive worsening of the lesion would be due to the hemodynamic and metabolic disturbances caused by the SDs. We have here investigated these events by simultaneously measuring:
• cerebral blood flow with laser speckle flowmetry (LSF)
• oxygen consumption with multispectral reflectometry (RGBR)
• glucose utilization with FDG-PET
• glucose and lactate concentrations in the extracellular space with online microdialysis (rsMD)
These data were then integrated into a kinetic model of brain metabolism that accounted for the rates of glucose transport, glycolysis, mitochondrial oxidative metabolism, lactate production and use, glycogen breakdown and synthesis.
Methods: LSF and RGBR were continuously acquired in male Wistar rats through thinned skull. A microdialysis probe was implanted via a small craniotomy and the dialysate was analysed for glucose and lactate concentrations every minute by rsMD. After recovery from implantation and baseline measurement, FDG was injected. At 20 minutes post-injection, SDs were elicited either by needle prick (single SD) or KCl infusion (multiple SDs). The hemodynamic and metabolic responses to SDs were observed for 70 minutes.
Results: SD waves were detected by LSF as propagating hyperemic waves. The response to one

single SD wave was biphasic: 1) within 5 minutes, glucose dropped by 20%, lactate increased by 60%, oxygen consumption increased by 20% and FDG uptake rose above control, 2) during the next 65 minutes, glucose and oxygen consumption remained below baseline, lactate fully recovered and FDG uptake gradually declined to normal values. This response could be modelled by a transient surge for ATP followed by a prolonged reduced energy demand. During phase 1, glycogen is rapidly broken down and glucose is fully catabolised via glycolysis and mitochondrial oxidative metabolism. During phase 2, cells are repolarised and glycogen stores are re-filled.
Following multiple SD waves, we observed a sustained increase in energy demand, with an ever-decreasing extracellular glucose, a prolonged elevated lactate level, and an up to 40% increase in FDG uptake. This indicates a prolonged period of high energy demand that might lead to irreversible damage.
Conclusion: We suggest that the energy requirements to recover from a single SD wave are largely fulfilled in physiological conditions. However frequent SDs could lead to an energy crisis that endangers tissue viability even in a well-perfused brain.

Disclosures: D. Feuerstein: None. **H. Backes:** None. **M. Gramer:** None. **M. Takagaki:** None. **A.J. Strong:** None. **R. Graf:** None.

Poster

903. Energy Metabolism

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Program#/Poster#:903.12/XX13

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: Variation in cerebral glucose transporter expression across the estrous cycle

Authors: *C. S. HARRELL[1], S. MALVIYA[1], S. D. KELLY[1], G. N. NEIGH[2]
[2]Physiol. and Psychiatry, [1]Emory Univ., Atlanta, GA

Abstract: Glucose is the primary fuel of the brain and alterations in glucose availability directly impact neuronal function. Cerebral glucose metabolism is disrupted in several neuropsychiatric and neurodegenerative disorders, and can further be affected by peripheral disturbances of glucose metabolism such as diabetes. Variation in glucose transport is influenced by hormonal state, yet models of cerebral glucose transport focus on the male and ovariectomized female and fail to explore how physiological fluctuations in sex steroids influence glucose transporters and insulin signaling. Female sex steroids impact glucose metabolism in contrasting ways; estradiol enhances glucose uptake and insulin sensitivity, while progesterone decreases glucose uptake

and promotes insulin resistance. Cerebral glucose uptake varies during the estrous cycle, with uptake highest during proestrus in the rat, when estradiol levels peak. Cerebral glucose uptake is mediated by a family of facilitative glucose transporters (GLUTs) that show isoform-specific distribution and regulation. We examined four members of this family: GLUT1, GLUT3, GLUT4, and GLUT8. GLUT1 primarily regulates blood-brain barrier glucose transport while GLUT3 is responsible for the majority of glucose uptake into neurons. GLUT4 and GLUT8 are both insulin-sensitive transporters; insulin receptor activation results in GLUT4 translocation to the plasma membrane and GLUT8 translocation to the endoplasmic reticulum. Our studies demonstrate that natural variation in sex steroids across the estrous cycle co-occur with changes in glucose transporter expression. Estrous cycles in adult female rats were monitored daily, and tissue was collected based on cycle stage. Uterine weights and serum estradiol and progesterone were used to further confirm cytologic staging. The hippocampus, hypothalamus, and prefrontal cortex were dissected for examination of glucose transporter expression. Our experiments demonstrated that each GLUT examined peaked in proestrus and with lowest expression in diestrus 1 (D1). Further, GLUT1 also showed significant elevations between D1 and diestrus 2 (D2), suggesting that this transporter may be most responsive to the rise in estradiol beginning in D2. These studies confirm the importance of hormonal state in regulating cerebral glucose transport, and provide an important baseline for further work on cerebral glucose metabolism in the female.

Disclosures: C.S. Harrell: None. **S. Malviya:** None. **S.D. Kelly:** None. **G.N. Neigh:** None.

Poster

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: AHA 10GRNT4540000

Tulane BIRCWH2K12HD043451

Title: Activation of TRPV1 in the paraventricular nucleus of hypothalamus regulates systemic blood glucose levels

Authors: *K. MIYATA, J. O'HARE, T. FOURRIER, A. KRANTZ, A. DERBENEV, A. ZSOMBOK
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Abstract: The autonomic nervous system plays a pivotal role in the regulation of glucose homeostasis. Transient receptor potential vanilloid type 1 (TRPV1) receptors colocalize with liver-related neurons in the paraventricular nucleus (PVN) of hypothalamus. Furthermore, our previous study demonstrated TRPV1-dependent excitation of liver-related PVN neurons. However, the effect of TRPV1 activation on whole body glucose homeostasis remains to be elucidated. The aim of this study was to demonstrate whether TRPV1 activation in the PVN mediates whole body glucose homeostasis. We examined systemic blood glucose levels, serum insulin levels, expression of hepatic gluconeogenic enzymes and Akt phosphorylation in male CD1 mice. Activation of TRPV1 by injecting capsaicin (0.5 nmol) bilaterally into the PVN significantly decreased systemic blood glucose levels. The reduction was detectable up to 30 min, with a maximum approximately at 15 min. The glucose lowering effect of TRPV1 was suppressed in the presence of the glutamate receptor antagonist, kynurenic acid. Serum insulin levels detected with ELISA did not show significant difference between groups. Moreover, activation of TRPV1 in the PVN decreased hepatic PEPCK levels, while there was no difference in G6Pase, and Akt phosphorylation levels. Our results demonstrate that systemic blood glucose levels are controlled by activation of TRPV1 in the PVN. Supported by AHA 10GRNT4540000, Tulane BIRCWH 2K12HD043451.

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Poster

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: Treadmill exercise rapidly induces the expression of hif-1α in the hippocampus of the adult rat

Authors: *K. L. BERGGREN, B. S. AHUJA, J. J. M. KAY, B. POCHINSKI, R. A. SWAIN
Psychology, Univ. of Wisconsin - Milwaukee, Milwaukee, WI

Abstract: Exercise induces a myriad of effects on the brain from the growth of new blood vessels (angiogenesis) and neurons (neurogenesis), to improvements in cognitive performance. A very recent study has found that in addition to angiogenesis and neurogenesis, commencement of an exercise regimen also causes cell death. This apoptosis also occurs shortly after the start of exercise, with levels of caspase-3 increasing at 12 hours and peaking within 24 hours of

voluntarily exercising. It is possible that exercise-induced increases in oxygen demand cause the brain to transiently experience a mild state of hypoxia causing cells to die. To investigate this hypothesis, we exposed male Long Evans rats to a six-day handling and pre-exposure procedure followed by a single bout of moderately intense treadmill exercise (20 minutes at ~15 meters/minute). Following the exercise session, animals were sacrificed and transcardially perfused at one of four time points: immediately (EX0), two hours (EX2), four hours (EX4), or six hours (EX6) after exercise. Additional groups of animals (inactive controls – IC0, IC2, IC4, & IC6) that did not exercise followed the same handling and pre-exposure procedures and were sacrificed at the same time points as the exercising animals. To determine if exercise induces hypoxia in the hippocampus, we measured via immunohistochemistry, protein levels of the hypoxia-inducible factor 1-alpha (HIF-1α), a transcription factor known to be upregulated in the brain under conditions of low oxygenation such as during and after ischemia, or exposure to an hypoxic environment. Following immunohistochemistry, tissue slices were imaged and analyzed using Image J software to determine the area fraction of HIF-1α-positive cells in the whole hippocampus as well as in three distinct hippocampal regions: CA1, CA2/3, and the dentate gyrus (DG). Preliminary data show that animals that were sacrificed immediately following exercise had significantly greater HIF-1α expression in the whole hippocampus compared to inactive animals. Furthermore, results indicate that EX0 animals showed greater HIF-1α expression in area CA1 of the hippocampus. These findings indicate that a single bout of moderately strenuous exercise induces hypoxia in the hippocampus. Furthermore, because HIF-1α is a common molecule of interest in stroke and hypoxia research, exercise may be considered another method with which to study the molecular pathways involved in alterations of brain oxygenation, and the mechanisms activated in an attempt to return the brain to normal oxygen homeostasis.

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Poster

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Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: EPB-200/2009 LSRB-DRDO

Title: Neuroprotective effect of flavonoids in hypobaric hypoxia in murine model

Authors: *A. SARKAR, S. M. ANGELINE, K. ANAND, P. ASTHANA, P. CHATTERJEE, R. K. AMBASTA, P. KUMAR
VIT Univ., Vellore, India

Abstract: Exposure to high-altitude results in hypobaric hypoxia which is considered as an acute physiological stress. This condition often leads to high-altitude illnesses such as high-altitude cerebral edema (HACE), high altitude pulmonary edema (HAPE) and hypoxic muscle weakness. One way to prevent hypoxic injuries is preconditioning with cobalt chloride, and another is treatment with effective drugs. Herein, we evaluated the effect of naringenin and quercetin against behavioral impairment and neuronal damage in hypoxia induced murine model. Hypoxia models were generated using *Swiss albino* mice. Oral dosage of quercetin and naringenin was administrated before every hypoxia treatment. Drug treated groups exhibited improved histopathological morphology validated by amino-cupric silver staining. We further checked the expression level of HIF1α, caspase-3 and ubiquitin. Drug treated brain showed decline in HIF1α level. Decreased expression of caspase 3 and ubiquitin in treated brain suggests the neuro protective effect of naringenin and quercetin in hypoxia induced damages.

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Poster

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Program#/Poster#:903.16/XX17

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: EPB-200/2009 LSRB-DRDO to Pravir Kumar.

Title: Protective effect of naringenin on hypoxia induced muscle degeneration

Authors: *P. ASTHANA[1,2], A. SARKAR[2], S. ANGELINE[2], K. ANAND[2], N. JAISWAL[2], S. K. JHA[2], N. K. JHA[2], P. CHATTTERJEE[2], R. K. AMBASTA[2], P. KUMAR[2]
[1]GHAZIABAD, India; [2]VIT Univ., Vellore, India

Abstract: Large number of people (especially army or rock climber) travel to high altitudes, entering an environment of hypobaric hypoxia. It has been known that high-altitude exposure is an aggressive physiological stress that induces several cellular deleterious effects. Previous findings have shown that high-altitude hypoxia triggers increased oxidative stress which is a

favourable condition for the activation of apoptotic cellular death pathways. In response to this hypobaric hypoxia several systemic and peripheral physiological responses are acutely and chronically triggered out to counteract the body overall hypoxic status. Hypoxic injuries can be prevented by either preconditioning or treatment with drugs. In the present study, the effect of naringenin against behavioural impairment and muscle damage in the *in vivo* acute and chronic hypobaric hypoxic mice model has been investigated. Male mice were randomly divided into the five groups and drug was administrated orally. In contrast with a regular skeletal muscle pattern observed in normoxia group, acute and chronic hypoxic group revealed profound structural alterations in the skeletal muscle tissue organization. Whereas treatment with naringenin substantially improved hypoxia induced skeletal muscle dysfunction confirmed by hematoxylin and eosin staining and behavioral test. Further, the protective nature was corroborated with immunoblot and immunoflurescence results where we confirmed the modulation of HIF1α and apoptotic markers. To conclude, the results suggest that naringenin prevents the loss of skeletal muscle integrity and function, against acute and severe hypobaric hypoxia.
Keywords : Hypoxia, bioflavonoid, muscle, caspase
Funding: EPB-200/2009 LSRB-DRDO to Pravir Kumar.
Conflict of interest: None

Disclosures: **P. Asthana:** Research Grant; EPB-200/2009 LSRB-DRDO. **A. Sarkar:** Research Grant; EPB-200/2009 LSRB-DRDO. **S. Angeline:** Research Grant; sonia_angem@yahoo.co.in. **K. Anand:** Research Grant; anand.kushi@gmail.com. **N. Jaiswal:** Research Grant; EPB-200/2009 LSRB-DRDO. **S.K. Jha:** Research Grant; EPB-200/2009 LSRB-DRDO. **N.K. Jha:** Research Grant; EPB-200/2009 LSRB-DRDO. **P. Chattterjee:** Research Grant; EPB-200/2009 LSRB-DRDO. **R.K. Ambasta:** Research Grant; EPB-200/2009 LSRB-DRDO. **P. Kumar:** Research Grant; EPB-200/2009 LSRB-DRDO.

Poster

903. Energy Metabolism

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:903.17/XX18

Topic: B.11. Glia-Neuron Interactions

Support: NIH RO1 AG29461

NIH T32-HL04776-29

Title: Thyroid hormone increases astrocyte survival in response to oxidative stress

Authors: N. L. SAYRE, D. HOLSTEIN, *J. D. LECHLEITER
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Abstract: In tissue surrounding ischemic stroke, disrupted energy metabolism leads to inability to maintain ionic gradients, in turn causing swelling and rupture of cells. Astrocyte metabolism represents a particularly attractive therapeutic target during stroke because astrocytes play numerous supportive roles and can significantly influence neuronal health. Indeed, our laboratory has previously demonstrated that enhancing astrocyte mitochondrial metabolism is neuroprotective in stroke[1] and in aging[2]. Similarly, our laboratory has shown that mitochondrial energy production can be increased via thyroid hormone receptor (TR) activation by treatment with the thyroid hormone 3,3'5 triiodo-L-thyronine (T3) [3].

The effect of thyroid hormones on energy metabolism is traditionally attributed to changes in gene expression, however we discovered a novel, non-genomic function of T3. Specifically, T3 treatment stabilizes the association of subunits within mitochondrial trifunctional protein in a TR dependent manner, which in turn increases fatty acid oxidation (FAO) and energy production. We previously showed that acute treatment with T3 increases FAO in fibroblasts[4]. Astrocytes are also capable of FAO. **We hypothesize that T3 increases astrocyte FAO and energy production, therefore increasing astrocyte survival after oxidative stress.**
Here we describe the effect of T3 treatment on astrocyte survival and fatty acid oxidation. Importantly, astrocyte survival after *tert*-butyl hydrogen peroxide treatment significantly increased with nanomolar concentrations of T3. Similarly, fatty axid oxidation of palmitate also increases. Future directions include determining whether the T3-mediated survival of astrocytes after oxidative damage is dependent on the increased fatty acid oxidation, and whether T3 mediates survival after stroke *in vivo.*

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[4]Chocron, E.S., NL; Holstein, D; Saelim, B; Ibdah, J; Dong, LQ; Zhu, X; Cheng, SY; Lechleiter, JD, *The Trifunctional Protein Mediates Thyroid Hormone Receptor Dependent Stimulation of Mitochondrial Metabolism.* Mol Endocrinol, 2011. **in press**.

Disclosures: N.L. Sayre: None. **D. Holstein:** None. **J.D. Lechleiter:** None.

Poster

903. Energy Metabolism

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:903.18/XX19

Topic: B.11. Glia-Neuron Interactions

Title: Frequency of autonomous electrical activity is modulated by extracellular glucose concentration in a cultured neuronal network.

Authors: *W. MINOSHIMA, S. N. KUDOH, H. ITOH
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Abstract: Cultured rat hippocampal neurons on a multi electrode array (MEA) dish are useful system for analyzing network electrical dynamics and its developmental changes. Neurons autonomously form a complex network on a MEA dish and spontaneous electrical activity is often observed without any input from the external world. The spontaneous activity is generated by synaptic interactions between neurons and reflects internal biochemical state of neurons in a whole neuronal network. The origin of electrical activity is electrochemical potential generated by active transport of ions, which requires catabolism of ATP. We elucidated relationship between autonomous electrical activity and external glucose concentration. The spatiotemporal pattern of autonomous activity changed transiently, depending on glucose concentrations, and we analyzed the number of spikes in autonomous activity. Electrical spikes increases in frequency depending on the concentration of external glucose. Interestingly, this increase is not suppressed but turn to decrease. In the case of glucose concentration is 17.5 mM, the number of activity is the most, and then it decreases in the case of glucose concentration is more than 17.5 mM. In addition, the decrease of neuronal activity at high glucose concentration is not influenced by the blockade of inhibitory synaptic activity, suggesting the decrease is intrinsic in activity generating process. A cultured neuronal network may have an optimal glucose concentration, which corresponds to the concentration of glucose in a culture medium (17.5 mM).

Disclosures: W. Minoshima: None. **S.N. Kudoh:** None. **H. Itoh:** None.

Poster

903. Energy Metabolism

Location: Hall F-J

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Program#/Poster#:903.19/XX20

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant 1RC24L101625

USAMRMC Grant W81XWH-11-1-0409

Title: Molecular mechanisms of natural neuroprotection in a hibernating mammal.

Authors: *C. P. SCHWARTZ[1], M. HAMPTON[2], M. T. ANDREWS[1]
[1]Biol., [2]Mathematics, Univ. of Minnesota Duluth, Duluth, MN

Abstract: During hibernation, thirteen-lined ground squirrels undergo rapid and extreme changes in body temperature and brain perfusion as they cycle between lengthy torpor bouts (7-10 days) and brief periods of euthermia called interbout arousals (IBAs, <24 hours). Arousal from torpor to IBA, where body temperature increases from 5 to 37ºC and heart rate explodes from 3 to 300-400 beats/min, takes just 2-3 hours. Amazingly, the hibernator brain tolerates these rapid fluctuations with no residual damage, suggesting a sophisticated neuroprotective strategy, along with a robust central control system to coordinate the changes. Adding even more complexity, different areas of the brain show very different activity profiles during hibernation. The hypothalamus remains active throughout the season, even in deep torpor, and is thought to play an important role in the induction and maintenance of hibernation. In contrast, the cerebral cortex is the first area of the brain to lose activity upon entrance to torpor, and the last to regain activity upon arousal. Examining the molecular changes occurring in these two differentially regulated areas of the ground squirrel brain during hibernation could provide valuable insight into how hibernation is centrally controlled, and also reveal neuroprotective mechanisms that allow variable brain activity. To do this, we used the Illumina HiSeq 2000 system to sequence the transcriptome of the hypothalamus and cortex at four time points during the hibernation season: October, Torpor, IBA, and April. Over 10 million raw cDNA sequence reads per sample were identified and quantified using species-specific contigs and an analysis of deviance was performed for each gene to determine differential expression across time points. Of 15,318 genes identified in the hypothalamic transcriptome, 1,554 showed differential expression ($p<0.01$). In the cortex, 15,184 genes were identified and 977 were differentially expressed ($p<0.01$). An overrepresentation analysis revealed that transcripts associated with neuron morphogenesis, plasticity, and ion homeostasis were significantly upregulated during torpor in the cortex. In the hypothalamus, transcripts involved in basic cell functions (transcription, translation, cellular respiration), along with DNA repair and neurogenesis were significantly upregulated in both torpor and IBA. The data from this transcriptomic study has revealed many genes and pathways underlying the physiological changes associated with hibernation and mechanisms of protection promoting survival in the brain during these rapid and extreme physiological changes.

Disclosures: C.P. Schwartz: None. **M. Hampton:** None. **M.T. Andrews:** None.

Poster

903. Energy Metabolism

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:903.20/YY1

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: PSC-CUNY Award Cycle 42

Title: Behavioral and physiological changes in naked mole-rats in environmental hypoxia/hypercapnia.

Authors: *D. P. MCCLOSKEY[1], X. GEOFFROY[2], S. ABRAHAM[3], R. ASSINI[3], I. RABINOVICH[4]
[1]Dept of Psychology and Program in Developmental Neurosci., [2]Dept of Psychology and Ctr. for Developmental Neurosci., [3]Master's Program in Developmental Neurosci., [4]Dept. of Psychology, Col. Staten Island/ CUNY, STATEN ISLAND, NY

Abstract: The naked mole-rat (Heterocephalus Glaber) is a eusocial, exclusively subterranean, species of rodent that lives in extensive burrow systems in eastern Africa, with colonies as large as 300 animals. This ecological niche can present a hypoxic/hypercapnic environment which field studies suggest these animals can tolerate very well, and previous in vitro work shows the adaptation is present at the neuronal level. The purpose of this study was to measure the impact of a reduced O2 (18.5%), elevated CO2 (3.5%) environment on behavioral and physiological measures in laboratory housed naked mole-rats to further understand this adaptation. A colony of 22 animals was measured under normal conditions, followed by 96 hours of hypercapnic/hypoxia. Animal movements were tracked in a complex housing system using a custom radio frequency identification (RFID) equipment. Common tunnel paths between interconnected cages in the housing environment were periodically closed to measure learning effects in adapting to altered environments. Brain levels of growth factors (VEGF, BDNF) and hormones (cortisol) were measured in a subset of animals. Our results suggest that animals in the hypoxic/hypercapnic environment move slower, but are more social, more active, and less disrupted by closing of tunnels. Video recordings showed robust seizure-like behavior in multiple animals as normoxia was being restored to the environment. Follow up studies suggest that these animals are prone to seizure activity in normoxia, which we have confirmed in hippocampal slice electrophysiology recordings. Together, these data suggest that the naked mole rats are sensitive to gaseous changes in the local environment, but in different ways than other mammals.

Disclosures: D.P. McCloskey: Research Grant; PSC-CUNY Welfare Fund. **X. Geoffroy:** None. **S. Abraham:** None. **R. Assini:** None. **I. Rabinovich:** None.

Poster

903. Energy Metabolism

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:903.21/YY2

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: Hypoxia alters metabolic rate, core body temperature and c-fos expression in the naked mole rat

Authors: *T. I. NATHANIEL[1], E. OTUKONYONG[2], J. SOYINKA[3]
[1]Biomed. Sci., Univ. of South Carolina Sch. of Med. At Greenville, Greenville, SC; [2]Dept. of Hlth. Sci., East Tennessee State Univ., Johnson City, TN 37614., TN; [3]Dept. of Pharmaceut. Chemistry,, Obafemi Awolowo Univ., IleIfe, Nigeria

Abstract: Recent investigations of hypoxia physiology in the naked mole rat have opened up an interesting line of research into the basic physiological and genomic alterations that accompany hypoxia survival. The extent to which such findings connect the effect of hypoxia to metabolic rate (O2 consumption), core body temperature (Tb) and transcripts encoding the immediate early gene product (such as c-fos) under a constant ambient temperature (Ta) is not well known. We investigated this issue in the current study. Our first sets of experiments measured Tb and metabolic rates during exposure of naked mole rats to hypoxia over a constant Ta. Hypoxia significantly decreased metabolic rates in the naked mole rat. Although core Tb also decreased during hypoxia, the effect of hypoxia in suppressing core Tb was not significant. The second series of experiments revealed that c-fos protein expression in the hippocampus neurons (CA1) increased in naked mole rats that were repeatedly exposed to 3% O2 for 60 min each day for 5 days when compared to normoxia condition. Our findings provide evidence for the up-regulation of c-fos protein, suppression of Tb, and metabolic rate in hypoxia tolerating naked mole rats under constant ambient temperature.

Disclosures: T.I. Nathaniel: None. **E. Otukonyong:** None. **J. Soyinka:** None.

Poster

904. Human Learning: Motor and Sequence Learning

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:904.01/YY3

Topic: F.01. Human Cognition and Behavior

Support: NSF Cyber-Enabled Discovery and Innovation Type I (Idea) grant # 094158 to EBT "A novel qtitative framework to study lack of social interactions in Autism Spectrum Disorders"

New Jersey Governor's Council for Medical Research and Treatment of Autism grant # 10-403-SCH-E-0 "Perceptual Motor Anticipation in ASD"

ONR MURI #10124957 to HP

Title: Spontaneity in the task-incidental segments of the pointing gesture emerges when visual guidance or movement imagination replace proprioception

Authors: *E. B. TORRES[1], J. COLE[2], H. POIZNER[3]
[1]Psychology Dept, Rutgers Univ., Piscataway, NJ; [2]Ctr. for Neurosci., Univ. of Southampton, Poole, United Kingdom; [3]Inst. for Neural Computation, UCSD, La Jolla, CA

Abstract: Patient IW lost his proprioception at the age of 19 due to a large fiber sensory neuropathy that led to the loss of the sensation of movement/position sense and light touch, below the neck (Cole, 1995). He eventually taught himself how to move using mental concentration and visual supervision; later he used mental rehearsal and imagery to prepare and launch movements. He developed fluid, spontaneous gestures that masked his lack of kinesthetic feedback rather well and made his motions look effortlessly natural. It seems puzzling that voluntary visual guidance resulted in such fluid spontaneous motions. However, both visual supervision and the mental processes involved in imagining and deciding over proper muscle configurations to attain a movement have two co-existing modes: slow-deliberate-conscious and fast-spontaneous-unconscious. Therefore, it would not be entirely surprising that his gestures eventually evolved in correspondence with other mental operations that came to replace his loss of kinesthetic sensation. Here we asked if the deliberate-spontaneous dichotomy would also manifest in the kinematics of the pointing movements of IW as it typically does in normal Controls (NC). To this end, participants made full pointing motions in a continuous forward and back loop with a deliberate, task-relevant segment intended to touch a target in space and a retracting task-incidental segment spontaneously transitioning to bring the hand back to rest. The target in some blocks was turned OFF and had to be memorized, while in others it remained ON and guided the motion throughout. In other blocks, we instructed subjects to move at different speeds to probe the effects of speed change on the hand trajectories -since the stochastic signatures of speed modulation and its effects on the hand trajectories can be used as an objective criterion to separate deliberate task-relevant from spontaneous task-incidental gestures (Torres, 2011). We found that the kinematics of his visually guided reaches in a fully lit room manifested the signatures of variability leading to the deliberate-incidental dichotomy that we also quantified in the NC. Remarkably, these features were quantified even in the dark (albeit more variable), suggesting that the fluidity of his retracting motions spontaneously emerged despite his voluntary control. Thus, both visual supervision and the imagination of movements integrated with his

efferent motor output can indeed lead to the two-movement class dichotomy, slow-deliberate and fast-spontaneous, a dichotomy that is also present in our cognitive mental processes.

Disclosures: E.B. Torres: Employment; Rutgers, The State University of New Jersey, Psychology. Research Grant; NSF Cyber-Enabled Discovery and Innovation Type I (Idea) grant # 094158 to EBT and DNM "A novel quantitative framework to study lack of social interactions in Autism Spectrum Disorders", New Jersey Governor's Council for Medical Research and Treatment of Autism grant # 10-403-SCH-E-0 "Perceptual Motor Anticipation in ASD". **J. Cole:** None. **H. Poizner:** Research Grant; ONR MURI #10124957.

Poster

904. Human Learning: Motor and Sequence Learning

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:904.02/YY4

Topic: F.01. Human Cognition and Behavior

Support: NHMRC Training Fellowship 1012153

Title: Motor cortical plasticity is enhanced by passive observation of mirror-matched limb movements.

Authors: *M. V. SALE[1], J. B. MATTINGLEY[2]
[1]Univ. of Queensland, St Lucia, Australia; [2]Queensland Brain Inst., Univ. of Queensland, Brisbane, Australia

Abstract: Watching others learn a motor task can enhance an observer's own later performance when learning the same motor task. This is thought to be due to activation of the action observation (or mirror neuron) network. Here we show that the effectiveness of plasticity induced in human motor cortex (M1) is significantly influenced by the nature of prior action observation. In separate sessions, 17 participants watched a video showing repeated goal-directed movements (action
observation) involving either the right hand (congruent condition) or the same video mirror-reversed to simulate the left hand (incongruent condition). Participants then repeatedly performed the movements observed in the congruent video, while receiving pulses of transcranial magnetic stimulation over the hand area of M1 paired with median nerve stimulation of the right hand (paired associative stimulation; PAS). The resting motor-evoked potential (MEP) in right abductor pollicis
brevis (APB) increased significantly 20 minutes after PAS, but only when participants had previously watched the congruent video. In this condition, all participants showed an increase in

MEP amplitude at 20 minutes post-PAS. There was no change in MEP amplitude following PAS when participants watched the incongruent video. We conclude that prior action observation is a potent modulator of subsequent PAS-induced neuroplasticity, which may have important therapeutic applications.

Disclosures: M.V. Sale: None. **J.B. Mattingley:** None.

Poster

904. Human Learning: Motor and Sequence Learning

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:904.03/YY5

Topic: F.01. Human Cognition and Behavior

Title: Delayed visual feedback does not prevent updating of an internal model, but slows error reduction in PD patients

Authors: *L.-A. LEOW[1], A. M. LOFTUS[2], G. HAMMOND[1]
[1]Sch. of Psychology, Univ. of Western Australia, Perth, Australia; [2]Facultry of Hlth. Sci., Sch. of Psychology & Speech Pathology, Curtin Univ., Perth, Australia

Abstract: It has been argued that simultaneous presentation of visual feedback during visuomotor adaptation is necessary to update an internal model, as delaying presentation of visual feedback resulted in small aftereffects, lengthened reaction time and large trial-to-trial variability in directional error, which suggests error reduction through compensatory strategies (Shabbott and Sainburg 2010; Hinder et al. 2010; Hinder et al. 2008). Delayed visual feedback is a demanding adaptation condition: increasing delays systematically slows the rate of error reduction (Kitazawa et al. 1995). Previous findings may be due to a combination of multiple demanding adaptation conditions (delayed feedback, large perturbation and multiple targets) which provoke the use of compensatory strategies to reduce error. It is hypothesized that delayed visual feedback combined with less demanding adaptation conditions allows error reduction by updating an internal model, evident through large aftereffects which decay trial-by-trial. As Parkinson's Disease (PD) patients are more reliant on visual feedback for movement, it is hypothesized that adaptation performance in PD patients may be more sensitive to delayed visual feedback. Method: Less demanding adaptation conditions (30° rotation, a single target) were used throughout this study. Participants (PD patients and neurologically intact young and older adults) completed 50 adaptation trials with concurrent or delayed visual feedback, followed by 15 veridical feedback trials in which aftereffects were assessed. Subsequently, participants completed 50 adaptation trials with concurrent or delayed feedback, followed by another 15

veridical feedback trials. The testing order was counterbalanced. Results: In controls, delayed and concurrent feedback resulted in similar rate and extent of error reduction, and aftereffects of similar magnitude and persistence. In PD, error reduction with delayed feedback was slightly slower than with concurrent feedback, although both delayed and concurrent feedback resulted in aftereffects of similar magnitude and persistence. Conclusions: Delayed visual feedback combined with undemanding adaptation conditions (30° rotation, single target) resulted in large aftereffects that decayed trial-by-trial, suggesting the formation of an adapted internal model. Hence, the simultaneity of visual feedback during visuomotor adaptation may not be crucial to the updating of an internal model. The slower error reduction with delayed visual feedback in PD but not in controls suggests a greater sensitivity to delayed visual feedback in PD.

Disclosures: L. Leow: None. **A.M. Loftus:** None. **G. Hammond:** None.

Poster

904. Human Learning: Motor and Sequence Learning

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:904.04/YY6

Topic: F.01. Human Cognition and Behavior

Support: OTKA MB08A 84743

Title: Boosting implicit sequence learning by hypnosis

Authors: *D. NEMETH[1,2]
[1]Inst. of Psychology, Univ. of Szeged, Szeged, Hungary; [2]Imaging Res. Ctr., Univ. of Texas, Austin, Austin, TX

Abstract: Human learning and memory depend on multiple cognitive systems related to dissociable brain structures. These systems interact not only in cooperative but sometimes competitive ways in optimizing performance. It has been previously demonstrated that functional brain connectivity is reduced in hypnosis, and this is especially typical for frontal areas. The main question of our study was how the disruption of frontal lobe functions affects performance in basal-ganglia dependent sequence learning. Fourteen highly hypnotizable, healthy adults participated in the experiment. Sequence learning was measured by the Alternating Serial Reaction Time (ASRT) task. Participants performed the ASRT task in waking alert and hypnotic state. We found improved sequence learning in the hypnosis condition compared to the alert condition. Our result provides support for the idea that learning and memory processes may not only involve the engagement of specific neuroplastic mechanisms, but may also rely upon the disengagement of interacting brain systems. The improved sequence learning in hypnosis could

be attributed to the reduction of interconnectivity between brain areas, especially the connections of the frontal lobe, which results in the disruption of attentional control and executive system. This result sheds light not only on the competitive nature of brain systems in cognitive processes, but also could lead to further research on improving human skill learning.

Disclosures: D. Nemeth: None.

Poster

904. Human Learning: Motor and Sequence Learning

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:904.05/YY7

Topic: F.01. Human Cognition and Behavior

Support: DBOF

FWO

Title: Task-specific effect of tDCS on motor learning

Authors: *C. M. SAUCEDO[1], X. ZHANG[1], N. WENDEROTH[1,2]
[1]Katholieke Univ. Leuven, Heverlee, Belgium; [2]Dept. Hlth. Sci. and Technol., Zurich, Switzerland

Abstract: Anodal transcranial Direct Current Stimulation (tDCS) enhances motor skill learning when applied to the human primary motor cortex (M1) of healthy controls and in patients [1-4]. However, it remains unclear whether tDCS improves motor learning in a general manner or whether there is a task-specific effect. In this study, we tested the effect of tDCS in two different motor tasks: (1) sequential finger sequence task (SFTT) and (2) Sequential Isometric Pinch Force Task (SIPFT).
Thirty-two healthy subjects participated in this double-blind, sham-controlled cross-over designed study. All subjects were randomly assigned to an anodal-tDCS group or sham-group. We applied tDCS over the primary motor cortex (M1) while subjects performed the motor task. Two different sessions (session interval>1 month) were performed, with the task-order randomized across participants. Motor training of each task consisted of 20 min training for 3 continuous days. Retention tests were performed on the final training day and one week after the training.
Learning scores were calculated and compared using a mixed model ANOVA analysis. The results showed that for both tDCS groups there was an overall improvement of scores across training ($p<0.001$). Anodal tDCS showed more improvement compared to sham, in both motor

tasks, but this effect did not reach significance. In the SFTT task, a significant interaction between the TIME of stimulation (pre, training, post, RT) and TYPE of stimulation (anodal/sham) was found (p=0.01), with the greatest improvement by anodal tDCS being at the 20 min retention test (p=0.01). On the other hand, the SIPFT task showed the greatest improvement in the 1 week retention test (p=0.03).
These findings suggest that anodal tDCS applied over M1 appears to improve motor skill acquisition but there might be task dependent aspects as well. However, it is important to note that group differences across Anodal vs. Sham stimulation cannot be excluded in this study design. Therefore, to further confirm these results a cross-over design across types of tDCS stimulation will be included as well as a screening for the brain derived neurotrophic factor (BDNF) genotype of our participants to investigate if BDNF mediates the beneficial effect of tDCS.

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Disclosures: C.M. Saucedo: Employment; KU Leuven. Research Grant; DBOF. Other Research Support; FWO. **X. Zhang:** None. **N. Wenderoth:** None.

Poster

904. Human Learning: Motor and Sequence Learning

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:904.06/YY8

Topic: F.01. Human Cognition and Behavior

Title: The role of the primary motor cortex in implicit learning and the processing of reward.

Authors: *L. WILKINSON, E. MOOSHAGIAN, T. ZIMMERMANN, J. LEWIS, E. WASSERMANN
Natl. Inst. of Neurolog. Disorders and Stroke, NIH, Bethesda, MD

Abstract: The basal ganglia and its cortical connections, including the primary motor cortex (M1) play an important role in both implicit/unconscious motor sequence learning and processing of reward. Continuous burst transcranial magnetic stimulation (cTBS) reduces in motor cortex excitability. We examined the effect of cTBS over M1on implicit sequence learning with and without reward, using a probabilistic serial reaction time paradigm. We investigated

implicit sequence learning with and without reward in two healthy groups after cTBS to M1 or sham. There was no overall effect of reward on learning. However, cTBS produced more impairment of learning in the unrewarded than the rewarded condition. Reward appears to protect learning against the effect of cTBS to M1 and may be a way of overcoming learning impairments in clinical settings.

Disclosures: L. Wilkinson: None. **E. Mooshagian:** None. **T. ZImmermann:** None. **J. Lewis:** None. **E. Wassermann:** None.

Poster

904. Human Learning: Motor and Sequence Learning

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Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust Fellowship WT090955AIA

FCT SFRH / BD / 43862 / 2008

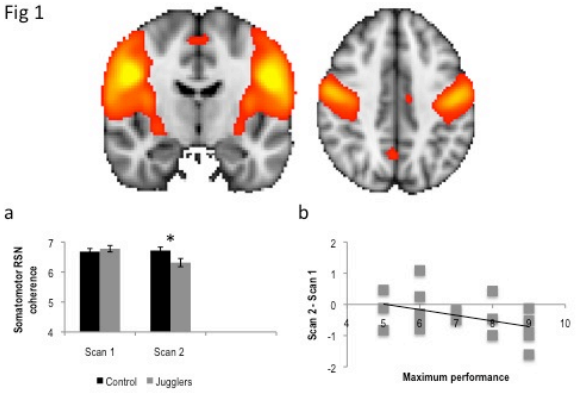
Title: Resting-state motor network coherence decreases with long-term motor learning

Authors: *C. SAMPAIO-BAPTISTA, N. FILIPPINI, J. SCHOLZ, H. JOHANSEN-BERG
Univ. of Oxford, Oxford, United Kingdom

Abstract: Introduction: Motor skill learning induces structural and functional plasticity in the brain (e.g. Scholz et al., 2009). While motor learning is known to involve several brain areas that work as a circuit (e.g. Doyon & Ungerleider, 2002), most fMRI studies have focused on discrete brain activation rather than interactive networks. The resting brain exhibits spontaneous activity that forms distinct temporally correlated networks. The functional meaning of these resting-state networks is unknown, although some have suggested a role in memory consolidation, rehearsal or future preparation (Buckner & Vincent, 2007) as well as in motor learning (Albert, et al., 2009; Ma et al., 2011). Here, we tested whether long-term motor learning alters resting state network coherence and whether this relates to performance.
Methods: Naïve participants (n=20) were scanned before and after 6 weeks of juggling practice (30 minutes/day, 5 days/week). A control group (n=20) was scanned twice 6 weeks apart. Whole-brain functional imaging was performed while participants lay at rest with eyes open. Preprocessed data was used to identify previously described resting-state networks with IndependentComponent Analysis (ICA) and thresholded group ICA outputs were used to define masks of the sensorimotor and default mode networks. A dual-regression approach was used to

extract subject-specific mean network values within these masks for each timepoint (Filippini et al., 2009). SPSS was used to test for differences between groups over time using a repeated measures ANOVA and to test for correlations with behaviour using Pearson's correlation.
Results:Long-term learning altered resting brain activity in a network specific manner.For the sensorimotor network only, learning decreased coherence (group x time interaction; $F(1,38) = 5.02$; $p < 0.05$) (Fig 1a). This decrease correlated with performance ($R = - 0.43$; $p < 0.05$) (Fig 1b).
Conclusion:Long-term motor learning decreases the resting-state motor network coherence in a behaviourally-relevant manner.

Fig 1



Disclosures: C. Sampaio-Baptista: None. **N. Filippini:** None. **J. Scholz:** None. **H. Johansen-Berg:** None.

Poster

904. Human Learning: Motor and Sequence Learning

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Program#/Poster#:904.08/YY10

Topic: F.01. Human Cognition and Behavior

Support: NIA/NIH Grant RO1AG036863

NIMH Grant MH088066

NIMH Grant MH086709

Title: Resting state connectivity predicts implicit sequence learning performance

Authors: *C. STILLMAN[1], E. M. GORDON[2], H. H. FELDMAN[1], J. R. SIMON[3], C. J. VAIDYA[1,4], J. H. HOWARD[5,1,6], D. V. HOWARD[1]
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Abstract: Our ability to rapidly acquire and integrate complex probabilistic associations is essential for many everyday activities. The neural basis of this type of learning has been explored using both functional MRI (fMRI) and diffusion tensor imaging techniques. These studies indicate that implicit associative learning is supported by the activation of a neural system including the striatum, hippocampus, and prefrontal cortex, and is mediated by the structural integrity of white matter tracts connecting these regions (Bennett et al., 2011, Simon, Vaidya, et al., 2011). Recent research using resting state fMRI suggests that brain regions which are both structurally connected and coactivated during cognitive tasks also form functional networks during rest. The present goal was to explore if the degree of coherence in the spontaneous activity in these regions at rest relates to implicit learning performance.
Twenty-two subjects completed three sessions of the Triplets Learning Task (TLT), a probabilistic sequence-learning task in which participants respond to the location of a target event in sequences of three events, or "triplets". Unbeknownst to participants, some triplets of events occur with high probability (HP) and others with low probability (LP). Implicit sequence learning is measured by subtracting response times to HP triplets from LP triplets. There was significant learning in all three sessions (all *p*s < .001), with most participants attaining their highest learning score in the final session. Resting state fMRI data were also collected for these subjects. Using the time series extracted from a bilateral dorsal caudate (DC) seed, a region where individual differences in functioning have been associated with learning performance in the TLT (Simon, Stollstorff, et al. 2011), voxel-wise whole brain analyses were conducted in SPM8 to generate a connectivity map of the DC for each subject. These maps were then entered into a regression model in which learning scores from the final session were entered as the sole predictor.
Higher learning scores were associated with stronger resting state connectivity between the DC and right hippocampus, bilateral parahippocampal gyri, and left inferior frontal gyrus. This pattern of results suggests that the DC, hippocampal and specific frontal regions form a functional network at rest that supports implicit associative learning during the TLT.

Disclosures: C. Stillman: None. **E.M. Gordon:** None. **H.H. Feldman:** None. **J.R. Simon:** None. **C.J. Vaidya:** None. **J.H. Howard:** None. **D.V. Howard:** None.

Poster

904. Human Learning: Motor and Sequence Learning

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Program#/Poster#:904.09/YY11

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant RO1AG036863

Title: The N400 reflects response predictability in an implicit sequence learning task

Authors: *S. A. KISER[1], R. FULLER[2], A. B. FORMAN-ALBERTI[2], K. M. O'NEIL[2], D. V. HOWARD[3], J. H. HOWARD, Jr.[2,3]
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Abstract: The N400 component of the ERP has been demonstrated in semantic priming (Kutas & Federmeier, 2011), masked priming (Misra & Holcomb, 2003) and recognition familiarity tasks (Rugg & Curran, 2007). However, there is debate about whether the N400 reflects explicit or implicit processes. In the present study we demonstrate a link between the N400 and response predictability in implicit sequence learning using the triplets learning task (TLT; Howard et al., 2008).
Fifteen college-age students completed three, 500-trial sessions of the TLT. On each trial in the TLT three consecutive “lights” appear on a computer screen. Participants observe the first two “cue” lights and respond to the last, “target” light by pressing a corresponding key. Unbeknownst to them, the first light predicts the target location on 80% of the trials (high frequency triplets) and another location on 20% of the trials (low frequency triplets). Event-related potentials were recorded and time-locked to the target stimulus. After the TLT, participants responded to a post-experimental interview and performed a recognition task to assess explicit knowledge.
A repeated-measures ANOVA conducted on reaction times revealed main effects of session and triplet type (high vs. low frequency triplets), as well as a significant interaction between session and triplet-type indicating that both overall skill learning and sequence-specific learning occurred. Results from the rating task and post experimental interview confirmed that learning was implicit.
ERP data were collected using the 64 channel Bio-Semi ActiveView System and analyzed offline using EMSE 5.2. Peak detection analysis was performed on the N400 component (300-500ms after stimulus onset) at electrode FCz using the 10-20 system. Analyses of mean amplitude revealed a significantly smaller N400 for the high frequency compared to low frequency triplets. Thus, the N400 magnitude corresponds to the predictive relationship learned

implicitly.
The present study contributes to the on-going debate as to the nature of the N400 component. Previous studies using semantic and masked priming as well as familiarity recognition tasks have shown that late occurring negativities are modulated by standard and deviant stimuli. The present study revealed a similar pattern for high and low frequency triplets in the TLT. These findings are the first to demonstrate N400 in a purely implicit learning task. This suggests that a common form of implicit priming might underlie N400 effects. Future research should use neuroimaging techniques to determine the generator of the N400.

Disclosures: S.A. Kiser: None. **R. Fuller:** None. **A.B. Forman-Alberti:** None. **K.M. O'Neil:** None. **D.V. Howard:** None. **J.H. Howard:** None.

Poster

904. Human Learning: Motor and Sequence Learning

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:904.10/YY12

Topic: F.01. Human Cognition and Behavior

Title: Cortical networks underlying vocal and instrumental musical training in Adults

Authors: *N. C. SINGH, M. SHARDA
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Abstract: INTRODUCTION: Long-term musical training involves development of skill-based learning and procedural memory and has a dynamic influence on the structure and function of the brain. It involves not only fine-tuning of sensorimotor processes but also an extensive refinement of the vocal apparatus in case of singers. The coincident presence of vocal and instrumental learning in the same individual gives us a unique opportunity to study the effect of this ‘dual-mode’ training on the brain.
METHODS: 29 participants performed a passive listening in an MRI scanner. The stimuli were presented in a sparse-sampled event related design with TR=10s and consisted of 3 conditions - spoken words, sung words and piano tones. Stimuli were bisyllabic verbs and nouns. 90 volumes were collected per participant along with a high-resolution T1 image. Processing was done using SPM5 and VBM8. The participants were adults with mean age 29.2 years and belonged to 3 groups - UNT(10): musically untrained, AMA(10): amateurs with childhood musical training and PRO(9):Professionals with atleast 10 years of training.
RESULTS: We used a 3 X 3 flexible factorial design to identify average and main effects of Condition (spoken word, sung word or piano tone) and Group (UNT, AMA, PRO). Average

effect of condition was seen extensively in bilateral temporal networks for both spoken and sung word perception. The activity was restricted to the right auditory cortex for perception of tones. The main effect of condition was localized to the bilateral temporal regions and right precentral gyrus. The average effect of group also differed across the three, with both the UNT and PRO recruiting bilateral temporal regions but the AMA only a small cluster in right STG. The main effect of group revealed peak activations in bilateral caudate. A subsequent structural analysis using voxel-based morphometry also revealed increased gray matter volume bilaterally in the caudate nucleus. A correlation between the parameter estimate values in the functional activation cluster from the main effect of group and the gray matter volume in the regions from the structural analysis revealed a significant relationship between the two.
CONCLUSIONS: Our main findings highlight the role of the caudate nucleus in extensive vocal and instrumental training in musicians. The caudate nucleus is a part of the basal ganglia and plays a role in many cognitive processes. However, this is the first report of a major role of the caudate in skill-based vocal and instrument learning in terms of both increased functional activation as well as increased gray matter volume. Future research will focus on understanding the role of the caudate in these processes.

Disclosures: N.C. Singh: None. **M. Sharda:** None.

Poster

904. Human Learning: Motor and Sequence Learning

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Program#/Poster#:904.11/YY13

Topic: F.01. Human Cognition and Behavior

Title: Muscle relaxation of the foot reduces the corticospinal excitability of the hand muscles

Authors: *K. KOUKI, ESQ[1], M. NOBUAKI[2], M. TETSURO[3], K. KAZUYUKI[4]
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Abstract: [Introduction]
Muscle relaxation is an important factor to make good performances in sports, but the mechanism has not been well understood. It has been suggested that muscle relaxation is accomplished by an active process in the brain, and not the mere end of contraction. Transcranial magnetic stimulation (TMS) study suggested that corticospinal excitability was reduced just prior to the voluntary muscle relaxation [1]. We have observed that EMG level of contraction in

one hand decreased during the relaxation of the ipsilateral foot. The purpose of the present study was to clarify the effects of relaxation of foot muscles on the corticospinal excitability of hand.
[Methods]
Nine subjects performed relaxation of the right foot dorsiflexor immediately in response to an audio cue. The completion of relaxation (offset-time) was defined by the offset of the EMG. TMS stimulation was delivered to the hand area of the left mortor cortex at -100, -50, 0, 50, and 100 ms (TMS stimuli-time) from the offset-time predetermined from 10 pre-trials without stimulation. Motor evoked potentials (MEPs) were recorded from the wrist extensor (ECR) and flexor muscles (FCR). Actual TMS timing was determined from the period between the offset-time of each trial to TMS stimuli-time. Corticospinal excitability was assessed by peak-to-peak amplitude of MEPs.
[Results]
The MEP amplitudes of ECR and FCR were higher in the periods of "~ -101 ms" than that in the resting condition. The MEP amplitude of ECR was smaller in the period of "100 ms~" than that in the resting condition. The MEP amplitude of FCR was smaller in the periods of "50~99 ms" and "100 ms~" than that in the resting condition.
[Discussion]
Corticospinal excitabilities of both ECR and FCR increased before ankle relaxation compared to resting condition. After ankle relaxation, on the other hand, corticospinal excitability decreased compared to resting condition. It is suggested that inhibitory commands were conveyed not only to the relaxing muscle (TA) but also to other resting muscles (ECR and FCR) during muscle relaxation. In conclusion, relaxation of foot dorsiflexor decreased in the excitabilities of hand muscle, both flexor and extensor.
[References]
1. Begum T et al., Neurosci Res. 53:428-435. 2005

Disclosures: K. Kouki: None. **M. Nobuaki:** None. **M. Tetsuro:** None. **K. Kazuyuki:** None.

Poster

904. Human Learning: Motor and Sequence Learning

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Program#/Poster#:904.12/YY14

Topic: F.01. Human Cognition and Behavior

Support: San Diego State University Grants Program

Title: Temporal order memory deficits in Huntington's disease

Authors: *D. R. NICOLL[1], A. E. COLLAZO[1], E. PIROGOVOSKY[2], J. GOLDSTEIN[3], A. EMAMI[1], J. COREY-BLOOM[3], P. E. GILBERT[2]
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Abstract: The frontal lobes play a critical role in temporal order memory for sequences of stimuli. Since Huntington's disease (HD) results in frontal-striatal circuit dysfunction, temporal order memory may be particularly sensitive to HD neuropathological degeneration. The current study examined temporal order memory in patients diagnosed with manifest HD and matched controls. Participants completed a visuospatial temporal order memory task involving manipulations of the temporal separation between two spatial locations in a random sequence of locations that varied on each trial. Studies suggest that there is more interference for temporally proximal stimuli relative to temporally distal stimuli. We found that the performance of HD patients and controls improved as a function of increased temporal separation. However, HD patients demonstrated significant impairments relative to controls, suggesting that temporal order memory is impaired in HD patients even when temporal interference is minimal. The findings identify a fundamental, yet relatively unexamined, processing deficit that may effect the execution of various daily living skills in individuals with HD.

Disclosures: D.R. Nicoll: Research Grant; San Diego State University Grants Program. **A.E. Collazo:** Research Grant; San Diego State University Grants Program. **E. Pirogovosky:** Research Grant; San Diego State University Grants Program. **J. Goldstein:** None. **A. Emami:** Research Grant; San Diego State University Grants Program. **J. Corey-Bloom:** None. **P.E. Gilbert:** Research Grant; San Diego State University Grants Program.

Poster

904. Human Learning: Motor and Sequence Learning

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:904.13/YY15

Topic: F.01. Human Cognition and Behavior

Title: Psychophysiological investigation of attentional processes during motor skill learning

Authors: *J. C. RIETSCHEL[1,2], R. N. GOODMAN[1], C. G. MCDONALD[4], M. W. MILLER[2], L. JONES-LUSH[3], G. F. WITTENBERG[3,1], B. D. HATFIELD[2]
[1]VA, Baltimore, MD; [2]Univ. of Maryland, College Park, MD; [3]Univ. of Maryland, Baltimore, MD; [4]George Mason Univ., Fairfax, VA

Abstract: As one becomes proficient at a motor task the attentional demand required to perform that task is reduced. Behavioral evidence suggests that experienced individuals possess greater attentional reserve during task execution compared to novices, such that, they are better able to cope with additional, possibly unexpected, challenges. This advantage may be the result of streamlining the neural processes underlying motor planning and execution over the course of learning. Such psychomotor efficiency reduces the demand on cortical resources imposed by the primary task such that they are available for coping with challenge beyond that of the task. The aim of this study was to provide neurobiological evidence of the positive relationship between motor skill and attentional reserve.

Twenty-one participants were randomly assigned to one of two groups, a group that learned a novel visuomotor distortion task, learning group (LG), and a control group (CG) that performed the same task with no distortion (i.e., no learning). For the duration of the task, event-related brain potentials (ERPs) elicited by a set of novel stimuli were recorded. The dynamic modulation of ERP component amplitude was used as an index of attentional reserve. We predicted that component amplitudes would initially be diminished in the LG relative to the CG, but that there would be a progressive increase in amplitude as a function of learning; by contrast, we predicted that ERP component amplitudes would remain relatively stable in the CG.

Importantly, task performance, as measured by initial directional error, was initially worse in the LG relative to CG and significantly improved over the course of exposure, whereas the CG's performance was stable. This suggests the visuomotor distortion task employed was successful in serving as a model of motor skill acquisition. Analyses of the ERPs elicited by the auditory probes revealed that the exogenous components, N1 and P2, were not different between the two groups and did not change over the course of learning suggesting that early sensory processing was comparable between the two groups. Notably, the novelty P3 component_an index of the involuntary orienting of attention_was initially attenuated in the LG relative to the CG, but progressively increased in amplitude as a function of learning in the LG only. This suggests that attentional reserve increased as a function of motor skill acquisition, such that greater attentional resources were available to process the auditory probes.

The current study provides psychophysiological evidence that attentional reserve increases as a function of motor skill acquisition.

Disclosures: J.C. Rietschel: None. **R.N. Goodman:** None. **C.G. McDonald:** None. **M.W. Miller:** None. **L. Jones-Lush:** None. **G.F. Wittenberg:** None. **B.D. Hatfield:** None.

Poster

904. Human Learning: Motor and Sequence Learning

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:904.14/YY16

Topic: F.01. Human Cognition and Behavior

Support: Intnal Group of Neuro (independent group)

Title: Rehabilitation Engineering for limb impairment using robots: Notes about rhythmic reinforcement learning for locomotion

Authors: *J. F. GOMEZ[1], A. TORRES[2], A. L. GOMEZ_MOLINA[3], A. RESTREPO[4]
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Abstract: INTRODUCTION. 1. Rhythms are essential in the formation of connectivity during development (Yuste 1992). 2. Bilateral movements permit inter-hemispheric facilitation (Parlow and Dewey, 1991). 3. Movement plays an important role in recovery. Movement and function are coupled in a positive feedback. Using the ideas of machine learning, we might consider activity not just an "action" but also a "goal". We then hypothesize that:
A. An important early drive to repair dysfunctional networks of lower and also upper limbs is by stimulating the production of locomotion-related rhythms. Since the circuitry of the limbs is similar, patterns of locomotion and turning might facilitate inter-limb reinforcement and transfer of information.
B. Robot-activated movements, if they are synchronized with cortical drive (using EEG/EMG, see Cheron G et.al 2012), generate proprioceptive and somatosentory signals related to locomotion, therefore helping in the recovery process. Increasing the movement of the dysfunctional hand in locomotion can restore the reconnection of their networks and the associated manipulation skills.
METHODS. Using theoretical tools (control and reinforcement learning algorithms) we present a sketch of a robotic system for rehabilitation assuming that A and B are correct.
RESULTS. (1) Portable robots that block/unblock movements in synchrony with locomotion can improve constraint-induced-movement therapies. (2) A robotic or biomechanical system that only allows repetitive movements and synergies between affected and intact sides creates a reinforcement learning scenario with an optimal police and an optimal-state-value function. Fig.1.
CONCLUSIONS. (1) Spontaneous bilateral rhythmic patterns based on inter-hemispheric facilitation can be stimulated using reinforcement learning. (2) Reinforcement facilitate persistent movement of affected side post-training (spontaneous arm use) if dose of therapy is above a threshold (see Han CE 2008). (3) Bilateral errors might increase transfer of training and interhemispheric reinforcement of activity.

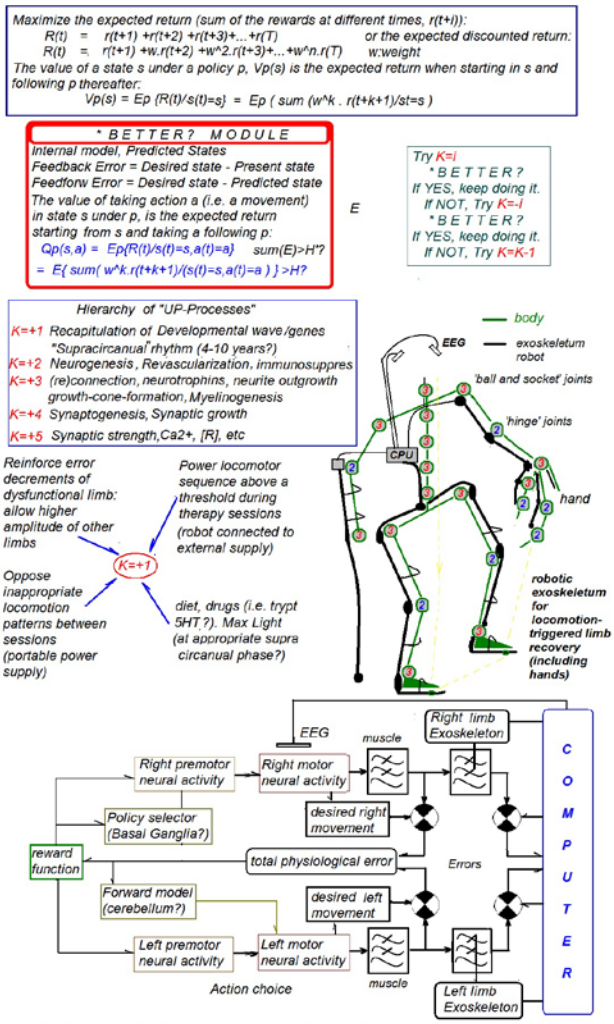


Fig 1. Informal notes to discuss about a design of a robot-therapy system for limb dysfunction based on reinforcement learning.

Disclosures: J.F. Gomez: None. **A. Torres:** None. **A.L. Gomez_Molina:** None. **A. Restrepo:** None.

Poster

904. Human Learning: Motor and Sequence Learning

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Program#/Poster#:904.15/YY17

Topic: F.01. Human Cognition and Behavior

Support: Foundation for Physical Therapy

Title: The contribution of exploration to learning in young infants

Authors: *B. A. SARGENT, S. S. KIM, N. SCHWEIGHOFER, L. FETTERS
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Abstract: A critical issue in the study of infant development is to identify the processes by which task-specific action emerges. It has been previously
suggested that spontaneous movements allow infants to learn task-specific action via reward-based learning. In theoretical models of reward-based learning, maximizing future rewards requires goal-directed exploration: exploration of task-unspecific actions should rapidly decrease and exploration of task-specific action should be modulated as a function of the change in rate of return. To test whether young infants exhibit such a behavior, we designed a two-day experiment in which 3 month old infants were placed supine under a musical mobile. For the 6 minutes immediately following a 2 minute baseline, kicking over a threshold activated music and movement from the mobile. The threshold was calibrated for each infant from baseline data along the dimension perpendicular to the support surface (z). All infants learned to activate the mobile with increasing frequency over the two days. There was a significant increase in the variance in the z dimension (task-specific) accompanied by a decrease in the variance in the x dimension (non-task-specific, parallel to the support surface). To further dissociate the changes in variance in the z direction due to learning to changes in variance due to exploration, we modeled the leg as a rod with 2 degrees of freedom at the hip, and developed and compared a number of reward-based learning models (such as policy-gradient models and value-based models). The difference between the best fitting model output and movement trajectory data from each infant at each time step was then taken as a combination of exploration and motor noise-related variance. Our findings provide the first quantification of the contribution of exploration to the early learning of task-specific leg action of 3 month old infants.

Disclosures: B.A. Sargent: None. **S.S. Kim:** None. **N. Schweighofer:** None. **L. Fetters:** None.

Poster

904. Human Learning: Motor and Sequence Learning

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:904.16/YY18

Topic: F.01. Human Cognition and Behavior

Title: Emotion is dissociable from error during motor learning

Authors: S. FESTINI, S. PRESTON, P. REUTER-LORENZ, *R. D. SEIDLER
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Abstract: Two prominent theories of motor learning include error-based learning and use-dependent learning. With error-based learning, the magnitude of the error that an individual produces while performing a movement tends to influence similar subsequent movements because the individual tries to correct the error on future attempts. Use-dependent learning posits that the current movement is influenced by the most recently executed movement, and it is shown to be distinct from error-based learning. Neither theory takes into account the affective state of the learner, however, even though emotion is known to influence cognitive and motor performance. For instance, positive affect can reduce the size of the error-related negativity (ERN), an electrophysiological measure of error-processing. Thus, in addition to error-based learning and use-dependent learning, the learner's affective state may also influence subsequent motor behavior. The current study aimed to determine if emotion is dissociable from error during motor learning, and whether emotion uniquely contributes to motor learning.

Using prism adaptation, we assessed the influence of emotion on motor learning in a trial-by-trial fashion. Participants performed a dart-throwing task, in which they earned points for accurate throws. The target location was assigned the highest number of points, and two nearby, but non-adjacent, locations were assigned smaller point values. Individuals were instructed to try to maximize their score. Error and affect were measured on each trial. Specifically, participants rated how ecstatic, disappointed, and frustrated they felt after each throw. After performing a set of baseline trials, participants donned prism lenses that shifted their visual field 30 degrees to the left. Participants had to learn to adjust their dart-throwing to the visual perturbation and had to readjust after the prism lenses were removed.

Results suggest that the subjective emotional response to motor performance is dissociable from the size of the error made. Affective ratings were not strongly correlated with error magnitude. Instead, emotional ratings were more closely associated with the points earned on a given trial. Error-based learning was also evident, as the size of the error on a given trial was correlated with the change in error on the next trial. The influence of the learner's affective state on prism adaptation and aftereffects will also be discussed.

Disclosures: S. Festini: None. **S. Preston:** None. **P. Reuter-Lorenz:** None. **R.D. Seidler:** None.

Poster

904. Human Learning: Motor and Sequence Learning

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Program#/Poster#:904.17/YY19

Topic: F.01. Human Cognition and Behavior

Title: Mirror neurons and P3 while observing and executing a visual oddball task.

Authors: L. E. PEÑA[1], *O. A. DIEZ-MARTINEZ[2]
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Abstract: Mirror neurons (MNs) transform specific sensory information into motor formats (Fabbri-Destro & Rizzolatti, 2008). They discharge both when an individual executes a specific motor act and when he observes a similar act being executed by others (Fabbri-Destro & Rizzolatti, 2008; Gallese & Goldman, 1998; Oberman et al., 2007; Rizzolatti, 2005). MNs allow recognition of actions performed by others (Buccino et al., 2001) and comprehension of action and intention, imitation, speech and appreciation of emotions (Calvo-Merino et al., 2005; Fabbri-Destro & Rizzolatti, 2008). We studied the influence of MNs upon event related potentials (ERPs). Our aim was to determine if MNs contribute to generate the P3 component in individuals that observe others performing a visual oddball task (VOT). We analyzed if the ERPs during observation were similar to those generated while executing the task. The sample consisted of 17 students (5 women, 12 men). We recorded their ERPs in three different conditions (observation: participants observed another individual executing a VOT; execution: the subject performed the VOT; and control: participants observed animation videos). We analyzed the data using a dependent samples t-test and an ANOVA for repeated measures. We studied P3 potentials obtained from the posterior middle frontal (PMF), middle central (MC) and middle parietal (MP) regions. Spectral analysis of the μ rhythm was also done. Subjects were able to understand the VOT and execute it correctly without receiving instructions. We recorded P3s in PMF and MC regions in subjects observing others executing the task. P3s were not found in MP region. We infer that, when the subject was an observer, he was trying to figure out what the performer was doing in order to imitate him. Thus, he was more focused on correctly representing motor acts of actions to be imitated, than in maintenance of working memory. No P3s were observed during the control condition ($F_{2,15}$=70.332, $p<0.05$). Except for its increased latency, the P3 recorded during observation in PMF and MC regions was not significantly different from that obtained during execution. Furthermore, suppression of μ rhythm during task observation and execution suggests that our findings are a result of the activation of MNs.

Disclosures: L.E. Peña: None. **O.A. Diez-Martinez:** None.

Poster

904. Human Learning: Motor and Sequence Learning

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Program#/Poster#:904.19/ZZ1

Topic: F.01. Human Cognition and Behavior

Support: Phd Fellowship (Research Foundation Flanders)

Title: Sleep restores the potential to undergo learning induced increases of corticomotor excitability: New evidence in line with the synaptic downscaling hypothesis

Authors: *T. T. DE BEUKELAAR[1], J. VAN SOOM[2], N. WENDEROTH[3,1]
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Abstract: "Synaptic downscaling" is hypothesized to be an important function of sleep such that previously potentiated synapses are reset. This is necessary for the acquisition of new memories during subsequent wakefulness. Currently, however, there is little experimental evidence available supporting this hypothesis. Here we used Transcranial Magnetic Stimulation (TMS) to quantify learning induced increases of corticomotor excitability, i.e. a marker of neuroplasticity. We asked whether (i) neuroplasticity would be large when acquiring a new motor task in the morning and reduced when doing so in the evening, and whether (ii) the potential to undergo learning related changes would be restored again after one night of sleep.
19 right-handed subjects were randomly assigned to one of two groups: the *WAKE-group* acquired a 5-element sequence at 8 a.m. and a different 5-element sequence at 8 p.m. The *SLEEP*-group followed the identical protocol but acquired the first sequence at 8 p.m. and the second sequence at 8 a.m. after one night of sleep. The sequences had to be performed as quickly and accurately as possible. Neuroplasticity was quantified by measuring Input-Output (I/O) curves before and after the learning task. Therefore, TMS was applied over the hotspot of First Dorsal Interosseus (FDI) with intensities ranging from 90% to 190% of Rest Motor Threshold (RMT) in steps of 25%. In the *WAKE-group*, repeated measures ANOVA revealed a significantly larger increase from pre- to post-learning in the morning than in the evening. Moreover, pre-learning excitability was higher in the evening than in the morning ($p<.05$). These findings are in line with the hypothesis that synapses become potentiated during the day; such that the potential for synaptic plasticity becomes smaller the longer subjects are awake.
In the *SLEEP-group*, the opposite pattern of neuroplasticity was observed ($p<.05$) such that changes of corticomotor excitability from pre- to post-learning were smaller in the evening than in the morning when tested after a sufficient amount of sleep. This indicates that the potential for synaptic plasticity was restored during a night of sleep.
At the behavioral level, both groups improved significantly during training with no differences between groups or between learning sessions. In conclusion, in line with the synaptic downscaling hypothesis, our neural data provide new evidence on the interaction between sleep and neuroplasticity. Even though quantifiable at the neural level, these mechanisms do not appear to induce significant effects at the behavioral level, at least not when probed with a relatively simple task as in the present study.

Disclosures: **T.T. De Beukelaar:** Employment; KU LEUVEN. Research Grant; Phd Fellowship (Research Foundation Flanders). **J. Van Soom:** None. **N. Wenderoth:** Employment; ETH Zurich.

Poster

904. Human Learning: Motor and Sequence Learning

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:904.20/ZZ2

Topic: F.01. Human Cognition and Behavior

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Title: Learning a spatial sequence activates a fronto-parietal network and leaves local traces in the resting state EEG

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Abstract: There is strong evidence from imaging studies that activity in frontal and parietal regions plays a fundamental role in the attentional and memory processes involved in the acquisition of spatial sequences. However, little is known about the fine dynamics of neural activations and interactions underlying such complex process. Here, we used high-density EEG and determined: first, the temporal evolution of spectral changes during different stages of sequence acquisition; second, whether learning induces changes in the resting state oscillatory activity in the areas that participated in the learning process, i.e. a local trace in the resting state EEG.
High density-EEG was recorded in 21 normal subjects before, during and after the acquisition of the order of a fixed spatial target sequence in five blocks (VSEQ) and during the presentation of targets in random order (VRAN). The comparison of the average spectral power of the VSEQ blocks to VRAN blocks revealed significant differences in two main regions, a right parieto-occipital and a centro-frontal region, for the alpha and theta bands. Specifically, in the right

parieto-occipital area, VSEQ compared to VRAN showed: 1. theta power decrease before the stimulus; 2. alpha power decrease, 750 to 1000 ms after the stimulus. Over the centro-frontal regions, theta power significantly increased in VSEQ compared to VRAN from 250 to 500 ms post-stimulus. In general, such differences were strongest in the first VSEQ block and decreased in the last VSEQ block. The changes in frontal theta power during the five blocks of VSEQ were positively correlated with the speed of learning, measured from verbal reports collected at the end of each VSEQ block. After the task, resting-state eyes-open recordings showed an increase of alpha activity in the right parieto-occipital region and a decrease of theta activity in the centro-frontal region.
We conclude that the alpha and theta changes during learning, in central frontal region and right parieto-occipital regions, are related to the attentional and control processes that are higher in the early stages of sequence acquisition and decrease when the sequence has been fully acquired. On the other hand, the changes in those same regions in the resting state after the task may represent an EEG trace of learning and a hallmark of use-dependent plasticity.

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Poster

904. Human Learning: Motor and Sequence Learning

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Program#/Poster#:904.21/ZZ3

Topic: F.01. Human Cognition and Behavior

Title: Implicit visuomotor sequence learning in a target-directed postural control task

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Abstract: In the serial reaction time (SRT) task participants press repeatedly keys that spatially correspond with the location of sequential targets. Practice will improve anticipation of the target location, thus improving motor preparation and hence faster reaction times for the next response in the sequence. This learning can occur implicitly and is therefore, in contrast to explicit learning, thought to be preserved with aging and when performing cognitively demanding dual tasks. Nevertheless, the SRT tasks involve only simple key presses making the merits of implicit sequence learning for motor tasks that involve more complex movement dynamics in elderly

unknown. A motor version, involving postural control, of the SRT task was conducted in a Virtual Reality lab (CAREN) with both young (age 23.9 ± 4.3) and older subjects (age 67.9 ± 2.5 years). A dot representing centre of pressure displacements on a screen in front of the person had to be moved by redistributing weight while standing on a force platform, from a central position on the screen to a target in one of five locations and back. In the explicit and implicit learning phase targets were presented in a 45 times repeating five-target fixed sequence. Different sequence learning conditions were performed, an implicit condition with a concurrent spatial dual task and two explicit conditions, one with and one without a dual-task. The participants improved their performance over sequence blocks, also in the implicit condition where the included participants were all completely unaware of the presented sequence. When a random block was introduced all movement parameters returned to baseline, indicating that the performance improvement was related to the sequence and not a general practice effect. Interestingly, target location anticipation (initiation time and accuracy of initial direction) was positively influenced only when people were aware of the target sequence and weren't distracted by a dual task. When progressing towards the target the speed and accuracy of the movement increased over time in all three learning conditions. So, movement execution, which is considered a more automatic process, was improved by implicit sequence learning whereas target anticipation was only improved by explicit sequence learning. Although the older participants moved generally slower and less accurate, learning effects were similar compared to the younger participants in all sequence learning conditions. So, we could not confirm more age-related impairments in explicit than in implicit motor sequence learning on this postural control task.

Disclosures: S.R. Caljouw: None. **S. Woldhek:** None. **H.G. Van Keeken:** None. **R.M. Bongers:** None. **C.J.C. Lamoth:** None.

Poster

904. Human Learning: Motor and Sequence Learning

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Topic: D.05. Visual Sensory-motor Processing

Support: ONR MURI Award No.: N00014-10-1-0072

NIH grant #2 R01 NS036449

NSF grant #SBE-0542013

NSF ENG-1137279 (EFRI M3C)

Title: Prospective optimization by reallocation of computational resources.

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Abstract: We investigated the human ability to optimize action over multiple future steps in rapidly changing risky environments. Subjects sat in front of a touch screen on which a grid of disks of different sizes (the "stimulus") scrolled down at a variable speed. The task was to maximize the score by touching one disk at a time in a rapid sequence. The positive score from touching a disk was proportional to disk size, while missing a disk incurred a negative score. Auditory and visual feedback conveyed outcomes of every action. Subjects were allowed to touch one disk per row, moving up on the screen. Only one of two disks could be touched in row n+1, adjacent to the disk just touched in row n, such that every choice constrained the part of the stimulus that could be touched in the future. This way, the ability to take into account multiple upcoming rows would yield a better total score. To infer the number of upcoming rows (“depth of computation” D) used in action selection, we counted how often human choices overlapped with choices of ideal planners characterized by different values of D and by different rate of recomputation (R) of action sequences. We found that evidence for D was a decreasing function of distance from the present row to upcoming rows: subjects relied on the immediately upcoming rows more than the remote rows. But as the time pressure decreased (at a lower speed of the stimulus), subjects were able to increase D and thus obtain larger winnings. Notably, the increased evidence for large D was associated with decreased evidence for small D, as if subjects reallocated their computational resources across D. We measured the utility of different disk sizes by comparing evidence for D in actual stimuli with the evidence computed for stimuli where only the largest disk sizes were present, or only the two largest sizes were present, etc. This analysis showed that subjects did not exclusively rely on the larger disks. Indeed, subjects used almost all disk sizes, although large disks had a greater weight in decision making than small disks. The results demonstrate that the ability for rapid decision making is flexible in two respects. First, humans can rapidly recompute the course of action over multiple future steps as new information enters the scope of computation. Second, humans can expand their depth of computation as task difficulty decreases, but the expansion has a cost: a decreased ability to use immediate information, revealing a limit to computational resources that are selectively allocated to different aspects of future actions.

Disclosures: D. Lee: None. **J. Snider:** None. **H. Poizner:** None. **S. Gepshtein:** None.

Poster

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Topic: D.05. Visual Sensory-motor Processing

Support: ASU FSE Grand Challenges Seed grant

Title: Learning joint actions: Behavioral correlates

Authors: *D. A. GUFFREY, S. HELMS TILLERY, C. A. BUNEO
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Abstract: Joint actions involve two or more agents coordinating their behavior in space and time to perform a particular task. Despite the fact that these types of actions are very commonly performed in everyday life, surprisingly little is known about how joint actions are learned and represented in the brain. Here we addressed the following question: How are joint motor actions learned in the absence of direct visual, auditory and haptic cues provided by another agent? Two pairs of human subjects were examined during the learning of a virtual reality based joint action task. The task involved moving a cursor from the center of a visual display outward to one of four peripheral targets, and returning to center within a two second time period. Importantly, motion of the cursor depended on the arm movements of both subjects, i.e. the mean of their simultaneous (but not necessarily synchronous) individual actions. Subjects were provided with cursor feedback but biomotion cues, i.e. visual cues of the moving arms and other body parts, were withheld. Subjects were also instructed not to provide auditory cues to one another. However, auditory cues indicating trial success/failure were provided. In this task, the mapping between subject motion and cursor motion was not veridical but involved a visuomotor rotation of 30 degrees that was applied to either one or both subjects in separate blocks. Subjects also performed an initial control block and three intervening deadaptation blocks where no rotation was applied. Each block required the successful completion of 15 trials per target. Directional error on each trial was calculated as the angle between the ideal outward trajectory (vector connecting center of display to target), and the actual cursor trajectory (center to point of peak outward velocity). Learning was assessed by comparing the mean directional errors during the first and last 15 trials of each block. No differences in directional error were observed in the control block or the deadaptation blocks for either pair of subjects. In contrast, for 3/6 rotated blocks, directional errors were smaller in the last 15 trials than in the first 15 trials (t-test, $p<0.05$). The results suggest that pairs of subjects are able to adapt their coordinated movements under visuomotor rotation, even when the relationship between cursor movement and each subject's individual movements are ambiguous. Effects of rotation angle magnitude and direction, as well as temporal lag will be pursued in future experiments.

Disclosures: D.A. Guffrey: None. **S. Helms Tillery:** None. **C.A. Buneo:** None.

Poster

905. Neural Correlates of Long-Term Memory Retrieval

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Topic: F.01. Human Cognition and Behavior

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Title: Neural correlates of the retrieval of temporal order memory

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Abstract: Everyday episodes typically consist of a series of mini-events that unfold over time. Thus, accurate memory of an episode requires retrieval not only of its content and context, but also of the temporal relations between its constituent components. The hippocampus has been proposed to play a role in forming associations between temporally discontiguous events. The basal forebrain, along with the lateral prefrontal and orbitofrontal cortices, is also known to be important for the memory of temporal order between events. It remains unclear, however, how these regions contribute to neurocognitive processes involved in the retrieval of temporal order memory. To investigate this issue, we conducted an fMRI study with a temporal order judgment task, in which two test items with varying numbers of intervening trials at study were presented. On each study trial, subjects were presented with two words in succession (SOA = 3 seconds) and were asked to judge which item fits better with a picture of unfamiliar scene. They were then given a surprise memory test with fMRI. Test stimuli consisted of intact pairs (words identical to those in each study trial), rearranged pairs (words from different study trials), or new pairs. The temporal lags between words in the rearranged pairs varied from 36 to 144 study trials. Subjects were asked to determine which word in the test pair was presented more recently. We found that activities in different hippocampal regions were associated with the retrieval of temporal order information between items within the same study pair (intact) and across the different study pair (rearranged). In addition, correlation analyses revealed that the activity in the right MTL region decreased with the number of temporal lag, and that the activity in the right hippocampus and the left basal forebrain increased with the number of temporal lag. The present findings indicate that

the retrieval of temporal order memory is supported by dissociable neurocognitive processes depending on the difference in temporal lag between events.

Disclosures: M. Suzuki: None. **J. Okuda:** None. **A. Ueno:** None. **Y. Matsue:** None. **T. Fujii:** None.

Poster

905. Neural Correlates of Long-Term Memory Retrieval

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Topic: F.01. Human Cognition and Behavior

Support: Grant-in-Aid for JSPS Fellows

Title: Roles of the prefrontal, precuneus and amygdala regions in remembering words encoded in the survival situations

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Abstract: Memory is improved when effective strategies are employed during encoding. Recent psychological studies have reported that stimuli encoded with the strategy of survival judgments, in which participants judge whether the stimuli are necessary in the survival situation, are better remembered than stimuli encoded with other strategies. However, little is known about the neural mechanisms underlying the effects of survival judgments on memory. The current fMRI study investigated retrieval-related activations of memories encoded with the strategy of survival judgments. During encoding, 23 participants (10 females, mean age=20.6) were presented with Japanese words in four conditions, in each of which participants rated how the words were needed in the survival (S) or moving (M) situations, how the words were pleasant (P), or how many numbers of letters the words had (L). During retrieval, participants were presented with old and new words, and performed an old/new recognition test with confidence ratings. The fMRI analyses focused on data from the retrieval. Successful retrieval activity (SRA) for words encoded in each condition was identified by comparing activities between hits with high confidence and all misses. Behavioral data demonstrated that words encoded in S were

remembered more accurately than those in other conditions of encoding. fMRI data yielded three main findings. First, SRAs in the right middle frontal gyrus and bilateral precuneus were identified selectively in the retrieval of words encoded in S. Second, the left amygdala showed significant SRAs for words encoded in both S and P. Third, in the hippocampus, we found significant SRAs for words encoded in all conditions. These results suggest that possible network including the middle frontal gyrus, precuneus, amygdala and hippocampus could contribute to the memory enhancement by the encoding strategy of survival judgments.

Disclosures: R. Nouchi: None. **T. Tsukiura:** None. **Y. Shigemune:** None. **T. Kambara:** None. **M. Sugiura:** None. **R. Kawashima:** None.

Poster

905. Neural Correlates of Long-Term Memory Retrieval

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Topic: F.01. Human Cognition and Behavior

Support: NIH grant 1R01 NS0783961

Title: Neural populations in human posteromedial cortex display opposing responses during memory and numerical processing

Authors: *B. L. FOSTER, M. DASTJERDI, J. PARVIZI
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Abstract: Studies of the human default mode network (DMN) using functional brain imaging have revealed overlapping activation patterns within this network across various conditions of 'internal' self-referential processing. Conversely, overlapping deactivation is also observed for this network during tasks most simply classified as requiring 'externally' directed attention. Despite this progress in the functional imaging of the DMN, similar advances have not been made in the electrophysiological domain.
We addressed this limitation by obtaining interhemispheric intracranial recordings from the human posteromedial cortex (PMC), the central node of the DMN. Eight subjects, balanced for gender and hemisphere, performed a simple task that required true/false judgments of either internally directed memory statements (3 subtypes: i) self-episodic; ii) self-semantic; iii) self-judgment) or arithmetic equations. These stimuli were self-paced and randomly interleaved with five-second periods of rest fixation.
We observed late onset (>400 ms) increases in broad high-gamma (HG; 70-180 Hz) power within PMC subregions during episodic memory retrieval. These response features were not

significantly different between episodic events judged to be true or false. However, HG power was significantly reduced or absent when subjects retrieved self-referential semantic memories or responded to self-judgment statements, respectively. Conversely, a significant deactivation of HG power was observed during arithmetic calculation, the duration of which correlated with reaction time at the signal trial level. Strikingly, at each recording site, the magnitude of activation during episodic memory retrieval predicted the degree of suppression during arithmetic calculation.
These findings provide important anatomical and temporal details, at the neural population level, of PMC engagement during episodic memory retrieval and how the same populations show a significant reduction of activity for other conditions of self-referential content. Our findings are consistent with the view that those DMN regions suppressed during external attentional are indeed the same neural populations activated during more internally focused processes.

Disclosures: B.L. Foster: None. **M. Dastjerdi:** None. **J. Parvizi:** None.

Poster

905. Neural Correlates of Long-Term Memory Retrieval

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Topic: F.01. Human Cognition and Behavior

Support: NSF Grant BCS0745880

Title: The sensory cortical basis of true and false memory for motion

Authors: *J. M. KARANIAN, S. D. SLOTNICK
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Abstract: Previous evidence has suggested that true memories can activate early sensory cortical processing regions to a greater degree than false memories, while both true memories and false memories can activate later sensory cortical processing regions. However, such evidence is very limited and, to our knowledge, this pattern of sensory cortical activation has only been reported at the modality-specific (visual/non-detailed) level of processing. The present functional magnetic resonance imaging (fMRI) study was conducted to assess whether similar results would also be observed during memory for feature-specific (motion) information. During the encoding phase, moving or stationary abstract shapes were presented to the left or right of fixation. During the retrieval phase, shapes from encoding were presented at fixation and participants classified each item as previously "moving" or "stationary" within the "left" or "right" visual field. A random effect general linear model analysis was conducted, collapsed over spatial location (as it

has been shown that memory for motion in either hemifield activates MT+ bilaterally). Motion processing region MT+, within the ascending limb of the inferior temporal sulcus, was localized by contrasting moving versus stationary items at encoding (encoding-moving > encoding-stationary). True memory for motion was isolated by contrasting accurate memory for moving items versus inaccurate memory for moving items (moving-hits > moving-misses). False memory for motion was isolated by contrasting inaccurate memory for stationary items versus accurate memory for stationary items (stationary-misses > stationary-hits). Critically, both of the retrieval contrasts corresponded to the subjective experience of "moving" versus "stationary". Furthermore, item type was constant for each retrieval contrast, which avoided stimulus confounds. Similar to previous findings, true memory for motion activated MT+ but false memory for motion did not activate this region (even at a lenient statistical threshold), and both true memory for motion and false memory for motion activated more anterior temporal regions. These results bolster previous findings that have suggested true memory can activate early sensory cortical processing regions to a greater degree than false memory, while both true memory and false memory can activate later sensory cortical processing regions. Moreover, the present results extend previous work as sensory cortical activation associated with true memory reflected feature-specific (detailed) processing.

Disclosures: J.M. Karanian: None. **S.D. Slotnick:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NEXT Program (LZ001), Cabinet Office, Government of Japan

Title: Age-related differences in the prefronto-parietal and hippocampal network during correct rejections of new items

Authors: *T. TSUKIURA[1], Y. SHIGEMUNE[1], R. NOUCHI[2,3], T. KAMBARA[3,4], R. KAWASHIMA[2]
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Abstract: Older adults are more likely than young adults to show age-related declines in correctly rejecting events that never occurred (correct rejections) as well as in remembering events that were experienced (hits). Although previous functional neuroimaging studies have often demonstrated the aging effects on neural activations during the successful retrieval of events that happened in the past, little is known how the neural mechanisms related to the correct rejections are affected by healthy aging. The current fMRI study investigated age-related differences in activations during correctly rejecting new items. During encoding, young (23 females; mean age=21.8) and older adults (23 females; mean age=66.1) were presented with unfamiliar faces one by one, and rated the trustworthiness of impression for each face. No reference was made to a subsequent memory test, and therefore encoding was incidental. During retrieval, the participants were presented with old and new faces, and performed a surprising old/new recognition test with confidence ratings (high or low). The fMRI analyses in this study focused only on data during the retrieval of new faces. Activity associated with the correct rejection (CRA) was identified by comparing activities between high- and low-confidence correct rejections for new faces, and the CRAs were compared between young and older adults. Three main findings emerged from this study. First, in behavioral data, older adults than young adults showed higher false alarm rates, but the elevated false alarms in older adults were observed only in high-confidence responses. Second, CRAs in the inferior frontal gyrus (IFG), supramarginal gyrus (SMG), and hippocampus (HIP) was greater in young adults than in older adults. Third, correlations, which were computed by data of individual trials in each subgroup, between IFG, SMG and HIP were significantly higher in young adults than in older adults. These findings suggest that the age-related increase of false alarms could be caused by a lower interaction between the prefonto-parietal region and hippocampal region.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Effects of emotion on retrieval-related activations in implicit memories

Authors: *C. TERAO[1], T. TSUKIURA[2], R. NOUCHI[3,4], Y. AKIMOTO[5], R. YAMAZAKI[1], M. SUGIURA[5,6], R. KAWASHIMA[3,7]
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Abstract: Emotion is known to enhance human memory functions. Experiences which are undergone in an emotional situation are remembered more distinctly than those in neutral situations. Although the beneficial effects of emotion on remembering memories have often been identified in the explicit retrieval, little is known about memory enhancement during implicit retrieval. In addition, evidence of the neural mechanisms during implicit retrieval of memories encoded in the emotional context is still unavailable. The current fMRI study investigated the neural mechanisms underlying the implicit retrieval of memory items encoded in an emotional context. In this study, we employed the masked priming method during encoding and the implicit retrieval method during retrieval. These methods are often used to create an implicit memory situation. For the encoding procedures outside the scanner, line-drawn pictures were presented as targets on emotional or neutral backgrounds which were chosen from IAPS. The presentation time of the targets was 12 ms. Presentation was preceded and followed by 40 ms masks so that, participants could not explicitly recognize the target pictures (the masked priming method). During retrieval in the fMRI scanner, previously learned and new line-drawn pictures were encoded one by one in a fragmented form. Participants were required to judge the semantic categories (animal, tool, and other) of the pictures (the implicit retrieval). Behavioral data showed that response times for judging categories of pictures encoded on emotional backgrounds were longer than those of pictures encoded on emotionally neutral backgrounds. fMRI data yielded two main findings. First, the right dorsolateral prefrontal cortex (DLPFC) showed greater activity during judging the category judgment of pictures encoded on negative backgrounds than of those on positive or neutral backgrounds. Based on our behavioral data, which showed the suppression of pictures encoded on negative backgrounds, the activation of the DLPFC would be related to the emotional regulation. Second, activity in the left anterior parahippocampal gyrus (APHG) was greater during the category judgment of old pictures than during that of new pictures. Taken together with previous findings, activity in the DLPFC could be associated with emotional regulation and activity in the APHG could be involved in the automatic processing of oldness for memory items.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Theta oscillations orchestrate hippocampus and neocortex in remembering autobiographical memories

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Abstract: Remembering autobiographical events can be associated with detailed visual imagery. The hippocampus, precuneus and prefrontal cortex are held to jointly enable such vivid retrieval, but how these regions are orchestrated with each other remains unclear. An influential prediction from animal physiology is that neural oscillations in the theta range may be important. In this experiment, participants prospectively collected audio recordings of personal events over 2 to 7 months. These were later replayed as autobiographical memory cues while recording brain activity with magnetoencephalography (MEG). General knowledge retrieval served as a control condition. We observed increased theta power in the left hippocampus during autobiographic retrieval. This hippocampal region was selectively phase-synchronized with theta oscillations in precuneus and ventro-medial prefrontal cortex. The strength of hippocampal-precuneus synchrony predicted the amount of visual imagery during retrieval. Thus, theta phase-synchrony orchestrates the hippocampus with the neocortical components of a distributed memory network when remembering autobiographic experiences.

Disclosures: L. Fuentemilla: None. **G. Barnes:** None. **E. Düzel:** None. **B. Levine:** None.

Poster

905. Neural Correlates of Long-Term Memory Retrieval

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:905.08/ZZ13

Topic: F.01. Human Cognition and Behavior

Title: Expectation and evidence as two components of the frontoparietal recognition memory response.

Authors: *A. R. O'CONNOR
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Abstract: A frontoparietal network of regions is recruited when we detect a stimulus whose mnemonic status contradicts its anticipated status (e.g. the presence of an ‘old’ item when a ‘new’ item was expected). Computer-generated ‘hints’ (e.g. “likely old”) can be used to generate mnemonic expectations on a trial-by-trial basis and allow the effects of different hints on the expectation violation response to be characterised. In an fMRI experiment with 18 cognitively healthy participants, I show that the strength of this frontoparietal expectancy violation response is influenced by two components of the hint-stimulus pairing: (1) the salience of the hint; and (2) the degree to which the mnemonic status of the stimulus contradicts its hinted status. More specifically, varying the wording of the hint alters the salience and behavioural consequences of the same hint e.g. “likely old” (high-salience ‘old’ expectation) vs. “unlikely new” (low-salience ‘old’ expectation). The magnitude of neural response to expectation violation reflects the salience of the initial hint, with high salience hints leading to a greater frontoparietal response when they are opposed by recovered memory evidence. High confidence in memory evidence (or lack thereof) is also associated with a comparable expectation violation response in both ‘old’ and ‘new’ items. This suggests the potential for dissociable contributors to the expectation violation response driven by an interaction between the hint and the participant’s confidence in their memory judgement. This interaction does not appear to be dependent on the status of the stimulus itself - ‘old’ items do not lead to a larger frontoparietal recognition response when expectation and confidence are accounted for. These findings suggest that the ‘oldness’ of a stimulus may be less influential on activation in the neural correlates of recognition memory than was previously thought.

Disclosures: A.R. O'Connor: None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: DOE grant DE-FG02-90ER-40577

Title: Two-population model for medial temporal lobe neurons

Authors: A. MAGYAR, *J. COLLINS
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Abstract: Recordings in the human MTL (Quian Quiroga et al. Nature 435, 1102 (2005) and many subsequent papers) have found many neurons that within the limits of the measurements respond to pictures of just one particular person out of around 100. How selective are these neurons in reality? Direct measurements would require too many stimuli. We present the results of a new method, which gives a more sensitive measure of the selectivity of the responses. The method exploits the large number of sampled neurons, and can determine the response sparsity even when it is much less than one response for the number of presented stimuli.
A previous analysis (e.g., Waydo et al., J. Neurosci. 26, 10232 (2008)) assumed a single neural population with a single response sparsity. We find that such a model gives an extremely poor fit to the data. Instead we propose to use two (or more) populations; one motivation is data from the HVC area of zebra finches, where three populations are distinguished anatomically and have dramatically different firing characteristics during song production.
We analyze the data of Mormann et al. J. Neurosci. 28, 8865 (2008), which give the numbers of neurons responding to different numbers of distinct pictures. We find that two populations give an excellent fit to data from the hippocampus and entorhinal cortex; a more complicated situation occurs for the functionally different amygdala and parahippocampal cortex. The two populations are dramatically different: In the hippocampus, one population has 7% of the cells with a 2.5% sparsity. But a much larger fraction 93% respond ultra sparsely to only 0.1% of the stimuli. This results in an extreme bias in analysis of neural responsiveness.

Disclosures: A. Magyar: None. **J. Collins:** None.

Poster

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Program#/Poster#:905.10/ZZ15

Topic: F.01. Human Cognition and Behavior

Support: NS18741

AA016624

Title: Patterns of cortical theta during autobiographical and semantic memory probed with magnetoencephalography.

Authors: *E. KAESTNER[1], B. Q. ROSEN[1], S. STEINVORTH[2], S. KOVACEVIC[1], K. MARINKOVIC[1], H. POIZNER[1], E. HALGREN[1]
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Abstract: Autobiographical memory (AM) and semantic memory (SM) are both supported by widely distributed brain networks. Studies have implicated theta activity in organizing these networks during encoding and retrieval, but studies of theta in humans during autobiographical memory are lacking. This study estimated the time-course of theta activity across the cortical surface during the retrieval and reminiscence of AM and SM. Since education is taking an episodic instance of learning and turning it into semantic memory, study of the episodic/semantic intersection has implications for learning.
Task stimuli were sentences displayed one word at a time, terminating in a cue to respond with a remember/not button press. The initial words of each sentence allowed the participant to identify the question as autobiographical or semantic. After the button press, participants reminisced on the memory for 6s. To ensure that the participants did not re-encode their memories before the test, AMs were obtained by interviewing their family/friends. Semantic memories were well known objects that are not often currently displayed in the media. Whole-head MEG signals were decomposed for each trial with Morlet wavelets in the theta (4-7 Hz) range. For theta source localization, each subject's cortical surface was reconstructed from their structural MRI which was used to constrain noise-normalized minimum norm estimates.
Reaction times were consistent between AM and SM. The distribution of cortical theta observed was broadly consistent with previous findings from fMRI of AM and SM (NeuroImage, 2006). Further, AM and SM evoked significantly different patterns of theta power. During presentation of the initial words, SM evoked significantly more theta than AM in the precuneus. In contrast, in the 1000ms before the remember/don't button press, successful AMs had greater response locked theta power relative to successfully recalled SMs in the left medial temporal lobe and left orbitofrontal cortex.
fMRI studies suggest that the precuneus is involved in tasks when participants visualize what they remember. The early theta activity suggests that from information in the initial words the brain begins retrieving semantic but not autobiographical memories. Fronto-temporal activation during AM is consistent with lesion and neuroimaging studies. Overall, the current study adds to emerging data supporting a central role of theta activity in the large-scale organization of cortical activity during memory retrieval, and further suggests that theta may contribute to both AM and SM but in different cortical locations and time-periods.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: Washington University McDonnell Center for Systems Neuroscience

James S. McDonnell Foundation

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Title: Separating signals in medial prefrontal cortex during episodic simulation using a catch-trial design

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Abstract: A robust observation in cognitive neuroscience is the finding that a distributed set of brain regions tends to be more active during periods of rest than during task performance. This default mode network (DMN) was originally discovered using PET (Shulman et al., 1997) and has since been verified in task-evoked fMRI studies and in resting-state functional connectivity analyses. Despite the initial characterization through task-induced deactivation, recent work has shown many default regions can demonstrate task-related increases in BOLD activity when one's attention is focused internally. In addition, while previous work has often treated the DMN as a generally homogeneous system, studies have now shown dissociations in activity across these regions. For example, Sestieri and colleagues (2011) recently demonstrated task-related deactivations in medial prefrontal cortex (mPFC) during a memory search task, while simultaneously showing task-related increases in posterior DMN regions. Such task-induced dissociation might allow for a deeper understanding of the role of discrete regions within the DMN. The present study sought to further investigate the activity of regions in mPFC during episodic simulation tasks using fMRI. Subjects were asked to recall events from their own personal pasts, envision events that might occur in their own futures, or imagine President Barack Obama participating in various events. By incorporating a catch-trial design (Ollinger et al., 2001), we were able to separately model BOLD activity associated with orienting toward one of these tasks (e.g., preparing to remember) and activity associated with event generation per se (e.g., remembering oneself at a barbecue). We hypothesized that separating the orientation component would provide a clearer picture of mPFC activity when engaging in the simulation task. We found that orientation cues led to significant task-induced deactivation in ventral mPFC regions, whereas the episodic simulation component showed significant positive activation in these same regions. Multiple, temporally overlapping processes may be supported in mPFC; by

separating different components of trials, one can better characterize the contributions of these regions to task performance.

Disclosures: A.W. Gilmore: None. **S.M. Nelson:** None. **K. McDermott:** None.

Poster

905. Neural Correlates of Long-Term Memory Retrieval

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Program#/Poster#:905.12/ZZ17

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant 1157432

Title: The dynamics of frontoparietal and medial temporal lobe networks during self-initiated memory search

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Abstract: In studies of source memory and recognition memory, a probe is presented to the participant, which facilitates time-locked statistical analyses of the brain networks involved in memory retrieval. Such studies have implicated lateral prefrontal cortex, hippocampus, and posterior parahippocampal gyrus in the recovery of contextual details associated with a previously studied probe. However, much less is known about the brain networks engaged during self-initiated memory search tasks such as free recall, where the timing and order of recall responses are variable and determined by the participant. This unconstrained nature of free recall complicates construction of an a-priori model of the cognitive processes underlying any given period of memory search. We collected functional magnetic resonance imaging (fMRI) data while 20 healthy, right-handed subjects (12 female) performed an immediate free-recall task. Independent component analysis was used to identify spatially distinct, temporally coherent networks within the collected data. To determine which networks contribute to recall, two regressors were constructed. A transient regressor was constructed to model activity to individual recall onsets, and a sustained regressor modeled activity maintained throughout the recall period. After rejecting artifactual components based on correlation to grey matter as well as spatial entropy, multiple regression provided a means to relate each component time series to the predicted response. Four transient components were detected, including a frontoparietal network, a cerebellar network, a premotor network, and an insula/temporal lobe network. One sustained component was found, a medial temporal lobe (MTL) network. Chronometric analysis of component time series revealed a cascade of activity, relative to the onset of individual recalls,

with both the frontoparietal and cerebellar components leading MTL and premotor activity. Testing the relationship between activity in these networks and task performance, the amplitude of the MTL component correlated with overall recall performance, while activity in frontoparietal cortex during recall was negatively correlated with recall performance, across subjects. Activity in the MTL network may reflect the retrieval of contextual information that is used to guide memory search in the absence of an external cue. Increased activity in the frontoparietal network may reflect increased control processes that are engaged to compensate for unsuccessful retrieval. These findings demonstrate how ICA can be used to gain leverage on the dynamics of distributed brain networks during self-initiated memory search.

Disclosures: J. Kragel: None. **S.M. Polyn:** None.

Poster

905. Neural Correlates of Long-Term Memory Retrieval

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Topic: F.01. Human Cognition and Behavior

Support: MacArthur Foundation's Law and Neuroscience Project

Title: Strategic influences on memory expression: Effects of countermeasures and memory strength on the neural decoding of past experience

Authors: *M. R. UNCAPHER[1], T. CHOW[1], J. RISSMAN[3], J. EBERHARDT[1], A. D. WAGNER[1,2]
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Abstract: Using fMRI and multivoxel pattern analyses (MVPA), we recently demonstrated that individual memories can be detected from distributed patterns of brain activity (Rissman et al., 2010). At the same time, a rich literature indicates that strategic processes at retrieval can impact the neural responses elicited by cues, raising the possibility that people might be able to willfully manipulate their brain responses when motivated to conceal or feign recognition of a given stimulus. To examine how retrieval strategies alter memory-related neural patterns, we investigated (a) whether MVPA decoding of memory states is affected by strategic efforts to conceal memory (i.e., countermeasures), and (b) how the effects of countermeasure strategies interact with memory strength. Following the study of a large set of photographs of faces (Day 1), participants underwent two fMRI retrieval phases (Day 2). During the first retrieval phase,

participants made ‘old/new’ recognition decisions about studied and novel faces. During the second retrieval phase, participants were instructed on how to employ countermeasures that might mask their true memory states. Specifically, for a recognized face, they were instructed to immediately disengage from attending to their memory for that face, instead attending to the surface qualities of the photograph. For an unrecognized face, they were instructed to think of a person that face reminded them of and recollect past experiences with that person. From the ‘old/new’ retrieval data, MVPA classification of correctly recognized old faces vs. correctly rejected new faces was well above chance. By contrast, classifier performance dropped to chance levels when tested on the data from the countermeasures retrieval phase. Moreover, the ability to strategically regulate one’s memory patterns (as assessed by classification performance on the countermeasure data) interacted with memory performance, as evidenced by a significant positive correlation between d’ during the old/new retrieval test and classifier performance during the countermeasures phase. Collectively, these findings demonstrate that relatively simple countermeasure retrieval strategies can significantly alter distributed neural signatures of memory retrieval and/or novelty detection. Although these data highlight the possibility that individuals may be capable of effectively concealing their memory states, participants’ ability to do so was diminished when they had stronger memories for the probed stimulus materials. Thus, the ability to strategically regulate memory-related neural patterns partly depends on the strength of to-be-regulated memories.

Disclosures: M.R. Uncapher: None. **T. Chow:** None. **J. Rissman:** None. **J. Eberhardt:** None. **A.D. Wagner:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: R01 076492

NIH NRSA F31MH094109

Title: Temporal profiles of episodic reactivation: An MEG study

Authors: *S. M. TUBRIDY[1,2], A. C. HEUSSER[2], L. DAVACHI[2,3]
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Abstract: The ability to bind, store, and retrieve complex multi-modal associations is a critical feature of human memory. This ability requires mechanisms by which the brain can link information processed in distinct, non-overlapping cortical. A common element of many theories of hippocampal function is the bidirectional flow of information between distributed cortical regions and the hippocampus. Although details of the theories differ, the basic framework adopted by much of the cognitive neuroscientific community holds that during an experience, distributed patterns of cortical activity are projected into the hippocampus via the medial temporal lobe (MTL) resulting in the formation of a hippocampal representation of elements of an episode. Recurrent connections between hippocampal neurons enable the reinstatement of the hippocampal ensemble given a partial input leading in turn, via backprojections through the MTL, to reactivation of cortical regions in which the contents of the memory are processed. Although empirical work provides support for pieces of the framework, there is currently no direct systems-level evidence demonstrating a temporal profile of hippocampus/MTL memory responses preceding content-specific cortical responses. Here, we leverage the millisecond resolution of Magnetoencephalography (MEG), coupled with emerging techniques for source localization, to assess the temporal relationship between medial temporal lobe signals and reactivation of cortical regions during associative retrieval. During MEG recording participants learn and are tested on a series of word-picture and word-sound associations. During retrieval trials, participants are presented with a word and asked to report the modality of its' associate. Behavioral results indicate successful memory formation, with performance well above chance on retrieval trials. MEG data identifies expected differences during encoding of sound relative to picture associations. Differences in the signal topography are also observed during retrieval trials for which the presented stimuli (words) are matched but the retrieved association differs (sound or picture), indicative of content-specific activity during associative retrieval. The timing of these cortical signal differences are related to memory signals in the MTL to address predictions of standard models of the hippocampal memory system.

Disclosures: S.M. Tubridy: None. **A.C. Heusser:** None. **L. Davachi:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Title: Vividness and the retrieval of recent and remote autobiographical memories: An fMRI investigation.

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Abstract: It is well acknowledged that the hippocampus is critical for retrieving autobiographical memories, however there is debate concerning the nature of this hippocampal involvement. Neuroimaging studies have suggested that the hippocampus is involved in both recent and remote autobiographical memory retrieval. Other studies have established that hippocampal contributions to autobiographical recall vary as a function of the quality of retrieval, particularly with how vividly a memory is retrieved. In the present study, we explored the interaction of a memory's age and vividness. We used a prospective collection of recent and remote events via audio diary and selected from this audio recordings that served as retrieval cues in an MRI scanner. Based on in-scanner subjective ratings of re-experiencing, all recent memories were classified as vivid and remote events were classified as either vivid or non-vivid. Using Partial Least Square (PLS) to analyze our results, three key findings emerged. First, a common network of brain regions that included the hippocampus was active for memories from both recent and remote time periods. Second, distinct spatial and temporal patterns of neural activity were found for vivid and non-vivid memories. Vivid memories preferentially recruited right frontal, left insular and bilateral posterior and thalamic regions, with this activity peaking early after stimuli onset. Less vivid memories recruited the left cingulate gyrus, right middle frontal gyrus, right temporal gyrus and the thalamus, with peak activation much later in retrieval. These temporal effects were also seen in a hippocampal ROI. Finally, the hippocampus showed different patterns of connectivity for vivid and non-vivid memories. Altogether these findings suggest that quality of retrieval (i.e. vividness) influences the regions involved in autobiographical retrieval, irrespective of a memory's age. The later onset of activity for less vivid memories may reflect that more effort is needed to recover these memories.

Disclosures: S. Sheldon: None. **B. Levine:** None.

Poster

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NSF GRFP

Title: EEG effects in the cognitive control of memory using an auditory directed forgetting task.

Authors: *M. E. CANO[1,2], R. T. KNIGHT[2,1]
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Abstract: The neural basis of directed forgetting is receiving increased attention, with a particular focus on the role of prefrontal cortex in memory suppression. Most research has focused on visual forgetting. To assess whether directed forgetting extends across sensory modalities we examined whether similar electrophysiological findings, as reported in visual electroencephalography (EEG) studies of directed forgetting, would be observed in the auditory domain. To address this issue, we recorded EEG in a modified auditory version of the Think/No-Think paradigm in young adults. Participants were instructed to first learn auditorily presented word pairs. Successful initial learning was followed by a recall test to confirm sufficient learning. In the next phase of the experiment, participants were again presented with the first word of each pair, accompanied by a visual instruction cue consisting of a red or green box indicating to either silently retrieve the second word of the pair (green box) or to inhibit recollection of the second word of the pair (red box). Subsequent memory was later tested with a same-probe and an independent-probe memory test to determine the effect of instruction and active control of memory on later memory. Here, we examine task-related EEG activity during covert intentional memory retrieval. Event-related potentials (ERP) revealed two main memory manipulation effects: No-think trials elicited an early frontal amplitude positivity decrease (200 to 300 ms) and a sustained late parietal amplitude increase (600 to 1000 ms) compared to Think trials. This ERP pattern is similar to that reported in visual studies and supports a modality non-specific mechanism for directed forgetting. The EEG data also provides evidence supporting differing roles and time courses of prefrontal and parietal cortices in the flexible control of auditory memory.

Disclosures: M.E. Cano: None. **R.T. Knight:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Title: ERP correlates of recognition without identification across real-world visual stimuli

Authors: *S. R. STALEY[1], A. J. RYALS[2], S. J. LEONARD[2], M. J. COWEN[2], W. J. MAHER[2], T. J. HAWKINS[2], A. M. CLEARY[2]
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Abstract: Recognition without identification (RWI) is observed when one is able to recognize an object as familiar despite an inability to retrieve the identity of the object. Traditionally, RWI has been studied in the laboratory using list-learning paradigms, showing that participants can discriminate studied from unstudied items among those that fail to be identified. Work from our laboratory suggests that the RWI effect can be applied to real-world stimuli as well. Participants are able to discriminate through familiarity ratings between famous and non-famous faces, famous and non-famous scenes, and living threats vs. non-threats in color photographs filtered with a monochromatic noise filter even when they are unable to identify the stimuli. The literature points toward various potential ERP components correlating with this effect. The N170 shows face sensitivity and may involve structural coding specific to faces, and the N300 has been previously implicated in at least one type of list-learning RWI effect, displaying differences for items which participants are able to correctly identify as having been previously studied. The present study examined the electrophysiological correlates of the real-world-stimulus RWI effect with faces and scenes. In two experiments, participants judged photographs that had been filtered by a monochromatic noise filter while high-density EEG was being recorded. Experiment 1 consisted of filtered famous and non-famous faces, and Experiment 2 consisted of filtered famous and non-famous scenes. Participants rated familiarity and attempted to identify the face or scene for each filtered photograph. A final phase of each experiment consisted of presentation of the famous faces or scenes unfiltered in order to determine whether the subject would have known the famous face or scene had perception not been hindered through masking. A behavioral RWI effect was observed in both types of stimuli by which familiarity ratings were higher for filtered famous photographs that were later identified than for the filtered non-famous photographs. Electrophysiological recordings revealed higher negativity at 140-190 ms, consistent with the N170, for unidentified filtered non-famous faces than for unidentified filtered famous faces that were later identified when unfiltered. In addition, an N300 component was observed whereby filtered famous scenes that were later identified when unfiltered evoked a higher negativity at 250-350 ms than did non-famous scenes. These findings implicate early processing of familiarity even before conscious recognition of the stimuli.

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Poster

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Hellman Young Investigator Award

NIH CCTS KL2 RR0224149

NIBIB T32EB006350

Title: Low-frequency ECoG phase synchronization couples medial temporal lobe and neocortical activity during spatial and temporal human source memory retrieval

Authors: A. WATROUS[1], *N. TANDON[2], C. R. CONNER[3], T. A. PIETERS[3], T. ELLMORE[3], A. EKSTROM[1]
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Abstract: Episodic memory, remembering the when and where of prior experience, is primarily dependent on the medial temporal lobes (MTL), with frontal and parietal networks playing additional roles in this process. The exact contribution of these regions and their inter-regional interactions during spatiotemporal retrieval, though, remains unclear. To address this issue, patients performed a task in which they retrieved spatial and temporal details associated with an episode while we recorded intracranial EEG simultaneously at multiple lobular sites in the frontal, parietal, and medial temporal lobes. Results showed significant increases in low-frequency phase synchronization in the MTL-Frontal, MTL-Parietal, and Frontal-Parietal networks during correct versus incorrect episodic retrieval. The MTL acted a convergence hub for all aspects of episodic memory processing, with prefrontal-MTL interactions playing additional roles in spatiotemporal representation and subregions of parietal cortex (precuneus and inferior parietal cortex, respectively) playing more selective roles in aspects of spatial and temporal processing. Further, the spatial and temporal retrieval networks were dissociable based upon the frequency and timing of their network interactions. The spatial network was characterized by interactions in the delta band that occurred earlier in time compared to the temporal retrieval network, which showed interactions primarily in the theta band. These results emphasize the importance of dynamic network interactions as central to episodic memory retrieval, and demonstrate dissociated networks for the retrieval of spatial and temporal aspects of an experience.

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Poster

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Title: Memory binding in hippocampus predicts successful associative memory in children

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Abstract: Behavioural evidence suggests that the ability to use strategies in episodic memory (EM) develops from childhood through adulthood [1, 2]. In opposite to the protracted development of strategic memory functions, associative memory is used efficiently in early development [3]. Children's ability in building associations is beneficial for memory performance and reflects the involvement of binding processes that may become less proficient in adults [4, 5]. However, it is currently unknown which neural mechanisms support children's superior associative memory performance. It is well accepted that MTL structures are involved in single item and associative item recognition [6, 7, 8] but their roles during development are less clear. Piecing these notions together we tested 16 healthy adults (19-26 years) and 5 children (7-12 years) using a subsequent associative memory paradigm. After the study phase in which participants incidentally learned pairs of objects, EM recognition was tested inside the MR-scanner. A subsequent associative memory task followed in which they indicated the correct pair item to a target item. First data on memory performance data showed that children retrieved more correct pairs than adults (children: M=.51, SD= .07; adults: M=.38, SD= .11; T_{19}= 2.53, p=.021). Preliminary fMRI data revealed a significant activation within the right hippocampus (hc; local maximum at [39, -28, -11], z= 3.36, p<.05, unc.) and left hc (local maximum at [-39, -31, -8], z=3.43, p<.05, unc.) for correctly identified associative hits in children compared to adults. Additionally the adult group showed increased activity within the left MTL (local maximum at [-33 -22 -20], z=3.77, p<.001, unc.) and left intraparietal sulcus (IPS; local maximum at [-33 -52 40], z=4.28, p<.001, unc.), whereas children showed lower activation in parietal areas. These data provide evidence that mainly the hc is related to successful associative memory in children and contributes to outperformance in associative memory compared with adults. In addition we suggest that adults rely more on the use of attention driven memory retrieval associated with activity in the posterior parietal cortex. In the future we will examine

more children to get robust effects of memory processing in children.
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Poster

905. Neural Correlates of Long-Term Memory Retrieval

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:905.20/AAA5

Topic: F.01. Human Cognition and Behavior

Support: NIMH Grant R01-MH072966

Title: Is recollection-related activity in left lateral parietal cortex sensitive to the salience of the retrieved information?

Authors: *R. ELWARD[1,2], K. L. VILBERG[1,2], M. D. RUGG[1,2]
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Abstract: Recollection of episodic information is associated with enhanced neural activity in the ventral aspect of left parietal cortex, in the vicinity of the angular gyrus. The functional significance of this finding is a subject of debate. According to one proposal, recollection effects in the angular gyrus reflect the role of this region in supporting the representation or maintenance of recollected information. Alternatively, it has been proposed that the effects reflect the region's role in mediating the 'bottom-up' re-orienting of attention away from the retrieval cue and towards the products of recollection. The present study attempted to adjudicate between these proposals by manipulating the salience of recollected information. According to the representational account of left parietal recollection effects, the salience of the recollected content should not impact the magnitude of the effects. By contrast, the attentional re-orienting account predicts that the effects should co-vary with salience. Subjects studied a series of pictures of objects, each of which was co-presented with a picture of a coin, either a

Deutschmark or a Lira. Crucially, subjects were unaware during study that the coins would be assigned different reward-values prior to the test phase. Test items comprised studied (old) and unstudied (new) objects. The requirement was to discriminate between old and new items and, for any item judged old, to judge whether it had been studied along with a Deutschmark or a Lira. Two dollars were awarded for each correct 'Deutschmark' judgment, whereas only a 2 cent reward was associated with correct Lira judgments. Incorrect judgments were penalized at the same rate as the reward, and no feedback was given during the test. Consistent with numerous prior findings, successful source judgments were associated with enhanced activity in left angular gyrus. These source recollection effects were however unaffected by the reward value of the recollected information. Importantly, effects of reward were evident in other brain regions, including those - such as the nucleus acumbens - classically associated with reward processing. The findings are difficult to reconcile with the proposal that recollection-related activity in the angular gyrus is a reflection of attentional re-orienting, at least as this process is usually conceived.

Disclosures: R. Elward: None. **K.L. Vilberg:** None. **M.D. Rugg:** None.

Poster

905. Neural Correlates of Long-Term Memory Retrieval

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Topic: F.01. Human Cognition and Behavior

Support: NIH grant R01-MH072966

Title: Dissociating the neural correlates of recollection and maintenance of recollected content using fMRI

Authors: *K. L. VILBERG, M. D. RUGG
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Abstract: Prior studies have identified a network of brain regions involved in successful episodic retrieval (recollection). In the present study, we investigated whether these regions could be distinguished according to whether recollection-related activity was transient or sustained across the time that recollected information had to be maintained prior to a behavioral judgment. In experiment 1, subjects studied a series of word-image pairs under the requirement to judge which of the paired objects was smaller. Following each of 4 study sessions, a scanned test phase occurred in which a series of studied and unstudied words was presented. The requirement at test was to decide whether each test item was old or new and, if judged old, to

retrieve the associated study picture and hold it in mind until a question cue appeared. The item-cue interval varied between two and eight seconds. The cue instructed subjects which of three different judgments should be applied to the retrieved picture (bigger than a shoebox? household object? living?). Separate responses were required when test items were either deemed new or the associated picture could not be retrieved. fMRI data were modeled to separately estimate activity elicited by the test items (transient effects) as opposed to activity sustained over the item-cue interval ('delay activity'). Regions demonstrating transient recollection effects were identified in the retrosplenial cortex and left parahippocampal/fusiform cortex extending into the hippocampus. Sustained effects were identified in left posterior parietal cortex extending into the angular gyrus, the left inferior temporal cortex, and lateral and medial prefrontal cortex, among other regions. Experiment 2 was identical to the prior experiment except that pictures instead of words were employed as test items, and the cues presented at the end of the item-cue interval queried different properties of the retrieved word (number of 'E's, number of syllables, whether the first and last letters were in alphabetic order). Similarly to experiment 1, sustained recollection effects were identified in a fronto-parietal network that included the left angular gyrus, intraparietal sulcus, and inferior temporal cortex, while transient effects were identified in the posterior cingulate/retrosplenial cortex and left medial temporal cortex. Thus, independently of its content, maintenance of recollected information engages a characteristic network of brain regions. It is hypothesized that some of these regions, including the angular gyrus, contribute to the 'episodic buffer' proposed to mediate between episodic memory and executive processes.

Disclosures: K.L. Vilberg: None. **M.D. Rugg:** None.

Poster

906. Circuity and Connectivity Subserving Language

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Topic: F.01. Human Cognition and Behavior

Support: NIH RO1 NS64033 awarded to Eishi Asano (Advisor)

Robert J. Bernucci Travel Award from the Translational Neuroscience Program

Title: Electrocorticography-constrained evaluation of the arcuate fasciculus

Authors: *E. C. BROWN, J.-W. JEONG, O. MUZIK, R. ROTHERMEL, N. MATSUZAKI, C. JUHASZ, A. K. SHAH, S. MITTAL, S. SOOD, E. ASANO
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Abstract: Over a century after the Wernicke-Lichtheim model, language connectivity has focused on a link between Wernicke's region and Broca's area via the arcuate fasciculus (AF). However, no detailed anatomical study has yielded solid evidence that AF terminates within Broca's area. The inferior lateral precentral gyrus (ILPreC) has been proposed as an alternative AF termination. Using diffusion tensor imaging (DTI) tractography, we determined the anterior termination of AF in healthy individuals. We subsequently determined whether temporal language sites defined by electrocorticography (ECoG) in epilepsy patients show white matter connectivity favoring Broca's area or ILPreC as a termination.

We studied five focal epilepsy patients who underwent ECoG prior to resective surgery and eight healthy control subjects (age 9-26). All subjects received imaging including 55-direction DTI. Patients received an auditory naming task during ECoG to determine language sites. Landmark-based conformal cortical mapping of individuals to an MNI152 atlas brain was performed. Atlas-based seed (S) and target (T) regions were defined: S1 - posterior half of superior temporal gyrus (STG), S2 - posterior half of middle and inferior temporal gyri (MTG/ITG), T1 - posterior two thirds of inferior frontal gyrus (Broca), T2 - ILPreC, and T3 - inferior lateral postcentral gyrus (ILPostC). Probabilistic tractography used atlas-defined seeds in controls. In patients, seeds were defined as superficial white matter within 8mm of a temporal lobe ECoG electrode showing significant language-related gamma activity. Percentage of total fibers tracking to each target was determined.

Across controls, tracking from STG revealed ILPreC to be a more likely termination than ILPostC but not Broca ($p = 0.030$ by Friedman's ANOVA - median of ILPreC vs. ILPostC: 0.0065% to 0%, $p = 0.028$ by Wilcoxon Signed Ranks). ILPreC was the primary termination from MTG/ITG ($p = 0.017$ by Friedman - ILPreC vs. Broca: 0.3389% to 0.0023%, $p = 0.017$; ILPreC vs. ILPostC: 0.3389% to 0.0025%, $p = 0.018$). Across 41 language-related temporal ECoG electrodes, 14 associated with tractography fibers to the targets. Across these 14 sites, the ILPreC was the primary termination ($p < 0.001$ by Friedman - ILPreC vs. Broca: 0.097% to 0.0079%, $p = 0.001$; ILPreC vs. ILPostC: 0.097% to 0%, $p = 0.001$). In no case was connectivity to Broca different from that to ILPostC.

This is the first study to constrain tractography with language-related ECoG. We found the primary termination of AF to be the inferior lateral precentral gyrus. We failed to show preferential connectivity between canonical language sites of temporal and frontal areas in our small cohort.

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Poster

906. Circuity and Connectivity Subserving Language

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Program#/Poster#:906.02/AAA8

Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 23591273 and 23591275

Title: Basal temporal "semantic" area: Direct evidence from electrical cortical stimulation and local field potential recording

Authors: *A. SHIMOTAKE[1], R. MATSUMOTO[1], T. UENO[2], T. KUNIEDA[3], S. SAITO[5], H. FUKUYAMA[4], S. MIYAMOTO[3], R. TAKAHASHI[1], M. A. LAMBON RALPH[2], A. IKEDA[1]
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Abstract: **Introduction**: The role of the ventrolateral part of the anterior temporal lobe (vATL) varies across disciplines, ranging from language function (known as basal temporal language area) to higher-order visual cognition. These mixed ideas can be accommodated by an idea of the ATL as being crucial for pan-modal semantic processing. Recent neuropsychological (semantic dementia patients), neuroimaging, rTMS studies are consistent with this idea. However, the patients' lesion is not confined to this specific area; the fMRI does not demonstrate the necessity of this area; non-invasive rTMS cannot target this specific basal area due to its buried anatomical location. We had a rare opportunity to probe the functioning of this area directly in late-onset partial epileptic patients, whose semantic performance appeared to be normal.
Methods: Subjects are three intractable partial epilepsy patients who underwent invasive presurgical evaluation with subdural electrodes (IRB #C533). High frequency electrical cortical stimulation and recording of local field potential (LFP) during semantic tasks were performed in the left language-dominant temporal lobe. In two patients, additionally, we undertook a systematic assessment of the vATL in comprehension and naming. Electrical cortical stimulation with four levels of intensity [sham, 3, 6, and 9mA] was delivered to the electrodes at the vATL whilst picture naming and written word to picture matching were tested.
Results: The robust LFP was observed maximally in the left anterior fusiform gyrus. Electrical cortical stimulation indicated that this region was involved in pan-modality, semantic processing. The core region identified with these two methods converged at and around the anterior fusiform gyrus across the patients, and was in good concordance with the semantic activation foci in previous neuroimaging studies. In two patients, additional graded electrical stimulation was delivered to the core region. The reaction time became more prolonged in the naming task than in the matching task as the stimulus intensity increased. Severe anomia with semantic/omission errors were also observed. All of these mimicked the typical behavior of semantic dementia patients.

Conclusions: The vATL is crucial for pan-modal semantic cognition both in expressive and receptive tasks and thus could be referred to as the basal temporal "semantic" area.

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Poster

906. Circuity and Connectivity Subserving Language

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Program#/Poster#:906.03/AAA9

Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 23591273

Title: Link between the dorsal and ventral language pathways in the temporal lobe: A cortico-cortical evoked potential study

Authors: *R. MATSUMOTO[1], T. KUNIEDA[2], A. SHIMOTAKE[1], K. USAMI[1], K. KOBAYASHI[1], H. IMAMURA[1], N. MIKUNI[4], S. MIYAMOTO[2], H. FUKUYAMA[3], R. TAKAHASHI[1], A. IKEDA[1]
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Abstract: Introduction: Contemporary neuroscience indicates a dual-stream model of speech processing: the anterior, ventral stream from the primary auditory cortex to the anterior temporal lobe for speech comprehension, and the posterior, dorsal stream to the posterior temporal and then to the frontal lobe for auditory-motor integration (Scott & Johnsrude, 2003; Hickok & Poeppel, 2007; Ueno et al., 2011). The link, however, between these two pathways remains elusive. Although limited to invasive presurgical evaluation, cortico-cortical evoked potentials (CCEPs) can trace the connections *in vivo* by directly stimulating a part of the cortices and by recording CCEPs from the adjacent and remote cortices with excellent time and spatial resolution (Matsumoto et al., 2004). We aimed to trace cortico-cortical connections between the dual streams in the temporal lobe, with emphasis on the connections among the anterior temporal language area (ATLA) in the ventral stream, the posterior temporal language area (PTLA) in the dorsal stream, and the basal temporal language area (BTLA) in the ventral anterior temporal

region.
Methods: 9 epilepsy patients who underwent invasive presurgical evaluation with subdural electrodes participated in this study (IRB No.443). After cortical language mapping with 50 Hz electrical stimulation, repetitive electrical pulses (1 Hz) were applied to ATLA, PTLA and BTLA, and CCEPs time locked to the stimuli were recorded from the lateral and basal parts of the language-dominant, left temporal lobe. Stimulus and CCEP response sites were anatomically identified by MRI-electrode coregistration.
Results: Stimulation of PTLA (pSTG & pMTG, 7 patients) elicited remote CCEPs in the ventral stream [aSTG (ATLA, n=2) and aMTG (n=4)] and BTLA (aITG, n=6). Stimulation of ATLA (aSTG, n=4) showed CCEPs in the dorsal stream [pMTG (PTLA, n=2; no function, n=1)], and BTLA (aITG, n=3). BTLA stimulation (aITG or anterior fusiform, n=7) elicited CCEPs in the ventral stream [aSTG (ATLA, n=3; no function, n=1), aMTG (n=5)], and the dorsal stream [pMTG & pSTG (PTLA), n=6].
Conclusion: The present electrical tract-tracing study demonstrated bidirectional connections between ATLA and PTLA that link the dual streams. Both pathways also had access to BTLA in the ventral anterior temporal region presumably for semantic processing.

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Poster

906. Circuity and Connectivity Subserving Language

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Support: NIH R01 DC007169

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NIH R21RR025786

Title: Single-trial decoding of open class and closed class words using EEG

Authors: *M. S. MELLEM, R. B. FRIEDMAN, A. V. MEDVEDEV
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Abstract: The ability to classify single words based on brain signals has potential applications for neurofeedback and brain-computer interfacing. One classification of importance is that of open class vs. closed class. Open class words (content words) contain semantic content, while closed class words (function words), provide grammatical structure to the sentence. Whether a word is an open or closed class word is particularly important as it signifies the word's role in a sentence. While univariate analysis has provided evidence that the neural correlates of these classes are dissociated (e.g., Neville et al., 1992), it has not yet been determined whether these neural signals can be used for single-trial classification of word class. In this study, we demonstrate single-trial decoding of word class (open v. closed) using multivariate analysis. Nine subjects read sentences while their EEG was recorded. We performed multivariate classification on the ERPs between 200-800ms after the appearance of a word stimulus. After standard preprocessing including artifact removal, we used a binary Support Vector Machine (SVM) classification algorithm and a 5-fold cross-validation method implemented in the Fieldtrip and Donders Machine Learning toolboxes to train the classifiers on the two word classes and to test them on single trials. At chance, classification is 50%, but after training on the SVM classifier, classification averaged 58% correct (and ranged from 55% to 67%) across subjects. When testing individual classification accuracy, we found that it was significantly greater than chance classification (binomial test, $\alpha<0.05$) for 8 of the 9 subjects. Importantly, the features pivotal for classifying the words were concentrated in left frontal channels. This left anterior location is in line with previous univariate results showing a dissociation of word class across these channels. These results not only lend further evidence to the dissociation of words by class but do so with some success on a trial-by-trial level. Additionally, they demonstrate the capability of an EEG classifier to decode building blocks of sentences which may one day lead to real-time decoding of sentences from electrical potentials of the brain.

Disclosures: M.S. Mellem: None. **R.B. Friedman:** None. **A.V. Medvedev:** None.

Poster

906. Circuity and Connectivity Subserving Language

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant DC06220

NIH 2T32MH09118-19

Title: A cross-modality fMRI investigation of sentence context effects on speech perception

Authors: *S. A. GUEDICHE[1], Y. ZHU[2], D. MINICUCCI[1], S. E. BLUMSTEIN[1]
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Abstract: Until recently, neuroanatomical models of reading and speech processing have evolved independently despite evidence for interactions between the two systems. Most theoretical models of speech perception are hierarchically organized with increasing levels of abstraction when mapping from acoustic and phonetic processes to higher levels of lexical and semantic processes. While models of reading have suggested several different routes by which orthographic information can access meaning, the details regarding interactions between perceptual and semantic processes across different modalities remain unclear.
Current neuroanatomical models of speech postulate feedforward and feedback interactions along a ventral processing stream between perceptual and semantic processes. In an fMRI study, we examine the neural areas underlying potential interactions between an orthographically presented sentence context and the perception of an acoustically ambiguous target word. To this end, we presented orthographical sentence contexts where we manipulated the Sentence Type (biased, neutral), and then investigated the effects of Sentence Type on an acoustically presented word target where we manipulated the Target Type (ambiguous, unambiguous) (e.g. coat vs. goat).
Behavioral results show a significant effect of sentence context on the categorization of the acoustically ambiguous word target. Preliminary functional neuroimaging results indicate that interactions between Sentence Context and Target Type emerge in anterior superior temporal gyrus bilaterally, and in a right mid superior temporal gyrus area that extended into the parahippocampal gyrus. A correlation analysis on these functionally defined regions revealed connectivity between the mSTG region of interest and parietal and fusiform areas, and between the aSTG region of interest and parahippocampal and occipital areas.
These findings provide support for interactions between neural networks involved in reading and speech perception by showing that the integration of higher-level semantic information presented in the visual modality can influence acoustic speech perception of a subsequent target word. Interactions between Sentence Context and Target Type have also been observed in a previous fMRI study that used acoustically presented sentence contexts in temporal cortex, although the interaction emerged in a more posterior MTG/STG area. These sets of results suggest that while access to meaning and its influence on acoustic speech perception occurs through interactions along a ventral pathway, presentation modality may affect where this interaction takes place.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: BMBF Grant 01EO0801

Title: Anodal transcranial direct current stimulation (tDCS) of primary motor cortex improves word-retrieval

Authors: *M. MEINZER, R. LINDENBERG, M. SIEG, K. AVIRAME, A. FLÖEL
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Abstract: A tight link between linguistic functions and activation of motor areas has been consistently reported in numerous studies indicating that the two systems share functional neural resources (Pulvermüller, 2005, Nat Rev Neurosci). Mutual interactions of these two systems have been demonstrated in healthy subjects (Liuzzi et al., 2011, Curr Biol) and in patients with post-stroke language impairments (aphasia; e.g., Meinzer et al., 2011, Neurorehabil Neural Repair). However, neural correlates of beneficial effects of motor-cortex pre-activation on language functions have not been studied systematically. This issue was addressed in the present study by using functional magnetic resonance imaging (fMRI) and simultaneous non-invasive brain stimulation. In particular, 18 healthy right-handed healthy young subjects performed an overt semantic (category-based) word-generation task during fMRI at 3T. Behavioral and neural effects were assessed in the scanner in a cross-over design during simultaneous excitatory ("anodal") transcranial direct current stimulation (atDCS) or a placebo stimulation condition ("sham") that were both administered over the primary hand motor cortex. Similar to a previous study that found beneficial effects of atDCS administered over a core language region (inferior frontal gyrus, Meinzer et al., 2012, J Neurosci), motor cortex stimulation improved word-retrieval (expressed as a significantly smaller number of errors during the semantic word-generation task). Preliminary analysis of fMRI data suggests that behavioral effects may be mediated by activity modulations in primary motor and language areas (prefrontal and superior/middle temporal cortices). Thus, the positive findings in this proof-of-principle study provide a rationale for exploring the effectiveness of motor cortex stimulation in patients with acquired language disorders (aphasia).

Disclosures: M. Meinzer: None. **R. Lindenberg:** None. **M. Sieg:** None. **K. Avirame:** None. **A. Flöel:** None.

Poster

906. Circuity and Connectivity Subserving Language

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Topic: F.01. Human Cognition and Behavior

Support: WT074414MA

Title: Disentangling speech, spectrum and melody - An fMRI investigation of spectral complexity, intelligibility and intonation profile in the perception of degraded sentences.

Authors: *S. K. SCOTT[1], C. MCGETTIGAN[2], J. KYONG[5], T. HOWE[3], S. ROSEN[4]
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Abstract: A influential early PET study of speech perception identified a response to intelligible speech in left anterior superior temporal sulcus (STS), while a right-hemisphere homolog was preferentially responsive to sounds conveying a clear sense of pitch (Scott et al., 2000). The present fMRI study expanded the previous design to allow a factorial investigation of the neural correlates of speech intelligibility (vocoded vs. vocoded+spectrally-rotated speech), speech melody (normal vs. flattened) and spectral complexity (2, 4 or 6 vocoded channels). Nineteen healthy adult speakers of English were familiarised with example sentences from all the conditions before entering the scanner, where they listened passively to further examples and attempted to understand as much as possible from the sentences during two functional runs of data acquisition. Replicating the main findings of Scott et al. (2000), the analyses revealed left-dominant responses to increased intelligibility extending from posterior to anterior STS, for both intelligibility resultant from increased spectral complexity (2 < 4 < 6 channels) or a comparison of vocoded vs. rotated conditions. Also similar to the previous work, a right-dominant preference for dynamic pitch profiles was found in the right superior temporal gyrus (STG; all results at voxel height threshold p < .001, cluster correction p < .05). There was no evidence for an interaction between intelligibility and intonation processing, presumably reflecting the fact that intonation is not a dominant cue to lexical identity in English (compared with, for example, tonal languages such as Mandarin). However, nor did any voxels at the chosen threshold show an interaction between speech intelligibility and spectral complexity - that is, a significantly greater effect of increasing complexity for the vocoded conditions (where increased complexity yields greater intelligibility), than for the vocoded+rotated items (where items remain unintelligible regardless of complexity). There was a trend toward this pattern, where the vocoded items showed a more extensive response to increased complexity in left STS than that shown by the rotated items, but the prevailing effect was an overall profile of greater signal for the vocoded than the rotated conditions along the whole STS. We discuss these

findings in the context of hierarchical models of auditory processing and the potential role of top-down / feedback connections in speech comprehension.

Disclosures: **S.K. Scott:** Research Grant; WT074414MA. **C. McGettigan:** Research Grant; WT074414MA. **J. Kyong:** None. **T. Howe:** None. **S. Rosen:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Title: The ability to learn new words depends on the strength of functional and anatomical connectivity of the left arcuate fasciculus

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Abstract: In human language, learning is a continuous process that require a constant upgrade of our vocabulary even into adult life. Moreover, most individuals aim to learn a new language that also requires the learning of new words. A common observation is that there are large individual differences in language learning, and the neurobiological bases of this variability are unknown. In the present study, we combined Diffusion Tensor Imaging (DTI)-Tractography and functional MRI (fMRI) to study the correlation between anatomical and functional variability of language pathways and their association with interindividual differences in language learning. Twenty-seven right-handed subjects (12 females) were recruited for this study. Language learning consisted in a speech segmentation task (i. e. to segment the speech stream in order to isolate possible words for later memorization) that subjects performed in a 3T scanner while during the fMRI acquisition. Functional connectivity analyses between frontal, temporal and parietal language regions were estimated from functional MRI time series during the learning task. DTI was also acquired and virtual dissections of the three segments of the arcuate

fasciculus performed. Number of streamlines, fractional anisotropy and perpendicular diffusivity were measured for each tract.
Our results showed that microstructural properties of the left direct connections between classical Broca's and Wernicke's areas, as measured by radial diffusivity (RD) and number streamlines (Ns), predicted performances in word learning (RD: Pearson = -0.6; $p < 0.005$) and the strength of the functional connectivity between the activated language areas (Ns: Pearson = 0.45; $p < 0.037$). In addition, the strength of functional connectivity also predicted the word learning performances ($p < 0.036$). In conclusion, this study is the first to demonstrate that our ability to learn new words rely on a efficient and fast cross talk between temporal and frontal areas in the left hemisphere that is related also to their structural connectivity.

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Poster

906. Circuity and Connectivity Subserving Language

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:906.09/AAA15

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant RO1 HD056107

Title: Brain basis of chinese character and english word reading in children

Authors: *A. J. KRAFNICK[1,2], L.-H. TAN[3], M. M. LUETJE[1], E. M. NAPOLIELLO[1], G. F. EDEN[1]
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Abstract: Functional brain imaging studies of reading in adults have shown that the left occipito-temporal cortex, termed the visual word form area (VWFA; Cohen et al., 2002; see also Price and Delvin, 2003) is engaged during reading, independent of whether reading occurs in alphabetic or logographic writing systems (Bolger et al., 2005). The early developmental process that leads to this universal use of the VWFA has not yet been investigated. Here we examine processing of words, characters and objects in monolingual English reading children attending 1st grade in the U.S. and Chinese reading children in Beijing, to establish if the VWFA is observed in both groups of beginning readers.
17 American and 16 Chinese 6 and 7 year olds (matched for reading and non-verbal IQ) underwent three types of runs: English Words, Chinese Characters and Objects and viewed

alternating blocks of these stimuli and Scrambled versions thereof as a visual control. These were interspersed with a resting Fixation baseline condition. Stimuli appeared either just to the right or left of fixation and subjects responded with a button press to indicate which side of fixation cross the stimulus appeared. Scans were obtained on identical 3Tesla Siemens Trio whole-body MRI systems in both locations. Analyses were restricted to bilateral fusiform/inferior temporal gyri (AAL Atlas).
Within-group analyses revealed that relative to Fixation, native learners of English showed bilateral activation for Objects, left hemisphere activation for Words and right hemisphere activation for Characters (p Scrambled Objects, neither group showed activation for their native writing system > Scrambled condition, suggesting specificity to the native writing system has not yet developed. A conjunction analysis between the groups for each group's native writing system ($p<0.001$ uncorrected height threshold) resulted in a significant cluster centered at -42, -56, -12, suggesting that both group were learning to read using the VWFA (relative to Fixation), consistent with the neuronal recycling hypothesis (Dehaene and Cohen, 2007). However, while percent signal change (PSC) within this cluster was similar for all stimuli in the U.S. group, PSC for Chinese Characters was significantly higher than English Words in the Chinese students, indicative of early differentiation for native versus unknown writing systems in this group.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: National Science Foundation [SBE 883 0541953 Science of Learning Center]

National Institute of Child Health and Human Development [P50 HD40095]

Title: Effects of sensory and signed language experience on the neural basis of visual motion processing

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Abstract: Previous investigations have shown that the neural substrates mediating visual motion processing are altered by sensory experience (i.e. deafness) and/or American Sign Language

(ASL) experience. Specifically, Bavelier *et al*. (2001) found higher percent signal change in posterior parietal cortex and greater extent of activation in posterior superior temporal sulcus in deaf signers compared to both hearing signers and hearing non-signers. Subsequent studies of native signers (Fine *et al*., 2005; Sadato *et al*., 2005) replicated findings in superior temporal cortex, affirming that plasticity can occur with altered sensory experience. However, participants in this research have been native users of sign language and the question arises whether the sensory-induced plasticity would be the same for deaf people who are not native users of sign, which is the case for most deaf individuals (as 90% of deaf people are born to hearing parents). During the acquisition of fMRI data, deaf and hearing subjects performed a visual motion task, indicating the direction (right/left) of motion of coherent dots via button press in a dynamic condition. In a static (control) condition, subjects indicated which side of the screen (right/left) contained a greater density of dots. Single subject maps were generated for the dynamic > static condition, then two-sample t-tests were performed to assess between-group differences ($p < 0.005$ height threshold, FWE corrected $p < 0.05$).

We found significantly greater activation in right superior temporal gyrus (STG) extending into primary auditory cortex in deaf native signers (n=14) compared to hearing native signers (n=16) in accordance with previous findings. However, in deaf adults who were raised with oral or cued English and were not native users of ASL (n=14), we did not replicate this right STG difference and instead found greater left superior parietal lobe activity when compared to a group of hearing participants (n=15).

In the light of previous work demonstrating differences attributed to ASL experience (Bavelier *et al*., 2001) we also directly contrasted our hearing participants with and without signing experience, but revealed no differences. The parallel comparison in our deaf participants (deaf native signers vs. deaf oral/cued English group) however revealed significantly greater activation in right precuneus, precentral gyrus and bilateral cingulate/medial frontal gyri in deaf native signers.

These results indicate that sensory and language-dependent plasticity will differ for deaf individuals depending on their language background and that studies in native users of ASL cannot be generalized to the entire deaf population.

Disclosures: T.M. Evans: None. **O.A. Olulade:** None. **D.S. Koo:** None. **G.F. Eden:** None.

Poster

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Program#/Poster#:906.11/AAA17

Topic: F.01. Human Cognition and Behavior

Support: NIH 2R01DC05660

Title: The role of theta oscillations in parsing speech into syllables for speech decoding

Authors: K. B. DOELLING[1], L. H. ARNAL[1], O. GHITZA[2], *D. POEPPEL[1]
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Abstract: We investigate how the human brain parses speech signals into chunks of the appropriate temporal granularity to facilitate decoding of spoken language (Ghitza, 2011; Giraud and Poeppel, 2012). One hypothesis suggests delta-theta (3-8 Hz) oscillations in auditory cortex extract low frequency temporal cues in the auditory input, aiding in the process of parsing speech into roughly syllable-size representations.
To test this hypothesis, Ghitza (2012) measured intelligibility of speech signals with manipulated modulation spectra. Three manipulations are relevant to our study. In a "Stop" condition, the envelopes of each critical band of the stimuli (7-digit strings) were filtered with a stop band from 2-9 Hz, removing spectro-temporal information in the theta band range and removing low frequency temporal cues in the speech waveform. To assess the importance of such cues, in a "Peak Position Coding" (PPC) condition, each critical band signal was replaced by short (50 ms) band-limited noise bursts of uniform shape and amplitude which were placed at the peaks in each critical band's envelope, creating an artificial input rhythm derived from these temporal cues. In the "Stop&PPC" condition, the Stop and corresponding PPC stimuli were linearly summed. Thus, we deprive the theta oscillation of an input rhythm to track (Stop), artificially create one (PPC) and then sum these together (Stop&PPC) to aid theta oscillations in tracking the envelope of this extremely degraded stimulus. Our participants listened to these altered speech sounds and rated their intelligibility while undergoing MEG recording.
Consistent with Ghitza's (2012) findings, intelligibility of the Stop&PPC condition was significantly higher than both the Stop and PPC cases. Intertrial phase coherence analyses of the neural recordings (Luo & Poeppel, 2007) suggest that the ability of the auditory theta oscillation to track low-frequency transients in speech is impeded in the Stop condition, but is regained in the Stop&PPC condition. Remarkably, this ability correlates with intelligibility ratings for individual stimuli.
The noise bursts of the PPC condition, rather than masking the stimulus, reintroduce a relevant input rhythm allowing theta oscillations to correctly parse the degraded speech. When presented with the PPC condition alone, theta oscillations show strong coherence despite low intelligibility ratings. This suggests that the auditory cortex employs theta oscillations to track the speech stimulus, using low frequency rhythmic transients to parse the speech envelope into chunks of the temporal granularity optimized to license decoding.

Disclosures: K.B. Doelling: None. **L.H. Arnal:** None. **O. Ghitza:** None. **D. Poeppel:** None.

Poster

906. Circuity and Connectivity Subserving Language

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Topic: F.01. Human Cognition and Behavior

Support: VA CSR&D Merit Review Award

Title: Language, lesion-behavior correlations in aphasia and resting functional connectivity networks

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Abstract: The resting-state fMRI (RS-fMRI) technique provides detailed maps of functionally connected networks of brain regions. However, the interpretation of these findings in terms of domains of cognition is not always straightforward. Further evidence from other techniques, such as task-based fMRI, metaanalysis of neuroimaging literature, and lesion studies can provide important information for the validation and interpretation of RS-fMRI-defined networks. Here, we assessed the behavioral impairments resulting from neurological damage to resting functional connectivity networks and language-related brain regions with distinct connectivity profiles. Our goals were two-fold: to improve the understanding of the lesion anatomy of aphasia; and to identify the functionally connected networks associated with different language abilities. In order to address these goals, we used brain lesion and neuropsychological data from a large group of aphasic patients, as well as RS-fMRI data from healthy individuals. Neurobehavioral and structural neuroimaging (MRI and CT) data were acquired from 154 left-hemisphere chronic stroke patients (right-handed native speakers of English, no prior neurological or psychiatric history, single event, majority involving the MCA, at least one year post-injury). The Western Aphasia Battery was administered for a comprehensive assessment of speech and language abilities. Digitally reconstructed brain lesions were transformed to MNI space for subject and group-level analyses. The RS-fMRI datasets from 25 healthy subjects were used to delineate the functionally connected networks involving the lateral surface of the left hemisphere, to derive a connectivity-based parcellation of the left lateral cortex, and to compute graph theoretical connectivity metrics of key frontal, temporal and parietal regions implicated in language. The lesion loads for each node of each rs-fMRI-based network, as well as the functional connectivity metrics characterizing each lesioned area, were correlated with behavioral deficits in different language domains and with aphasia syndrome types. Consistent with the notion that language is subserved by a broadly distributed set of interacting brain regions that extends outside the areas traditionally associated with language, aphasic syndromes and domain-specific deficits were associated with lesion patterns that are best characterized at the network-level, with the severest

deficits involving damage to multiple networks and the nodes playing central nodes in their respective networks.

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Poster

906. Circuity and Connectivity Subserving Language

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Program#/Poster#:906.13/AAA19

Topic: F.01. Human Cognition and Behavior

Support: the Office of Naval Research

Title: The distinct performance of human brain for voice identification

Authors: *Y. S. LEE[1,2], M. HANKE[3], D. KRAEMER[2,4], S. LLOYD[3], R. GRANGER[3]
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[3]Psychological and Brain Sci., [4]Educ., Dartmouth Col., Hanover, NH

Abstract: How good is the human brain at distinguishing voices from other sounds? We performed machine-learning analyses of the categorization performance of twelve subjects who listened to several sound categories including human voices, animals, and man-made objects. Each sound was presented both intact, and in a spectrally rotated version in which low-level attributes were matched though the sounds overall were unrecognizable in behavioral tests.
A searchlight analysis (Kriegeskorte et al., 2006) tested for every voxel's "precision index," i.e., (true positive) / (true positive + false positive) for the category of the sound. With this stringent test, no voxels were found that were able to distinguish between the intact and rotated versions of any of the sounds except those of human voices. (Less inflexible tests did identify areas distinguishing between intact and spectrally rotated sounds at several brain loci.)
Locations in multiple brain regions were found that significantly distinguished intact from rotated human voice sounds, suggesting that (i) the ability to distinguish human voices outperforms any other auditory distinction in human brains; (ii) the distinctions were being made independent of low-level acoustic components, since these were identical in the intact and rotated sounds; (iii) many areas of the brain may participate in making these distinctions, and (iv) these findings may suggest reconsideration of the hypothesis that only very-restricted brain loci process human voices.

Disclosures: Y.S. Lee: None. **M. Hanke:** None. **D. Kraemer:** None. **S. Lloyd:** None. **R. Granger:** None.

Poster

906. Circuity and Connectivity Subserving Language

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Topic: F.01. Human Cognition and Behavior

Title: Tone 3 sandhi and tone sequencing in Mandarin Chinese: An fMRI study

Authors: *H.-C. CHANG, O. TZENG, W.-J. KUO
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Abstract: This study seeks to identify the neural correlates of Chinese tone production using functional magnetic resonance imaging (fMRI). It specifically addresses two questions: what are the neural mechanisms underlying (i) Tone 3 sandhi, a
language specific context variation acquired rather than driven on-line by ease of articulation, and (ii) lexical tone sequencing. We collected reaction time sound recording, and imaging data during the production of twelve different tone sequences. To address (i), neural responses during production of tone sequence that elicits Tone 3 sandhi, namely sequence 3333, were compared to those that do not. To address (ii), repeated and mixed tone sequences were compared, e.g. 2413 vs. 1111. To remove the effect of context dependent variation driven by ease of articulation, a context factor was derived based on the acoustic data and served as a covariate in the statistical model of the fMRI data.
The results showed that the processing of the Tone 3 sandhi involved right IFG (Brodmann area 45 & 46), right inula, right superior temporal gyru, right thalamus, and left postcentral gyrus. We believe that the right inferior frontal gyrus and insula were responsible for the core processing of Tone 3 sandhi in the preparation stage of speech production, which resulted in longer RT, while top-down modulation of the right superior temporal gyrus and the left postcentral gyrus benefited feedback monitoring. On the other hand, similar to previous study in syllable sequencing (Bohland & Guenther, 2006), mixed sequence revealed a broad left dominant network, implying that mixed sequence increased loading on both speech preparation and execution, which was reflected in longer RT and duration. Left SMA and pre-SMA, which are known to be involved in speech and motoric sequencing (Bohland & Guenther, 2006; Shima & Tanji, 1998, 2000) were included in this network. Our finding of the right lateralized network for Tone 3 sandhi supports that the advantage of right hemisphere in pitch processing extends beyond auditory analysis. We propose that right hemisphere is

specialized in processing suprasegmental signal, but once tone is combined to segments, their composite are processed in the left hemisphere. Following this proposal, the left dominant activation for mixed sequence indicates that,
unlike Tone 3 sandhi, sequencing of tonal sequence operates on the composites of tone and segment.

Disclosures: H. Chang: None. **O. Tzeng:** None. **W. Kuo:** None.

Poster

906. Circuity and Connectivity Subserving Language

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Program#/Poster#:906.15/BBB1

Topic: F.01. Human Cognition and Behavior

Title: Verbal fluency affects performance on Montreal Cognitive Assessment (MoCA) subtests

Authors: V. MATHUR KUMARASWAMY[1], M. NAIR[2], S. CHANDRASHEKAR[1], *A. K. NAIR[2]
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Abstract: Background: Fluency of speech is an important cognitive function. Fluency may affect components of the Montreal Cognitive Assessment (MoCA) that need verbal ability.
Methods: Using de-identified data from a memory clinic www.thealzcenter.org, we obtained baseline MoCA scores. We analyzed if fluency affected performance on verbal components of MoCA by univariate analysis and multivariate linear models including factors such as diagnosis (Alzheimer, MCI or Other), education, age, race and gender.
Results: 163 baseline MoCAs were analyzed. Fluency was related significantly to verbal subtests measuring abstraction, naming, digit span forwards and backwards, and serial subtractions (Table 1). Repetition was not significantly affected by fluency. These associations persisted in multivariate models even after adjusting for education, diagnosis, age, race, and gender as co-factors.
Conclusion: Fluency correlated with performance on abstraction, naming, digit span and serial subtraction, independent of factors such as educational status, diagnosis, age, race and gender.

Baseline Characteristics and Relationship of Verbal Fluency to Subtests of MoCA

Variable	Mean or (Number if Discrete Data)	SD or (Percentage if Discrete Data)	Univariate analysis: p-	Multivariate analysis: p-value

			value	
Age	74.81	0.81	NA	NA
Education	12.26	2.16	NA	NA
Gender (Female)	(87)	(53.37%)	NA	NA
Race (White)	(153)	(93.86%)	NA	NA
Diagnosis (AD)	(101)	(66.13%)	NA	NA
Verbal Fluency	8.09	5.14	NA	NA
Abstraction	0.97	0.78	<0.001	<0.001
Naming	2.24	0.83	<0.001	<0.001
Digit Span Forwards (Correct)	(110)	(82.70%)	<0.001	<0.01
Digit Span Backwards (Correct)	(89)	(66.92%)	<0.001	<0.001
Repetition	1.34	1.21	NS	NA
Serial Subtraction	2.37	1.90	<0.001	<0.001
*n= 143 patients with baseline MoCA scores. NS = not significant, NA = not applicable.				

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Poster

907. Working Memory: Functional Neuroimaging

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Program#/Poster#:907.01/BBB2

Topic: F.01. Human Cognition and Behavior

Support: SNF Grant CR 13I1_127115

Title: Hippocampal activity predicts successful object-location binding during short-term memory maintenance

Authors: *D. VON ALLMEN[1], K. WURMITZER[1], E. MARTIN[1], P. KLAVER[2,1,3]
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Abstract: In cognitive neuroscience, several studies have raised doubt on the traditional view that the hippocampus is exclusively involved in long-term memory processes [1, 2, 3]. Recent imaging studies have shown that this region might play a role in short-term maintenance of object-location associations [4, 5]. This claim has been supported by lesion studies [6, 7]. Jeneson and colleagues [8], however, proposed in another lesion study that performance in a change detection task could in fact rely on both short-term and long-term memory. According to their view the hippocampus supports long-term memory and is only used when memory load exceeds individual short-term memory capacity. These findings led us to the question whether neural activity in the hippocampus depends on object-location binding performance. In order to investigate this question, we measured maintenance-related BOLD activity in 16 healthy adults (age range 19-26 years, eight females) while they performed a visual short-term memory task probing object-location associations (set sizes 1, 2, 4 and 6; delay period = 900 ms). Two performance groups were formed (high- and low-performers; each group N=8), based on individual short-term memory capacity (Cowan's k formula [9]). We found a significant 'performance group' by 'set size' interaction within the left hippocampus (local maximum at [39, -10, -14], z=4.33, p<.001, unc.) and right hippocampus (local maximum at [36, -10, -23], z=3.54, p<.001, unc.). High-performers (mean capacity = 5.3) showed neural activity in the hippocampus at set size four and six, whereas low-performers (mean capacity = 3.8) showed activity only at set size two. At set size six, low-performers showed deactivation. These data demonstrate that neural activity within the hippocampus was related to object-location binding during successful visual short-term memory maintenance.

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3) Schon et al. (2004), J Neurosci, 11088-97
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5) Piekema et al. (2006), Neuroimage, 374-82
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7) Finke et al. (2008), Neuropsychologia, 614-23
8) Jeneson et al. (2012), J Neurosci, 3584-9
9) Cowan (2001), Behav Brain Sci, 87-114

Disclosures: D. Von Allmen: None. **K. Wurmitzer:** None. **E. Martin:** None. **P. Klaver:** None.

Poster

907. Working Memory: Functional Neuroimaging

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Program#/Poster#:907.02/BBB3

Topic: F.01. Human Cognition and Behavior

Support: BMBF 01GQ1003B

Title: Cognitive processing during a spatial working memory task in humans: A multivariate analysis of fMRI time series

Authors: *C. DEMANUELE[1,2], F. BÄHNER[1,2], M. M. PLICHTA[1], H. TOST[1], A. MEYER-LINDENBERG[1,2], D. DURSTEWITZ[1,2]
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Abstract: The radial arm maze (RAM) task has been intensely studied in rodents to elucidate neural correlates of spatial memory, decision making and reward processes. Here we employ a diverse set of multivariate statistical approaches on fMRI data recorded from a group of healthy adults during a virtual reality version of the RAM task, where subjects moved through a virtual park surrounded by various landmarks to find gold coins hidden at the end of 12 alleys.
First, various multivariate test statistics (such as Wilk's lambda) were computed separately for several regions of interest (ROIs) as a measure of the overall discriminability of different task stages (namely *encoding*, *decision*, *reward expectation*, *reward consumption*, and *delay*) within the blood oxygenation level dependent (BOLD) signal pattern. Mahalanobis distances were also calculated to identify pairwise differences between cognitively defined task epochs. Significance levels were determined non-parametrically through time series (block-permutation & time-shifting) bootstraps. Results demonstrate that those anatomical regions postulated to be involved in task execution, such as the DLPFC and ACC, and the visual areas (V1) contain significant information on the task stages which distinguishes them from non-task related (control) areas, such as the Heschl gyrus and insula. Furthermore, we found that the decoding capability of some ROIs varies with different classification schemes; e.g. the DLPFC yields the highest

discrimination between low/high memory load decisions. Neural trajectories were reconstructed from the BOLD signal using a penalized version of Fisher's linear discriminant analysis applied to delay coordinate maps constructed for each ROI. We found that within ROIs associated with cognitive load (DLPFC) or sensory (precuneus) processing, different task epochs occupied disparate regions of the state space.
Multivariate Hidden Markov Models were also employed on this self-paced task as a complementary unsupervised approach for identifying the neuronal processing stages and their sequential timing. The goodness of fit was highest for models derived from the DLPFC and visual areas. The models' output state sequences were significantly correlated with experimenter-defined behavioral labels as used in the supervised classification analysis, and this correlation was highest for those ROIs that also yield strongest separation of task epochs in the supervised approach. Thus, we conclude that different cognitive stages during performance of the RAM task can be identified and separated from BOLD time series using both supervised and, partly, unsupervised methods.

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Poster

907. Working Memory: Functional Neuroimaging

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Program#/Poster#:907.03/BBB4

Topic: F.01. Human Cognition and Behavior

Support: BMBF 01GQ1003B

Title: Recruitment of hippocampo-neocortical networks during a spatial working memory task in humans

Authors: *F. BAEHNER[1,2], C. DEMANUELE[1,2], M. M. PLICHTA[1], H. TOST[1], D. DURSTEWITZ[1,2], A. MEYER-LINDENBERG[1,2]
[1]Dept. of Psychiatry and Psychotherapy, Central Inst. of Mental Hlth., Mannheim, Germany; [2]Bernstein Ctr. for Computat. Neurosci. Heidelberg-Mannheim, Heidelberg University, Germany

Abstract: Hippocampal-prefrontal interactions are essential for spatial navigation and memory, and disturbed in patients with schizophrenia and subjects at genetic risk. In an effort to develop translational tasks that aid in investigating this system, we have established a virtual reality version of the radial arm maze, a reward-based spatial working memory task that is widely used in rodents. During the acquisition of fMRI blood oxygenation level dependent (BOLD) data

subjects navigated through a virtual park surrounded by various landmarks to find hidden gold coins at the end of 12 alleys. The paradigm was completely self-paced and consisted of three task (training/encoding, delay, test/retrieval) and matched control phases, respectively, in each trial. The task design enforced the use of a spatial, landmark-based strategy.
Analysis of the pilot data recorded from a group of healthy subjects showed a strong recruitment of a network associated with spatial working memory and navigation. In the blocked design, we observed activations of the dorsolateral prefrontal cortex, parietal and midbrain areas (Adcock RA Neuron 2006) during both training and test phases. While activation of (para-)hippocampal areas was specific to the training phase, activation of the ventral striatum was only observed during the test period. Behavioral data was analyzed to determine when subjects encoded and retrieved information about the location of hidden rewards during the training and test phase respectively. Event-related analysis revealed that hippocampal areas are selectively recruited during encoding, while activation of parietal areas was weak. During retrieval, strong activations were found in parietal and prefrontal areas as well as the ventral striatum. Furthermore, we employed a set of multivariate statistical methods to characterize task stage-specific activation patterns (see poster by Demanuele C et al.).
Our analyses suggest a contribution of neural signals for salience related to dopamine function to the navigation task we employed, further strengthening the usefulness of this approach as a translational method to examine disorders such as schizophrenia.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: MH68680

Title: Working memory demand and the modulation of dorsal anterior cingulate efferent pathways revealed by fMRI and dynamic causal modeling

Authors: *H. MARUSAK, R. WHITE, S. WADEHRA, V. DIWADKAR
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Abstract: Background: Parametric variations in working memory demand increase regional cortical-striatal responses in children and adolescents. Yet the impact on network function has

not been systematically addressed. Here we employed dynamic causal modeling (Friston et al., 2003) to investigate working memory demand-related modulation of cortical-striatal pathways with an emphasis on efferent pathways from the dorsal anterior cingulate (dACC), a primary control region in the brain.

Methods: Subjects participated in a parametric n-back (1-back + 2-back; Diwadkar et al., 2011). 64 competing model architectures were constructed with an emphasis on contextual modulation (by 1-back, 2-back or both) of pathways originating in the dACC. An *a priori* cortical-striatal network was assessed (Wager & Smith, 2003) including the dorsal prefrontal cortex (dPFC), superior parietal cortex (SPC), and basal ganglia.

Results: See Figure 1.

Discussion: Four equi-probable architectures suggest a complex implementation of a simple paradigm within cortical-striatal networks. Notably, the largest effects of contextual modulation were observed in the dACC à dPFC pathway regardless of demand (1-back & 2-back). This pattern suggests that efferent pathways originating in the dACC and to the executive core of the frontal lobe are preferentially excited regardless of demand. This pattern may reflect core mechanisms of working memory implementation, and when extended to adolescent clinical populations, these studies can provide novel characterizations of the network bases of psychiatric disorders in adolescence.

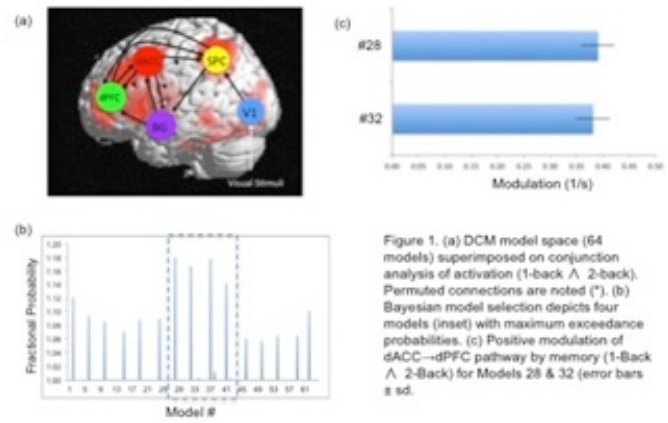


Figure 1. (a) DCM model space (64 models) superimposed on conjunction analysis of activation (1-back ∧ 2-back). Permuted connections are noted (*). (b) Bayesian model selection depicts four models (inset) with maximum exceedance probabilities. (c) Positive modulation of dACC→dPFC pathway by memory (1-Back ∧ 2-Back) for Models 28 & 32 (error bars ± sd.

Disclosures: H. Marusak: None. **R. White:** None. **S. Wadehra:** None. **V. Diwadkar:** None.

Poster

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Support: ISF Grant 1413/07 and 616/11

Bikura First Foundation Grant 942/09

Title: Activity of fronto-parietal network is reduced when subtle auditory regularities are detected.

Authors: *L. DAIKHIN, M. AHISSAR
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Abstract: Previously we found that when repetition or regularity is introduced to a simple discrimination task, participants' strategy quickly changed and their performance substantially improved. Rather than actually manipulating the presented stimuli, which requires working memory, they categorized them with respect to an internal reference. We now asked whether this quick implicit switch is accompanied by modifications in the neural networks underlying performance.
We used 2 tasks, each with 2 protocols: 1) 2-tone frequency discrimination, where participants heard sequentially presented pairs of tones and had to decide for each pair which tone was higher. We tested Reference 1st protocol - the first tone was always 1000Hz and the second was higher or lower, and No Reference - there was no repeated tone. 2) Phonological awareness task, spoonerism, where in each trial participants heard word pairs and had to switch between their first phonemes. We tested a With Repetition protocol, where a small set of word pairs was repeatedly used, and No Repetition protocol, where every word pair occurred only once. Each experiment was performed during 1 fMRI run using a block design. The protocols of each experiment were measured in an interleaved manner. Within each experiment we contrasted the 2 protocols to reveal repetition-related changes in activation. We then compared the 2 resulting contrasts for possible overlaps.
Beyond the bilateral activation of the auditory cortex, activity in both the tonal and verbal tasks was left lateralized. The [no-repeat - repeat] contrast in the tone discrimination task showed a reduction of activation in explicit working memory (WM) regions (lateral-inferior prefrontal; intra-parietal) and in regions associated with syntactic processing (posterior-superior temporal sulcus) and with audio-motor integration in speech (dorsal-premotor). In the equivalent verbal contrast we found a partial overlap with the tonal contrast in WM networks in addition to areas associated with processing of verbal information (superior and middle temporal gyri), and regions associated with word processing (posterior-inferior temporal).
To conclude, we found that partially overlapping WM networks, which are left lateralized in both tasks, reduce their activity when regularity is detected. This reduction does not reflect a general decrease in task difficulty, which was equated for the frequency discrimination task by making the Reference 1st protocol more difficult. Rather, utilization of cross-trial temporal

patterns leads to a strategic switch from the laborious on-line calculations to a more automatic categorical retrieval processing.

Disclosures: L. Daikhin: None. **M. Ahissar:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

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Bikura First Foundation Grant 942/09

Title: ERP marker for automatic auditory regularity detection predicts behavioral success

Authors: *S. JAFFE-DAX, M. AHISSAR
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Abstract: Detection of repeated stimulation patterns (stimulus regularities) is an important learning mechanism, which is largely automatic. In the auditory system it is often tapped by ERP components as mismatch negativity (MMN) peaking ~150ms from regularity violation, and early left and right anterior negativities (ELAN and ERAN) peaking ~200ms from linguistic and musical structural violations, respectively. We now asked whether structural regularities introduced into a simple paradigm of 2-tone frequency discrimination can also be detected automatically, and whether the ability to automatically detect such a structural regularity predicts the individual's aptitude for regularity detection in other contexts.
We administered two protocols of 2-tone frequency discrimination to 35 participants, which were previously used for active discrimination. However now, participants watched a silent movie. Each protocol had a simple structural regularity - in each trail (separated by 1400ms) one of its two tones (separated by 600ms) was fixed across trials (1000Hz-reference) and the other was higher or lower. In protocol1 it was the 1st tone whereas in protocol 2 it was the 2nd. Our previous behavioral data (Nahum et al., J Neurosci 2010) indicated that protocol 1 was performed significantly better even though its objective complexity is equal to that of protocol 2. Participants' automatic ERP response at ~200ms from the onset of the 1st stimulus (P2) was larger in protocol 1, where the 1st tone is the repeated reference, than in protocol 2. This difference indicates that participants detected the structure of protocol 1, and produced a larger response to the repeated reference, even though it was locally surrounded by non reference

stimuli. No such difference was found in response to the 2nd tone, indicating that participants did not detect the structure of the "less favorable" protocol 2. Our participants were also tested with few cognitive tasks. Their automatic sensitivity to the auditory structure was correlated with their sensitivity to visual regularities in word spelling, i.e. their rate of discriminating correct versus incorrectly spelled frequent words. These findings reveal that automatic regularity detection can be tapped even without structural violation. Moreover, automatic sensitivity predicts behavioral success in a range of tasks, both at the group (protocol asymmetry) and at the individual level.

Disclosures: S. Jaffe-Dax: None. **M. Ahissar:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: Bikura First Foundation Grant 942/09

Title: Tapping working memory with sensorimotor synchronization

Authors: *N. JACOBY[1,2], H. JAKOBY[1], I. LIEDER[1], N. TISHBY[1], M. AHISSAR[1]
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Abstract: Traditionally, sensory
and motor tasks were considered related to low-level processing. Reverse
Hierarchy Theory, suggested by Ahissar and Hochstein (Nature 1997), challenges
this view by emphasizing the importance of top-down processes in sensory tasks
that were traditionally considered as low level. In this study, we give
evidences that a motor task -sensorimotor synchronization- is also linked with
high-level processes, mainly ones that involve working memory.
Sensorimotor
synchronization, the temporal coordination of a rhythmic movement with an
external rhythm, is studied experimentally by tapping experiments (Repp 2005)
in which subjects are required to finger tap in synchrony with an auditory
stimuli. One type of tapping experiments is the step-changes experiment,
where subjects are required to adapt their taps to a metronome alternating
between two tempi. Repp and Keller 2004 showed that this type of
experiment is modulated by cognitive load, and depends on explicit processing.
They conjectured that this could be explained by the importance of period

correction, a theoretical concept that models the adaptation of the internal time-keeper tempo in the sensorimotor control loop. Our motivation for this study was to trace cognitive processes that are linked to period correction. Subjects (N=30) performed
a battery of cognitive and sensory tasks, and two-step tapping experiments. We found high positive inter-subject correlations between successful performance in the tapping experiment (decreased tapping variance) and working memory related tasks, the highest correlation to an n-back auditory task, in which subjects are required to report whether the current tone matches a tone n-steps earlier (n is changed adaptively). We also found significant correlations to other tasks that pound on verbal or tonal memory. However, the Block Design task, a standard spatial-reasoning visual task, usually considered to measure general cognitive abilities and not directly related to working memory, did not show a significant correlation.
We propose that these
correlations can reflect the involvement of high-level working memory mechanisms for the representation of tempo in the sensory motor control loop, and therefore in line with the Reverse Hierarchy Theory's predictions.

Disclosures: **N. Jacoby:** Employment; Hebrew University. Research Grant; Bikura First Foundation Grant 942/09. Other; Bar Ilan University. **H. Jakoby:** None. **I. Lieder:** None. **N. Tishby:** None. **M. Ahissar:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Title: Short-term memory for visual features: inferior frontal junction initiates maintenance processes in the frontal eye fields

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Abstract: Short-term memory (STM) encoding and maintenance of simple visual features involve interactions between retinotopic visual cortex (VC) and a set of regions in frontal cortex. Specifically, the Inferior Frontal Junction (IFJ) has been associated with memory encoding, while the Frontal Eye Fields (FEF) show sustained activity during memory intervals, indicative of a role in maintenance of the memory trace. The patterns of influence between these areas during the different stages of a STM task are however poorly understood. In the present study, we adapted the delayed orientation discrimination task to enable separation of responses associated with memory encoding, maintenance, and retrieval in a fMRI experiment. The study also included measures of responses to identical stimuli either passively viewed, or actively encoded but not associated with a memory task. The aim was two-fold: (i) to investigate with univariate analyses how individual areas contribute to the different STM stages; (ii) to examine effective connectivity (Granger Causality (GC)) between regions of interest (ROI) to get further insight into the function of the different nodes.
The BOLD-response was significantly stronger during encoding than during retrieval and control conditions in a set of higher-tier visual areas and in IFJ, but not in FEF. Furthermore, FEF, but not IFJ or VC, showed significant sustained activity during the retention interval. No differences were found between retrieval and encoding of non-STM-related stimuli in any of the areas. These results suggest that the VC-FEF-IFJ network plays a central role in STM encoding and maintenance. To elucidate the temporal dynamics of the network, we extracted ROI time-series from task periods representing memory encoding and retrieval, and for periods following control stimuli. Bivariate GC analysis between ROI-pairs showed that IFJ and VC influenced FEF during the memory encoding stage. The pattern was significantly different from the influences during retrieval and control conditions, and could thus not be due to vascular properties of the ROIs. To investigate whether VC's influence on FEF during encoding was contingent on IFJ, we ran a second GC analysis, conditioning on IFJ. No significant GC was observed from VC to FEF when the variance explained by IFJ was removed from the calculations, indicating that the influence of VC on FEF is mediated by IFJ.
Our results suggest that IFJ together with VC is particularly involved during STM encoding. The observation that FEF shows maintenance-related activity, and is preceded by IFJ, fits well with the developing understanding of IFJ as a control region with an initiating role in STM operations.

Disclosures: M.H. Sneve: None. **S. Magnussen:** None. **D. Alnæs:** None. **T. Endestad:** None. **M. D'Esposito:** None.

Poster

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NIH P50 MH071702-01A2

NCRR P41RR14075

Title: A high-resolution fMRI study of hippocampal subfield and medial temporal lobe structures during the maintenance of overlapping and non-overlapping stimuli

Authors: *R. E. NEWMARK, K. SCHON, R. S. ROSS, B. JUN, C. E. STERN
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Abstract: Computational models and experimental studies suggest a critical role for medial temporal lobe (MTL) regions in working memory (WM) tasks. Experimental work also suggests a critical role for the hippocampus in disambiguation, an episodic memory process. However, whether delay period activity in the hippocampus and MTL cortex is related to WM processes or to episodic memory processes is unclear. Here, we probed WM with a load manipulation, and episodic memory with a manipulation of disambiguation during encoding. We used high-resolution fMRI (Siemens 3T; voxel size, 1.5mm3; TR, 2 s) to explore the recruitment of hippocampal subfields (CA1, CA3/DG, subiculum) and surrounding MTL subregions (amygdala, perirhinal, entorhinal, parahippocampal cortices) when participants maintained overlapping (OL) and non-overlapping (NOL) stimuli at two different WM loads. We predicted that MTL cortex, but not the hippocampus would show a load effect during the Sternberg task delay, while the hippocampus and MTL cortex would show greater delay activity for the OL than for the NOL condition. Nineteen participants (12 men; age, 23.0 ± 4.19) performed a modified Sternberg task (Schon et al., 2009). Participants encoded either 2 or 4 OL or NOL stimuli which they then maintained across a 10-s delay. After the delay, they indicated whether a test stimulus was a match or non-match to one of the stimuli seen before the delay. OL stimuli were pictures of unfamiliar, morphed composite faces sharing 30% of their features. NOL stimuli were distinct, unmorphed, unfamiliar faces. Analysis of accuracy and reaction time showed that participants performed better on load 2 than load 4 trials and on NOL than OL trials. During the delay period, a two-way ANOVA on the fMRI data (WM load - load 4 vs. load 2 by condition - OL vs. NOL) showed a main effect of condition (OL > NOL) in CA1 and MTL cortex, while a main effect of load (Load 4 > Load 2) showed extensive activation in the CA3/DG, CA1, and subiculum and in the surrounding MTL cortex. A significant interaction between load and condition was localized to CA1, the amygdala, and the parahippocampal cortex. An examination of parameter estimates showed that for NOL stimuli, CA1, and amygdala were engaged to maintain a greater WM load. In the parahippocampal cortex, increased activity was shown for maintaining OL information at a greater load. Our data shows that CA1 is bilaterally recruited to maintain larger WM loads of distinct, NOL representations. Our results provide evidence for a

role for hippocampal subfields and MTL subregions to support both WM and episodic memory processes during the WM task delay.

Disclosures: R.E. Newmark: None. **K. Schon:** None. **R.S. Ross:** None. **B. Jun:** None. **C.E. Stern:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: DFG TI 239/9-1

Eu

Title: Involvement of the cerebellar cortex and nuclei in verbal and visuospatial working memory: A 7T fMRI study

Authors: *M. THÜRLING[1,2], H. HAUTZEL[3,4], M. KÜPER[1], M. R. STEFANESCU[1,2], S. MADERWALD[2], M. E. LADD[2,1], D. TIMMANN[1]
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Abstract: The aim of the study was to investigate whether different areas of the cerebellar cortex and nuclei contribute to different working memory tasks (n-back vs. Sternberg tasks). Young and healthy subjects participated in two functional magnetic resonance imaging (fMRI) studies using a whole-body 7T MR scanner (Magnetom 7T, Siemens, Erlangen, Germany). One group of subjects (n = 21) performed an abstract and a verbal version of an n-back task contrasting a 2-back and 0-back condition. Another group of subjects (n = 23) performed an abstract and a verbal version of a Sternberg task contrasting a high load and a low load condition. A block design was used. Normalization of the cerebellar cortical data was done based on the spatially unbiased atlas template (SUIT) of the human cerebellum (Diedrichsen, 2006). For image processing of the dentate nuclei, a recently developed region of interest (ROI) driven normalization method was applied (Diedrichsen et al., 2011). Whereas activated areas of the cerebellar cortex and dentate nuclei were not significantly different comparing the abstract and verbal versions of the n-back task, activation in the abstract and verbal Sternberg tasks was significantly different. In both n-back tasks activation was most prominent at the border of lobules VI and Crus I, within lobule VII, and within the more caudal parts of the dentate nucleus

bilaterally. In Sternberg tasks the most prominent activations were found in lobule VI extending into Crus I on the right. In the verbal Sternberg task activation was significantly larger within right lobule VI compared to the abstract Sternberg task and compared to the verbal n-back task. Activations of rostral parts of the dentate were most prominent in the verbal Sternberg task, whereas activation of caudal parts predominated in the abstract Sternberg task. The lack of difference between abstract and verbal n-back tasks and the lack of significant lateralization suggest a more general contribution of the cerebellum to working memory regardless of the modality. In contrast, the focus of activation in right lobule VI in the verbal Sternberg task suggests specific cerebellar contributions to verbal working memory. The verbal Sternberg task emphasizes maintenance of stimuli via phonological rehearsal, whereas central executive demands prevail in n-back tasks. Based on the model of working memory by Baddeley and Hitch (1974), the present results show that different regions of the cerebellum support functions of the central executive system and one of the subsidiary systems, the phonological loop. No evidence, however, was found that the cerebellum supports the second slave system, the visuospatial sketch pad.

Disclosures: M. Thürling: None. **H. Hautzel:** None. **M. Küper:** None. **M.R. Stefanescu:** None. **S. Maderwald:** None. **M.E. Ladd:** None. **D. Timmann:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Title: Regional brain activity during updating in working memory

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Abstract: The network of brain regions supporting working memory (WM) has been well elucidated through neuroimaging research. However, the tasks commonly used in neuroimaging work on WM have limited the extent to which some component processes of WM can be isolated. Here we report preliminary results (N = 8) from a functional Magnetic Resonance Imaging study of a novel WM task that allows for separation of the process of updating the contents of WM from encoding and rehearsal of the target information. During the delay between target and probe stimuli in a letter item recognition task,

participants were instructed to either add or subtract an item from the target set or to simply rehearse the initially presented stimuli. Unlike a traditional item recognition task, this allows for analysis of brain regions involved when suppressing or adding a stimulus that is presented directly. Comparison of events in which participants added stimuli to the target set to simple rehearsal revealed activation in language processing and selection regions (left ventrolateral prefrontal cortex) as well as dorsal anterior cingulate and pre-supplementary motor area, supplementary motor areas, and bilateral insula. Comparison of subtracting items from the target set to simple rehearsal revealed no significant activation, suggesting a substantial difference in the neural demands of updating in WM between adding and dropping information. While other work has shown that dropping information results in additional activation, tasks used in those studies appear to place greater demand on cognitive control. Results of this preliminary investigation show that adding new items to working memory load induced activation in regions similar to those activated by simple WM maintenance, while subtracting items did not induce any significant activation, indicating that adding, but not dropping items engages brain regions understood to be involved in working memory.

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Poster

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DFG CIN

Title: Influence of response modality on fMRI activity in a working memory task

Authors: *A. PILACINSKI, M. WALLSCHEID, A. LINDNER
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Abstract: Most fMRI experiments investigating short term memory require certain behavioral measures, such as manual or verbal responses, in order to control for the memorized content. It remains unknown, however, if the memory-related activity observed by such studies solely reflects the current memory content or, in addition, depends on the effector used to respond. In

the present study we addressed this issue by investigating the modulation of working memory related BOLD signals as a function of response modality.
We scanned a total of 10 subjects, performing a spatial memory task. At the beginning of each trial, subjects were instructed that they will have to respond either verbally or by pressing an appropriate button of a button box. Then, a set of objects was presented for a limited amount of time. After a memory maintenance phase, subjects had to report whether a second set of objects, which was presented in the response phase, was identical to or different from the initial set of objects presented. This condition was contrasted with a simple control task, where subjects were also instructed initially about which effector to use but, instead of memorizing any spatial information, they had to only determine whether a set of dots presented in the response phase was symmetrical or not. Importantly, in both tasks the required motor responses were not predicted by the initial cues and thus could not be pre-planned.
We performed a region of interest-based analysis in areas that were significantly activated during the memory maintenance phase, as compared to a corresponding period of the control task. We then analyzed BOLD signals extracted from these regions and compared them between verbal and manual response modality, as well as for different memory loads (2/6 objects). Our analyses revealed a significant interaction between response modality and memory load in the right dorsal premotor cortex. Specifically, activity in this area was higher for “manual” than for “verbal” conditions, however for low memory loads, only. This effect did not reach significance for high memory load conditions. Importantly, we did not observe a similar effect in the control task, which argues against the idea that the observed difference could be simply due to unspecific motor preparation.
Our results demonstrate that the response modality affects BOLD-activity related to memory maintenance processes. This finding implies that encoding and maintenance of information within working memory may be optimized not only with respect to the content of information, but also with respect to the behavioral context in which this information is to be used.

Disclosures: A. Pilacinski: None. **M. Wallscheid:** None. **A. Lindner:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: CERC Program

Title: Working memory revisited - are category and process specific models orthogonal or mututally exclusive?

Authors: J. P. WONG, A. M. OWEN, *A. HAMPSHIRE
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Abstract: Working memory maintenance (WM) refers to the process of actively holding information on-line in mind. It forms one of the core components of goal-directed behaviour, being necessary for reasoning, comprehension and fluid intelligence with impairments evident in many neurological diseases. While the frontal and parietal cortices (FPC) are known to play a key role in WM the exact cognitive and neural architecture remains in dispute. In particular, one long standing debate that has never been fully resolved is what role specific frontal-lobe regions contribute to stimulus-specific processing (e.g. locations versus objects) versus stimulus-independent (category insensitive) processing.
To examine whether FPC regions are differentially active based on stimulus category, 19 participants took part in a novel WM fMRI study. Stimuli of three distinct types were displayed - positions, numbers and objects - each at three levels of load (3, 5 or 7 items). Participants were required to encode and maintain this information over a delay period and subsequently identify the non-encoded item in a probe array. Regions of FPC cortex that had previously been implicated in a broad range of cognitive task contexts (Multiple demand cortex - MD) were first examined using predefined regions of interest (ROI), including the anterior insula/frontal operculum, inferior frontal sulcus and inferior parietal cortex. These MD regions were highly active at all task stages regardless of stimulus type, supporting a process specific (stimulus-independent) model. However, voxel-wise whole brain analysis revealed that other FPC regions, including the superior frontal sulcus, inferior frontal gyrus and frontal polar cortex, were responsive specifically to the location or object properties, supporting a category specific model. Furthermore, parametrically modulating the number of items affected MD regions during encoding for all categories of stimuli, whereas other FP regions outside of MD showed category specific load effects. There were no significant effects of number of items during the delay anywhere within FPC cortex.
These results suggest that models of frontal-lobe control of WM that include category- and process- specific functional axes may not, as was previously believed, be mutually exclusive. More specifically, there appears to be a central core of MD regions that are 1) category unresponsive, 2) involved in encoding and maintenance, but 3) only sensitive to load during encoding. Concurrently, there exists another set of category responsive FP regions outside of MD that do conform to the posited ventral-dorsal/object-location dissociation.

Disclosures: J.P. Wong: None. **A.M. Owen:** None. **A. Hampshire:** None.

Poster

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Support: AFOSR Grant 001569300

Title: Distinct neural correlates of visual working memory number and resolution: An fMRI investigation

Authors: *H. SCHWARB[1], S. D. KEILHOLZ[1], R. A. MCKINLEY[3], L. TRIPP[3], E. H. SCHUMACHER[2]
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Abstract: Since antiquity, philosophers and scientists have been interested in human memory; today, researchers are still working to understand its boundaries and architecture. One popular theory of working memory capacity (WMC) identifies a limit of approximately four items (Cowan, 2001). Consistent with this theory, neuroimaging research shows that activity in the intraparietal and intraoccipital (IPS/IOS) sulci reflects visual WMC and reliably tracks the number of items stored (Todd & Marois, 2004). This capacity limit is not, however, universally accepted and some researchers have proposed that visual WMC is strongly influenced by the complexity of the items stored (Alvarez & Cavanagh, 2004). Furthermore, storing items of varying complexity results in distinct patterns of neural activity (Xu & Chun, 2006). Awh, Barton, and Vogel (2007) have recently proposed a unifying account of visual WMC suggesting that visual working memory is mediated by two independent factors: number (number of it ems stored) and resolution (amount of detail stored). The current study investigates the unique neural correlates of number and resolution using the change detection task. Data suggest that number is mediated by regions previously associated successful memory formation including right hippocampus and right IPS/IOS. Conversely, resolution is independently mediated by regions previously associated with sustained attention and working memory such as left dorsolateral prefrontal cortex. These results suggest distinct neural activity independently supporting the general number and resolution processes responsible for identifying changes among complex stimuli.

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Poster

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RCUK

Title: Distinct dorsal and ventral posterior cingulate responses to internally and externally directed memory processing

Authors: *R. LEECH, G. SCOTT, D. J. SHARP
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Abstract: Introduction
Attention can be directed internally or externally. During the retrieval of autobiographical memories attention is directed towards information from an individual's past. In contrast, when behavior is guided by external events, attention is focused on the environment. Mesulam argues that this distinction is reflected in neuroanatomy. Limbic structures process internally-directed information such as memories whereas sensory/motor cortices process external information. Paralimbic structures such as the posterior cingulate cortex (PCC) may form a link between regions involved in processing internally or externally derived information.
We use functional MRI to directly compare processing that is directed either internally or externally. Based on a hypothesis originating from our previous work (Leech et al. J Neurosci 2011) we test whether dorsal and ventral parts of the PCC show distinct responses when memory involves internally stored information (autobiographical and episodic memory) or external information (working memory).
Methods
Neural activity was studied in twenty subjects using functional magnetic resonance imaging. Four conditions with varying levels of internal and external attentional focus were performed: 1) autobiographical memory (AM) retrieval; 2) episodic memory retrieval (EM) of information presented around 1 hour prior to scanning; 3) a zero-back task, in which subjects responded to a specific word; 4) a two-back task, in which subjects responded when the word was the same as the one presented two previously.
Results
We have previously demonstrated distinct responses in dorsal and ventral PCC regions during performance of the externally-focused N-back (tasks 3 and 4). Our results extend this work by directly comparing PCC responses on the N-back with responses observed during internally-focused autobiographical and episodic memory retrieval. Relative to the undemanding zero-back

condition, activation in the whole of the PCC decreased during two-back performance. In contrast, activity in the ventral PCC and retrosplenial cortices increased during AM relative to zero-back, and was unchanged during EM.

Conclusions

The results support a functional distinction between dorsal and ventral parts of the PCC during different types of memory. Relative reduction in PCC activity is seen during working memory, where attention is directed externally. In contrast, activity increases in the ventral PCC for internally directed memory, in particular during autobiographical memory. This suggests the PCC is a heterogeneous region involved in regulating the balance between internally and externally directed attention.

Disclosures: R. Leech: None. **G. Scott:** None. **D.J. Sharp:** None.

Poster

907. Working Memory: Functional Neuroimaging

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:907.16/BBB17

Topic: F.01. Human Cognition and Behavior

Support: NIH IBSC center 1-P50-MH079485

Title: Responses, mappings, and dimensions distinguishing preparatory processes in the PFC.

Authors: *T. KRIETE[1], J. REYNOLDS[2], R. O'REILLY[3]
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Abstract: Recently, there has been substantial interest in identifying how different areas of the prefrontal cortex (PFC) may be involved in cognitive control processes. In particular, one popular hypothesis is that lateral PFC is organized such that posterior areas are sensitive to concrete pieces of information (such as information regarding a response), whereas more anterior areas are sensitive to abstract pieces of information (such as information regarding the dimension of a stimulus to attend to). However, recent data suggest that this type of gradient might interact with working memory demands; relatively anterior areas of PFC (e.g. mid-dorsolateral PFC) appear to be associated with high memory demand conditions, irrespective of how abstract the to-be-maintained information is. The goal of the current study was to directly investigate this issue using event-related functional magnetic resonance imaging (fMRI). Specifically, the current study used a cueing paradigm in which participants were presented with a four different types of preparatory cues prior to an imperative stimulus. They could be presented with: a) low-

abstraction information associated with the response (right vs. left hand), b) high-abstraction information associated with the relevant stimulus dimension (visual contrast vs. size), c) information associated with the relevant stimulus-response mapping ("large" values mapped onto index vs. middle finger), and d) a null cue that controls for visual stimulation and timing, but does not provide relevant information regarding the upcoming probe. Critically, the current paradigm used a catch-trial design in which cue-related and probe-related processes can be dissociated.
Results indicate that participants used each of the content cues to prepare for upcoming probes. Further, results indicated that, relative to the null cue, all of the content cues were associated with increased cue-related activity in various different areas of lateral PFC, including mid-dorsolateral PFC and areas of the inferior frontal junction (IFJ). The current data suggest that these areas are associated with preparatory working memory processes, irrespective of how abstract the information is.

Disclosures: T. Kriete: None. **J. Reynolds:** None. **R. O'Reilly:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: JSPS Grant 23800034

JSPS Grant 22220003

JSPS Grant 23240036

Title: Limited attentional capacity for emotional information with negative valence: An fMRI study with cognitive load

Authors: *T. MINAMOTO[1], M. OSAKA[3], N. OSAKA[2]
[2]Psychology, [1]Kyoto Univ., Kyoto, Japan; [3]Human Sci., Osaka Univ., Suita, Japan

Abstract: The present study challenged a research question that attentional resource for emotional information is limited as is the case for perceptual information. In the field of visual selective attention, it is shown that visual working memory load attenuates interference effect by a visual distractor when the category of load and that of distractor overlaps. The less interference effect is attributed to depletion of attentional resource available for processing of distractors.

Based on the two-dimensional framework for emotional experiences (valence and arousal), the present study manipulated the emotional valence and tested if distractor interference decreases as a function of emotional valence. Participants performed the emotional Stroop task (positive or negative valence) while maintaining negative emotional information in working memory. Three types of memory load was given (high negative, low negative, none), and its effect on the emotional Stroop task was measured. Brain activation was also obtained while participants performed the task. Given that attentional resource for emotion is valence-based, the interference effect in the emotional Stroop task with negative valence should be weakened as memory load increased. Behavioral analysis on the Stroop task showed a main effect of the emotion, but not a main effect of the load and an interaction between the load and emotion. However, fMRI data showed that the activation of the anterior cingulate cortex and the orbitofrontal cortex showed less activation when participants performed a Stroop task with negative valence under high memory load than under no memory load. Considering the fact that activation of the anterior cingulate cortex reflects emotional conflict, less activation of the region under high memory load means smaller interference effect. Such load effect was not observed in the emotional Stroop task with positive valance. Interestingly, small volume correction analysis focusing on the bilateral amygdala showed that high negative emotional load produced greater activation than no emotional load during emotional Stroop task with negative valence. Those results indicate that higher emotional load depletes attentional resource for processing emotional distractor, possibly consuming the resource of amygdala. The present study provides the evidence that attentional resource for negative emotional information is valence-based.

Disclosures: T. Minamoto: None. **M. Osaka:** None. **N. Osaka:** None.

Poster

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Program#/Poster#:907.18/BBB19

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant ROI MH075706

Title: Neural evidence for the flexible use of working memory and episodic memory in prospective remembering

Authors: *J. D. COHEN[1,2], J. A. LEWIS-PEACOCK[1,2], K. A. NORMAN[1,2]
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Abstract: How do we remember to execute a specific goal at the appropriate time while busily pursing other plans? We can use an effortful strategy -- actively maintaining the goal information in working memory -- or a reflexive strategy -- relying on external cues to trigger retrieval of the goal information when it is needed (McDaniel & Einstein, 2000). The flexible choice of strategy involves a tradeoff between effort and performance, and depends on many factors including the availability of cognitive resources and the likelihood of goal retrieval. We designed an fMRI study to assess whether brain activations can track, on a trial-to-trial basis, the use of working memory versus episodic memory to achieve a delayed goal. Each trial began with a target picture, followed by a variable-length sequence of 2-sec memory probes each containing two pictures and a letter string. Participants were required to balance two orthogonal goals: 1) to make repeated lexical judgments (about the letter strings that appeared in the sequence of probes); and 2) to identify when the picture target reappeared (between 2 and 42 sec after its introduction). For half the trials, the lexical judgment was simple, while for the other half it was more difficult. We reasoned that when a trial involved a series of lexical judgments that was more demanding, participants would be less likely to maintain the picture target in working memory, relying instead on retrieval from episodic memory. We also varied the degree of proactive interference associated with the target. In half the trials, it was drawn from a large heterogenous set of trial-unique pictures (low interference), while in the other half it was drawn from a small homogenous set that repeated frequently across trials (high interference). We reasoned that high proactive interference would interfere with episodic memory retrieval, thereby biasing participants to use working memory to maintain the target. We used multivariate pattern analysis of the fMRI data in ventral temporal cortex to identify the target-related activity during each trial. We also identified brain regions (e.g., inferior parietal cortex) for which activity reflected successful episodic memory retrieval of the target. In all trials, prospective memory success was associated with elevated estimates of target activation at the moment that it reappeared. Critically, sustained target activation prior to its reappearance was predictive of success only on those trials with low evidence of episodic retrieval activity. Together, these findings provide initial neural evidence for the flexible use of working memory and episodic memory in prospective remembering.

Disclosures: J.D. Cohen: None. **J.A. Lewis-Peacock:** None. **K.A. Norman:** None.

Poster

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Program#/Poster#:907.19/BBB20

Topic: F.01. Human Cognition and Behavior

Support: JSPS Grant 22220003 to NO

JSPS Grant 23240036 to MO

Title: Second-order false belief task needs working memory in normal adults: An event-related fmri study based on theory of mind

Authors: *N. OSAKA[1], K. YAOI[1], T. MINAMOTO[1], M. OSAKA[2]
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Abstract: Theory of Mind (ToM) allows us to attribute empathies, desires, and intentions to others to predict or explain their actions and to posit their intentions (mindreading). ToM testifies an ability to make inferences about others' mental state and allows us to understand what we believe to be true and what is true may be different (Perner, 1991). Children under the age of four, along with most autistic children of older ages are likely to fail to perform 1st order False Belief Task (FBT) like the Sally-Anne task. We assumed one of the causes of the failure would due to insufficient/immature executive function of working memory. Thus, we applied 2nd order FBT as more sophisticated mindreading task to normal adults to examine simultaneous DLPFC activation related to working memory using an event-related fMRI. We thought the higher-order meta-representations based on working memory are prerequisited to solve the task. Results showed brain activations in the dorsal working memory network involving bilateral DLPFC and posterior parietal cortex (PPC) as well as the cortical midline structures including bilateral MPFC and precuneus in under the 2nd-order FBT. Those results indicate that the dorsal working memory network plays a critical role in the higher-order mindreading.

Disclosures: N. Osaka: None. **K. Yaoi:** None. **T. Minamoto:** None. **M. Osaka:** None.

Poster

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Program#/Poster#:907.20/BBB21

Topic: F.01. Human Cognition and Behavior

Support: Rice University Start Up Funds

Title: In and out of focus in working memory: Neural evidence of role of long-term memory

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Abstract: Working memory has been argued to constitute of 2-tiers in memory updating paradigms- a core focus of attention (FoA) of 1 item, with representations outside the focus are activated long-term memory (LTM; Verhaeghen & Basak, 2005). Accessing the representations outside the focus involves focus switching. If the representations outside the FoA are unpredictable, a controlled search process is indulged; else, the focus switch is constant (Basak & Verhaeghen, 2011a, 2011b). In short-term memory paradigms, enhanced MTL activation has been found for items presented before the last item, provided support for focus of 1 and activated LTM outside the FoA (Ozetkin et al., 2008, 2010).
The goals of the current study is to establish a) whether representations outside the FoA are indeed activated LTM, b) neural evidence for the search process outside the focus, and c) whether neural activations of representations outside the focus correlate with success in various short-term memory/WM tasks outside the focus. We designed a novel memory updating paradigm where WM set-size (N) varied from1 to 3, and we manipulated the stimulus predictability across blocks for N>2.
Behavioral results support our prior results - faster retrieval speed and higher accuracy were observed for N=1; for N>1, retrieval speed increased with N for unpredictable, but remained constant for predictable probe sequence.
N=1, compared to N>1, yielded enhanced activations in dorsolateral PFC, cingulate gyrus, cuneus, and precuneus. For N>1, versus FoA, enhanced activations are observed in insula and parahippocampus/hippocampus (PHCG); activations of both brain regions were greater for unpredictable than predictable probe sequences. Brain-activation-by-task-complexity functions indicate that increases in PHCG were linear, with no increase in the most complex condition (unpredictable N=3). Individuals with higher short-term or working memory capacity (e.g., forward and backward digit span) and faster retrieval rates outside the focus also displayed greater activations in in left and right PHCG for N>1. The results suggest that representations outside the focus are activated LTM indicated by activations in MTL; these activations increase with more effortful search for active representations, and are correlated with both capacity and retrieval speed of WM.

Disclosures: C. Basak: None. **M.I. Cunha:** None. **C. Hamilton:** None.

Poster

907. Working Memory: Functional Neuroimaging

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:907.21/BBB22

Topic: F.01. Human Cognition and Behavior

Title: Evidence for a different role for the ipsilateral Area MT in human Visual Working Memory in motion discrimination

Authors: *P. KÖRTVELYESSY
Cognitive Neurol., Hertie Inst. For Clin. Brain Res., Tübingen, Germany

Abstract: Recent psychophysical experiments analysing motion discrimination in non-human monkeys and humans revealed a critical spatial separation resulting in a decline of the discrimination threshold in visual working memory (VWM). This critical spatial separation was conform to a putative receptive field of area MT neurons in non-human monkeys and maybe in humans also. These results pointed to a spatiotopic component in the directly activated Area MT in visual working memory, which could not be verified in human fMRI and non-human monkeys with single-cell recordings. The human fMRI results rather showed a holoistic activation including the ipsilateral Area MT; especially together with feature-based attention.
We conducted two psychophysical experiments (both with 4 participants and 500 trials each) to glimpse at the role of the ipsilateral Area MT in human VWM. We used different random-dot-kinematograms (RDK) with different noise levels being presented in both visual hemifields during a match-to-sample task and determined the level of 75% correct matching/motion discrimination per position. The positions of the RDK had different spatial separations. Eye position was monitored and recorded via an eyetracker. This data was filtered and trials with eye movements bigger than 3 degrees were deleted.
If the ipsilateral Area MT played the same part in human VWM as the contralateral it would show the same critical spatial separation as the directly activated contralateral Area MT. We could not find a critical spatial separation for the ipsilateral side in our first experiment (one factor repeated measures ANOVA for position p=0,405). In our second experiment we added a RDK as a cue, which should have activated the ipsilateral Area MT via feature- based-attention. Again no critical spatial separation could be found (one factor repeated measures ANOVA for position p=0,2886). Also, there was no significant difference between these two experiments in terms of the motion discrimination thresholds (ttest=0,4605).
Therefore we conclude that the human ipsilateral Area MT does not seem to play the same role in human VWM as the directly activated Area MT. In our experiments the ipsilateral Area MT does not have the same spatial component as stated for the directly activated Area MT. The relevance of the activity measured in the fMRI in the human ipsilateral Area MT remains elusive.

Disclosures: P. Körtvelyessy: None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH Intramural Research Program of the NIMH

Title: Representations of remembered objects depend on task

Authors: *S.-H. LEE, D. J. KRAVITZ, C. I. BAKER
NIH/NIMH, Bethesda, MD

Abstract: When we bring an object to mind, some aspects of the object are often emphasized whereas other aspects are ignored. For example, when remembering the same object, we can focus on its visual shape (i.e. physical property) or on its usage or category (i.e. semantic property). While prior studies have implicated prefrontal cortex in aspects of retrieval and working memory, it is unclear what role it and more caudal perceptual regions play in maintaining the retrieved information dependent on the emphasized properties. To address this issue, we used functional magnetic resonance imaging (fMRI) at 7 Tesla to investigate the representation of objects across whole brain regions during two delayed-match-to-sample tasks emphasizing either physical or semantic aspects of the objects. In the physical task, participants were asked to decide whether an object part presented after the delay period belonged to the remembered object. Whereas in the semantic task, they had to determine whether the test image was from the same category. We focused on two primary regions-of-interest identified using independent data: an object-selective region in ventral temporal cortex and a task-responsive region in lateral frontal cortex. In each region we measured whether the multi-voxel response pattern could be used to decode the remembered object during the delay period. In the physical task, the response of object-selective, but not prefrontal cortex, could be used to decode the identity of individual remembered objects. In contrast, during the semantic task, prefrontal, but not object-selective regions, showed significantly distinct representations for each remembered object. To examine what kind of information is actually maintained during the tasks, we performed a behavioral experiment using images or words as distracters during the delay period. The physical task was more impaired with image than word distracters, whereas the opposite was true in the semantic. This result suggests that participants mainly maintained the appropriate physical or semantic properties of the objects during the delay period. Altogether, the imaging and behavioral results suggest that the distribution of information about remembered objects is dependent on the nature of the information that the task demands. Object-selective areas are primarily involved in maintaining visual, but not semantic, information whereas prefrontal areas are primarily involved in maintaining semantic, but not visual, information.

Disclosures: S. Lee: None. **D.J. Kravitz:** None. **C.I. Baker:** None.

Poster

908. Childhood Socio-Economic Status and Brain Development

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant HD055689

NIH Grant DA022807

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NIH Grant DA014129

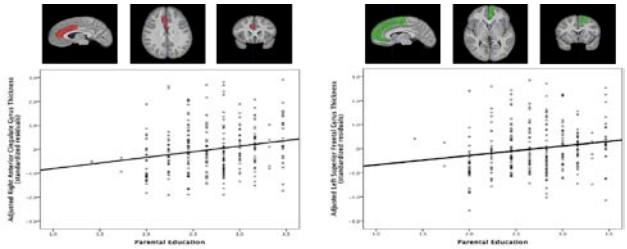
NIH Grant NS045839

Title: Associations between socioeconomic status and cortical thickness in prefrontal cortical subregions

Authors: *G. M. LAWSON, J. T. DUDA, J. WU, B. B. AVANTS, M. J. FARAH
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Abstract: Childhood socioeconomic status (SES) is associated with disparities in executive function performance, and SES differences have been observed in measures of prefrontal cortical function, but little is known about the structural correlates of childhood SES in prefrontal cortex. Structural MRI data from a sample of 283 healthy children from the first time point of data collection in the National Institute of Health (NIH) Magnetic Resonance Imaging (MRI) Study of Normal Brain Development were used to systematically investigate the association between childhood SES and cortical thickness in subregions of prefrontal cortex. T1-weighted MRI data were processed using Advanced Normalization Tools (ANTs) and the associated pipelining framework PipeDream. Cortical thickness, the shortest distance between the white matter surface and pial gray matter surface, was estimated within prefrontal regions of interest (ROIs) using the Diffeomorphic Registration Based Cortical Thickness (DiReCT) algorithm. Hierarchical linear regression was then used to assess the association between two principal measures of childhood SES, family income and parental education, on cortical thickness in ten prefrontal ROIs and three asymmetry measures. After correcting for multiple comparisons and controlling for age, sex, total brain volume, BMI, IQ, and race, higher parental education significantly predicted increased cortical thickness in the right anterior cingulate gyrus and left superior frontal gyrus. Scatterplots of parental education and cortical thickness in these regions, adjusted for covariates,

are shown in Figure 1. These results suggest that brain structure in frontal regions may provide a meaningful link between SES and cognitive function among healthy, typically developing children.



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Poster

908. Childhood Socio-Economic Status and Brain Development

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Topic: F.01. Human Cognition and Behavior

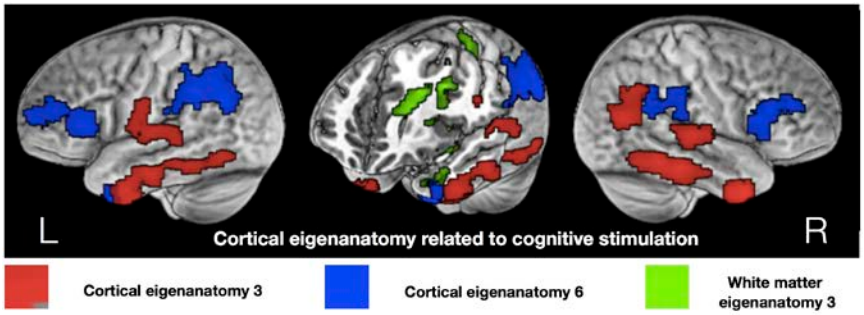
Support: NIDA 5R01DA014129-11

Title: Early childhood home environment predicts frontal and temporal cortical thickness in the young adult brain

Authors: *B. AVANTS[1], L. BETANCOURT[2], J. GIANNETTA[2], G. LAWSON[3], J. GEE[3], M. FARAH[3], H. HURT[2]
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Abstract: Introduction: Evidence from animal models suggests that the early environment has long-term consequences on later cognitive functioning and brain development. Little work has been performed in human subjects to test whether normal variation in early experience has measurable long term effects on the brain [1,2].
Methods: Our analysis leverages 64 participants from a low socioeconomic status background who were followed from birth and who underwent T1 and diffusion tensor (DT) MR imaging on a Siemens 3T Trio. Each subject underwent the HOME assessment at age 4. MRI was collected later in life in both 29 male (a.a. = 19.4 ± 1.17) and 35 female (a.a. = 19.1 ± 1.30) subjects. Differences in the age of the cohort distribution and in parental IQ between males and females

are not significantly different. Each subject also underwent the HOME assessment at age 4 which is a questionnaire that may be used to quantify a more cognitively stimulating and emotionally supportive home. In this work, we use neuroimaging and machine learning-based statistics to efficiently test the hypothesis that networks supporting cognition should be enhanced by increased stimulation and nurturance during childhood. Our statistical model, implemented in the R programming language, controls for age, gender and parental IQ.
Results and Discussion: Our results show, for the first time, that cortical thickness in early adulthood is reduced (independently from parental IQ) by increased quality of the home environment at age 4. These results, which identify a novel network of cortical regions that are directly influenced by parental behavior, appear in Figure 1. A further analysis revealed that early cognitive stimulation has a stronger effect on frontal lobe cortical thickness in female subjects ($p < 0.0069$) than in males ($p < 0.5000$) and that the temporal lobe regions are related to verbal comprehension as measured near the time of imaging. This is one of the few studies to confirm the effect of normal variability in the home on neuroanatomy.
[1] van Praag H et al Nat Rev Neurosci 1: 191–198.
[2] Duncan GJ et al Child Dev 65: 296–318.



Disclosures: B. Avants: None. **L. Betancourt:** None. **J. Giannetta:** None. **G. Lawson:** None. **J. Gee:** None. **M. Farah:** None. **H. Hurt:** None.

Poster

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John M. Driscoll, MD Scholars Program to KGN

Title: Neural correlates of socioeconomic status in the developing human brain

Authors: *S. HOUSTON[1,2,3], E. KAN[1,2], E. R. SOWELL[1,2], K. G. NOBLE[4,5,6]
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Abstract: Introduction: Socioeconomic disparities in childhood are associated with remarkable differences in cognitive and socio-emotional development during a time when dramatic changes are occurring in the brain. Yet, the neurobiological pathways through which socioeconomic status (SES) shapes development remain poorly understood. Behavioral evidence suggests that language, memory, social- emotional processing, and cognitive control exhibit relatively large differences across SES. Here we investigated whether volumetric differences could be observed across SES in neural regions that support these skills, including the hippocampus, the amygdala, the anterior cingulate cortex and several structures in a language-supporting network in the left temporal, temporo-occipital and frontal cortices.
Methods: Sixty typically developing, socioeconomically diverse children (31 female) were recruited, ranging in age from 5 to 17 years (Mean=11.4, SD=3.1). Participants had no history of neurological impairment, psychiatric disability, learning disability, language impairments, developmental delay, or significant exposure to prenatal teratogens such as alcohol. Two to four sagittal T1-weighted images were collected for each participant using the following parameters: TR= 1900 ms; TE= 4.38 ms; flip angle, 15; matrix size, 256x256; voxel size, 1 x 1 x 1 mm; acquisition time, 8 min 8s. Pre-processing and definition of cortical and subcortical gray matter regions on structural images were conducted using automated brain segmentation software (Freesurfer, 4.5). First, relationships between SES and regional cortical volume were explored. Next, we examined the degree to which the effects of SES factors on regional brain volume might change with age, by examining SES x age interactions in each ROI.
Results: Highly significant SES differences in regional brain volume were observed in the hippocampus and the amygdala. Specifically, higher parental education levels predicted larger amygdala volumes, whereas higher income-to-needs ratios predicted larger hippocampal volumes. In addition, SES x age interactions were observed in the left superior temporal gyrus and left inferior frontal gyrus, such that children from higher SES families showed relatively greater volumetric increases with age in these regions. These results were not explained by differences in gender, race or IQ. Likely mechanisms include differences in the home linguistic environment and exposure to stress, which may serve as targets for intervention at a time of high neural plasticity.

Disclosures: S. Houston: None. **E. Kan:** None. **E.R. Sowell:** None. **K.G. Noble:** None.

Poster

908. Childhood Socio-Economic Status and Brain Development

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Program#/Poster#:908.04/BBB27

Topic: F.01. Human Cognition and Behavior

Support: NIH NIDCD DC000128

Title: Effects of childhood socioeconomic status on cognition and related neural systems in adulthood

Authors: *E. PAKULAK, Y. YAMADA, A. HAMPTON WRAY, E. ISBELL, H. NEVILLE
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Abstract: A large and growing literature documents the profound impact of lower socioeconomic status (SES) on cognitive skills and brain structures and functions in children (Hackman, Farah, & Meaney, 2010). In previous event-related potential (ERP) studies we have reported that lower SES children display reduced neural modulation by selective attention compared to higher SES children (Stevens, Lauinger, & Neville, 2009) and that neural systems supporting attention are modifiable by training (Stevens et al., 2008; Neville et al., 2011, under review). We have also reported that the relationship between SES and cognition persists into adulthood, as childhood SES predicts both native language proficiency as well as the ERP response to syntactic processing in adult native speakers (Pakulak & Neville, 2010). Here we show that this relationship extends to other aspects of cognition and to a different, more ecologically valid ERP language processing paradigm. Finally, we present preliminary evidence suggesting that certain aspects of these systems may be malleable in adults from lower SES backgrounds. Typically developing adults from a wide range of SES backgrounds were administered a comprehensive battery including measures of working memory and language and three ERP paradigms assessing selective attention and different subsystems of language. Childhood SES was significantly predictive of working memory in adulthood. Employing the same ERP measures of attention previously employed (e.g., Stevens et al., 2009), we found that adults from higher SES backgrounds showed enhanced neural modulation by selective attention to linguistic probe stimuli compared to adults from lower SES backgrounds. Using an ERP language paradigm featuring a more ecologically valid context, we replicated and expanded our previous findings (Pakulak & Neville, 2010). Adults from higher SES backgrounds and who scored higher on standardized measures of language proficiency showed neural responses

consistent with greater maturity to both semantic and syntactic violations. Finally, we provide evidence that aspects of attention are trainable in adults. We measured attention/executive function abilities of parents of lower SES children randomly assigned to either an evidence-based parenting program or one of two control groups and observed improvements in specific aspects of attention after eight weeks in the parent training program only. These results suggest that the effects of lower childhood SES on the development of multiple cognitive systems extends into adulthood, but also that such effects may be ameliorated by training in adulthood.

Disclosures: E. Pakulak: None. **Y. Yamada:** None. **A. Hampton Wray:** None. **E. Isbell:** None. **H. Neville:** None.

Poster

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Program#/Poster#:908.05/BBB28

Topic: F.01. Human Cognition and Behavior

Support: NIH NIDCD DC00128

Title: Effects of SES on neural mechanisms supporting multiple subsystems for language processing

Authors: *H. J. NEVILLE, E. PAKULAK, Y. YAMADA, A. HAMPTON WRAY
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Abstract: A growing literature documents the profound impact of lower socioeconomic status (SES) on cognitive skills and brain structure and function in children (Hackman, Farah, & Meaney, 2010). Numerous studies have shown that children from lower SES families have reduced language abilities compared higher SES peers (Hart & Risely, 1995; Huttenlocher, 2002; Noble, McCandliss, & Farah, 2004). Previously we showed that early neural indices of selective attention, a key factor important for school readiness, are reduced in lower SES children (Stevens, Lauinger, & Neville, 2009). We have also demonstrated that attention systems in lower SES preschoolers are highly modifiable. Following an eight-week parenting training and attention training program for children, children's cognition and neural mechanisms of attention improved significantly compared to a passive or an active control group (Neville et al., 2011; under review).
Among the aspects of cognition to improve following training was receptive language, consistent with previous findings suggesting that brain functions for language are highly sensitive to differences in experience early in development. However, previous studies of neural systems

mediating language processing in young children are limited and few, if any, examine the relationships between neural indices of language and SES. We developed a novel, ecologically valid paradigm containing naturally spoken sentences accompanied by an animated video in a coherent narrative to assess language processing indexed by event-related potentials (ERPs). We examined the relationships between neural subsystems for language and SES in 3-5 year-old children. Our child-friendly paradigm contains semantic and phrase structure violations and canonical controls. Semantic violations elicited smaller N400 amplitudes in the higher compared to the lower SES group, consistent with a more mature pattern for semantic processing. Syntactic violations elicited a biphasic response. The late-onset precursor to the typical adult anterior negativity began earlier and was larger in amplitude in the higher compared to the lower SES children. This suggests SES-related differences in the maturation of systems hypothesized to underlie more automatic syntactic processing in adults. The later positive component resembling the P600 was comparable across groups. These results add to the literature by documenting that SES impacts neural mechanisms underlying multiple neurocognitive systems, including specific subsystems for language. There is an urgent need for evidence-based educational programs that can ameliorate the large and growing achievement gap in the US.

Disclosures: H.J. Neville: None. **E. Pakulak:** None. **Y. Yamada:** None. **A. Hampton Wray:** None.

Poster

908. Childhood Socio-Economic Status and Brain Development

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:908.06/BBB29

Topic: F.01. Human Cognition and Behavior

Title: Girls and boys with Autism Spectrum Disorders show different stochastic signatures of movement decision time and pointing kinematics

Authors: *J. V. JOSE[1,2], R. W. ISENHOWER[4], E. B. TORRES[5,3]
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Abstract: Current inventories used to diagnose Autism Spectrum Disorders (ASD) apply similar criteria to boys and girls despite well-established differences prevalent across the course of normal development between the genders. Recent work suggests that girls with ASD run the risk

of receiving a delayed diagnosis, potentially missing a critical window of opportunity for early intervention. Here we address this question in the context of natural behaviors. Children used variants of a simple computerized match-to-sample task using pointing gestures to communicate their choice. The forward, ***task-relevant*** component of the pointing gesture was contrasted with the retracting, ***task-incidental*** component. We assessed gestural kinematics in tandem with cognitive parameters of the decision making process over hundreds of repetitions in each of 30 children (12 girls, 18 boys, 4-16 years old). Sensory-motor gestural parameters included linear and angular speed timing (head, trunk, arm segments) towards and away from the target. Cognitive parameters included the speed and accuracy of the decision. The experiments took place in the naturalistic settings of the classroom. The random fluctuations of the gestures and cognitive parameters across hundreds of repetitions revealed highly skewed frequency distribution. Their stochastic signatures uniquely localized each child in the Gamma probability distribution parameter plane with 95% confidence level.. Across the broad ASD spectrum, from low-functioning to TD, the Gamma distribution captured the range of stochastic variability signatures from exponential to normal. Dramatic gender-specific and cognitive performance-specific effects separated the variability of each gender group and that of each individual. Specifically, the signatures of the boys with ASD were in the exponential range of the Gamma phase space. The TD fell intermediate in the skewed range and those of the girls with ASD were in the Gaussian range. Plotting the girl's parameter data in a log-log plot revealed a scaling effect with a well characterized by a power-law relation (linear regression R-square 0.99, rmse 10^-4, slope 1.002, intercept 0.005) whereby early in development girls with ASD fall closer to TD girls but as they age their signatures dramatically shift along the line. We discuss these results in terms of a marked gender-dependent sensory-motor difference. Our results invite reconsidering gender specificity in ASD relative to the broader spectrum of human performance: from autistic to typical, in particular, early subtle differences in girls' performance towards typicality should not mislead clinicians and delaying diagnosis.

Disclosures: J.V. Jose: None. **R.W. Isenhower:** None. **E.B. Torres:** None.

Poster

908. Childhood Socio-Economic Status and Brain Development

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Program#/Poster#:908.07/BBB30

Topic: F.01. Human Cognition and Behavior

Support: NSF 094158

New Jersey Governor's Council for Medical Research and Treatment of Autism 10-403-SCH-E-0

Title: Statistical classification tools for autism spectrum disorders: motor variability across the autism spectrum and beyond

Authors: *R. W. ISENHOWER, III[1], J. V. JOSÉ[2], E. B. TORRES[1]
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Abstract: Autism Spectrum Disorders (ASD) are characterized by a constellation of neurodevelopmental problems encompassing social, communicative, and sensory-motor impairments. Manifestations of the disorder vary from low-functioning to high-functioning/Asperger's (HFA/AS). Autism purportedly occurs more often in families of physicists, engineers, and mathematicians (Baron-Cohen et al., 1998) and instruments, such as the Autism Quotient (AQ), indicate that individuals in these fields are more likely to score similarly to those with HFA/AS (Baron-Cohen et al., 2001). Instead of exclusively relying on surveys that make inferences about social functioning, here we take an objective sensory-motor based approach and directly measure the statistical structure of the random fluctuations of the variability of physical movements, which serve as re-afferent sensory input. We reasoned that this input may be utilized differently by individual with different cognitive predispositions. To test this idea, we map stochastic signatures of several movement trajectory parameters and compared them across the human cognitive and sensory-motor continuum--from non-verbal low-function autism, to those with HFA/AS, to individuals from a variety of cognitive predispositions/backgrounds (literature, psychology, biology, engineering, computer science, physics, mathematics). We use the Gamma probability distribution family to characterize with 95% confidence the variability of pointing trajectories in each individual using maximum likelihood estimation. We find that the severity of ASD (or its absence) correlates well with an individual's location of his/her stochastic signatures in the Gamma-parameter plane. When deliberately pointing to a target, the variability of peak linear velocity of non-verbal individuals falls in the exponential range of the Gamma. While HFA/AS are in the skewed range, those mathematically inclined who scored high on the AQ, fall in the normal range of the Gamma. Assessment of the time to reach peak linear velocity of retracting task-incidental motions yielded the exact opposite trend. According to these objective sensory-motor metrics individuals who scored high on the AQ may not necessarily have HFA/AS, but rather fall along the broad spectrum of cognitive and sensory-motor behavior. Furthermore, differentiating individuals at this end of the spectrum may become more difficult after AS is subsumed under Autistic Disorder in the DSM-5. The present results therefore invite complementing current clinical evaluations with objective sensory-motor metrics of behavior to help resolve ambiguities at the upper end of the autism spectrum.

Disclosures: R.W. Isenhower: None. **J.V. José:** None. **E.B. Torres:** None.

Poster

908. Childhood Socio-Economic Status and Brain Development

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Program#/Poster#:908.08/BBB31

Topic: F.01. Human Cognition and Behavior

Support: 1R01HD067312-01

Title: Structural connectivity predicts risk for dyslexia in kindergarteners

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Abstract: Developmental dyslexia, defined as an unexplained difficulty in learning to read, is quite prevalent and has been associated in children and adults with alterations in white matter organization as measured by diffusion-weighted imaging (DWI). It is unknown, however, whether these differences in structural connectivity are related to the cause of dyslexia, or instead are consequences of reading difficulty (e.g., far less reading experience, or the development of compensatory brain organization). Here, we examined the relation of white-matter organization to major behavioral predictors of dyslexia, including phonological awareness (PA) for language sounds, in 20 kindergartners (mean age=5.6 years, 9F), who had little or no reading instruction. We examined white matter tracts known to connect critical components of language and reading networks, specifically the inferior longitudinal (ILF) and arcuate fasiculus (SLFa), using a tracts of interest (TOI) approach with probabilistic tractography and also at the whole brain level. TOIs were defined using a new automated method (TRACULA/FreeSurfer/FSL) that reliably defines major tracts in each participant's native diffusion images using tractography and anatomical priors based on manual labeling. We measured volume, fractional anisotropy (FA), radial (RD) and axial diffusivity (AD) in three TOIs bilaterally: ILF, SLFa, and a control tract (parietal section of SLF). The relationship between these diffusion measures and behavioral assessments, including PA, were tested by means of cross-validation correlations. Whole brain regressions of behavioral scores with diffusion measures were also performed.
Superior composite PA scores were significantly and positively correlated with the volume and FA of left SLFa, but not with any other tracts/measures, including controls of head size and SNR variance. The correlation was selective, since other behavioral measures associated with risk for dyslexia, such as naming fluency, did not correlate with SLFa volume or FA. We next explored which PA tasks drove the correlation with left SLFa. Left SLFa tract volume, AD, and FA,

correlated only with Blending Words (and not with Elision or Non-Word Repetition). Whole-brain models of these diffusion measures were consistent with the TOI analyses.
These findings reveal that the left SLFa, which has been frequently associated with poor reading in previous studies, is already smaller and has lower FA and AD in kindergarteners at risk for dyslexia because of poor PA. This suggests a structural basis of risk for dyslexia that predates reading instruction.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: National Institutes of Health HD047520, HD059205, K01MH092288

Netherlands Organization for Scientific Research NWO 446.10.010

Title: Hippocampal and prefrontal reorganization underlies the maturation of fact retrieval: A longitudinal fMRI study in young children

Authors: *S. QIN, S. CHO, M. ROSENBERG-LEE, D. GEARY, V. MENON
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Abstract: Basic fact knowledge lies at the foundation of human cognitive development. The ability to efficiently retrieve basic facts from memory and bring them to bear in constantly changing situations is a key characteristic of mature problem solving in children. During development, children's math problem solving skills become gradually dependent on memory-based strategies such as direct retrieval, and less dependent on effortful procedures such as counting. Little, however, is known about neurodevelopmental mechanisms underlying these transitions in children.
We conducted a longitudinal functional magnetic imaging (fMRI) study to investigate neurodevelopmental changes associated with increased use of retrieval strategies in children's mathematical learning. Participants included thirty 7 to 9-y-old children who were scanned twice ('Time 1' & 'Time 2') separated by 18 months. During scanning, children were instructed to solve a set of single-digit addition problems. Each child's mix of strategies for solving problems was assessed using a standardized and well-validated measure that classifies strategies based on

experimenter observation and child self-reports. Functional brain images were acquired on a 3T GE Signa scanner using a T2* weighted gradient echo spiral in-out pulse sequence.
We found that the use of retrieval strategies in children increased over time whereas counting strategies decreased over time. These behavioral results suggest that with time children became more reliant on memory-based strategies. fMRI analyses of the two time points replicated a widely distributed frontoparietal network involved in solving addition problems. Importantly, comparing 'Time 2' with 'Time 1' for solving addition problems revealed significantly greater activation in the bilateral hippocampus. In contrast, there was reduced activation in the bilateral dorsolateral prefrontal cortex (DLPFC) and right angular gyrus (AG) over time.
Over an 18 month period children became less dependent on counting strategies and more dependent on retrieval strategies while solving addition problems. This transition was paralleled by increased hippocampal and decreased DLPFC and AG engagement over time. Our behavioral and neuroimaging findings provide important evidence that the transition from counting to more efficient retrieval-based strategies is characterized by greater reliance on the hippocampal memory system and decreased reliance on the prefrontal cortex. Our findings have important implications for understanding knowledge acquisition in early childhood.

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Poster

908. Childhood Socio-Economic Status and Brain Development

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Program#/Poster#:908.10/BBB33

Topic: F.01. Human Cognition and Behavior

Title: Individual differences in adolescents' ability to filter items for working memory predict neural structure and function.

Authors: *A. S. FINN[1], M. A. SHERIDAN[4], J. SALVATORE[2], J. LEONARD[2], J. GABRIELI[3]
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Abstract: The ability to filter information into or out of working memory (WM) predicts an individual's WM capacity or the amount of information (no longer present in the environment) that one can hold in mind (Vogel, McCollough, & Machizawa, 2005). Evidence from both infrahuman primate neurophysiology and human neuroimaging has highlighted the importance

of basal ganglia structures, including the caudate and putamen, in the filtering of information into WM (Pasupathy & Miller, 2005; McNab & Klingberg, 2008). WM ability has been shown to be related to myriad measures of learning, school outcomes, and general fluid intelligence, so the ability to filter-or gate access to WM-may be important in development and education. In the present study, we asked whether the basal ganglia plays a similar role in the brains of adolescents, and whether variation in basal ganglia function and structure is related to WM variation among adolescents. We administered a WM filtering task during functional MRI in which adolescents were asked to remember either 4 or 2 stars that were presented on a screen. Adolescents were told that a cue (a circle or a square) would indicate whether they would need to keep all of the stars in mind over a delay or whether they would have to remember a subset of the stars (i.e., filter irrelevant information out of WM). Data from 15 adolescents (aged 13 to 16, mean 14.8; 6 females) reveals that individual differences in the ability to filter information are related to 1) two separate measures of working memory ability (nback: r=.57 , p=.067; count span: r=.4, p =.1), 2) the degree to which the prefrontal cortex is recruited during the delay period for working memory (r=.554, p =.032), 3) the degree to which the basal ganglia are recruited while holding information in mind (r=.569, p=.027) and 4) the volume of the caudate (r= -.481, p =.070) across individuals (anatomically defined). These behavioral, functional and structural relationships converge to highlight the importance of filtering for working memory in adolescents. These data suggest that ongoing development of the basal ganglia-and not just the prefrontal cortex-should be considered in thinking about the development of WM ability during adolescence.

Disclosures: A.S. Finn: None. **M.A. Sheridan:** None. **J. Salvatore:** None. **J. Leonard:** None. **J. Gabrieli:** None.

Poster

908. Childhood Socio-Economic Status and Brain Development

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Program#/Poster#:908.11/BBB34

Topic: F.01. Human Cognition and Behavior

Title: Emotional memory consolidation over mid-day sleep in early development

Authors: *L. B. KURDZIEL, J. KENT, R. M. C. SPENCER
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Abstract: The circadian sleep-wakefulness rhythm changes throughout development. Serum cortisol levels demonstrate a distinct circadian rhythmicity, with adult-like amplitudes, by the

age of 6 months; however, children do not develop the adult-like monophasic sleep pattern, consolidating sleep to one nocturnal bout, until 4-6 years of age. The mid-day nap must therefore play an important role in development. Children 33-60 months of age ('preschool children') rapidly develop emotional processing skills. A recent study and decades of anecdotal evidence, suggests that mid-day sleep plays a role in emotional processing at this age: Children are emotionally dysregulated in the absence of a mid-day nap. In young adults, it has been suggested that emotional memories are prioritized for consolidation over sleep. As such, we hypothesized that mid-day naps benefit emotional regulation by consolidating recent emotional memories. To examine this, preschool children (mean age = 51.7 +/- 6.7 months) were tested on their memory for faces described as either "mean," or "nice." Children were tested following a typical mid-day nap and an equivalent period of wake. All were tested in both conditions, separated by 1-3 weeks, to control for differences in brain maturation and only children who napped at least 60 minutes were included in the analysis (n = 28). Memory performance was not different immediately following a nap and wake period. However, when tested the following day, a delayed benefit of the prior day's nap was seen: Memory for faces was significantly better when children had napped the day before ($F(1,22) = 4.22$, $p = 0.05$). Nap-dependent consolidation was greatest for mean faces ($t(22) = 1.68$ $p = 0.05$). Moreover, the total time spent napping for all children correlated with overall memory performance improvement ($r = 0.33$, $p = 0.03$). Finally, the benefit of sleep on memory was exaggerated in children with initial low memory performance, and this was true for both mean ($t(33) = 4.03$, $p < 0.001$) and nice ($t(33) = 2.14$, $p = 0.04$) faces. These results support a role of mid-day sleep in emotional memory consolidation during development. The delay in observing the benefit of sleep is similar to that reported in song birds: Juvenile zebra finches decreased song performance following sleep yet those that performed worse over sleep actually learned the song the best overall in the long-term.

Disclosures: L.B. Kurdziel: None. **J. Kent:** None. **R.M.C. Spencer:** None.

Poster

908. Childhood Socio-Economic Status and Brain Development

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Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust WT096195

NIMH Grant MH087510

Title: Lateralisation of myelination during postnatal development relates to language ability

Authors: *J. O'MUIRCHEARTAIGH[1,2], D. DEAN, III[1], H. DIRKS[1], N. WASKIEWICZ[1], K. LEHMAN[1], B. JERKSKY[1], S. DEONI[1]
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Abstract: Background: The human cerebral hemispheres are functionally lateralized for specific cognitive domains, e.g. language, spatial processing. This is reflected by gross anatomical and functional asymmetry in adulthood. Although functional lateralization develops over time from infancy to childhood to adulthood, structural connectivity is likely to mediate this lateralization through more efficiently myelinated axonal bundles. The representation of this asymmetry at birth and its development remains unclear.
Objective: Here we use a quantitative myelin-specific MRI technique to index white matter asymmetry over the whole brain in a cohort of children under the age of 6, hypothesizing that the degree of structural lateralization will increase with age.
Methods: 109 children (66 Male) between the ages of 12 and 72 months (mean 37.3m) took part in this study. MRI data was collected using the mcDespot sequence (Deoni et al, 2008), a relaxometry technique that allows calculation of the myelin water fraction, a more specific, quantitative measure of myelin content. Each dataset was flipped on the sagittal axis and both images were spatially normalized to a custom pediatric template in approximate MNI space using ANTS (Avants et al, 2008), spatially smoothed with a 6mm Gaussian kernel, and a scaled lateralization index was created for every subject (L-R)/(L+R). A general linear model modeling for mean lateralization, age and gender was designed. The resulting model was estimated voxelwise by permutation and considered significant at $p<0.05$ corrected for multiple comparisons.
Results: Significant leftward asymmetries were detected in the anterior cingulum bundle and mesial frontal regions, anterior thalamus and caudate, as well as visual, temporal, and motor (localized over the hand knob) cortical areas. Rightward asymmetry was detected in the external capsule, as well as Heschl's gyrus. Frontal asymmetry significantly predicted receptive language scores ($F=6.363$, $df=1,92$, $p<0.013$).
Discussion: The resulting asymmetry of myelin volumetric quantity indicates that myelination differences are occurring in both focal cortical areas and basal ganglial cortical loops (anterior thalamus, caudate and mesial frontal; putamen, ventral frontal). The frontal leftward lateralization specifically was functionally meaningful, relating significantly to receptive language ability. However, this lateralization didn't significantly change with age or gender, though actual myelin quantity increases with age. This indicates that cerebral lateralization may be biologically constrained prior to functional development, and may predict cognitive ability.

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Poster

909. Individual Differences II

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant GM103526

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NIH Grant EB006589

Title: Does the resting state connectivity have hemispheric asymmetry? A near-infrared spectroscopy study

Authors: *A. V. MEDVEDEV
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Abstract: Near-infrared spectroscopy (NIRS) is a promising new technology for low-cost noninvasive brain imaging suitable for use in virtually all subject and patient populations. Numerous studies of brain functional connectivity using fMRI, and recently NIRS, suggest new tools for the assessment of cognitive functions during task performance and the resting state (RS). We analyzed functional connectivity and its possible hemispheric asymmetry measuring coherence of optical signals at low frequencies (0.01-0.1 Hz) in the prefrontal cortex in 13 right-handed (RH) and 2 left-handed (LH) healthy subjects at rest (4-8 min) using a continuous-wave NIRS instrument CW5 (TechEn, Milford, MA). Two optical probes were placed bilaterally over the inferior frontal gyrus (IFG) and the middle frontal gyrus (MFG) using anatomical landmarks of the 10-20 system. As a result, 28 optical channels (14 for each hemisphere) were recorded for changes in oxygenated (HbO) and deoxygenated (HbR) hemoglobin. Global physiological signals (respiratory and cardiac) were removed using Principal and Independent Component Analyses. Inter-channel coherences for HbO and HbR signals were calculated using Morlet wavelets along with correlation coefficients. Both coherence- and correlation-based Fisher-transformed connectivity matrices showed qualitatively similar patterns of connectivity which was higher within each anatomical region (IFG and MFG) and between hemispheres (e.g., left IFG <-> right IFG) than between IFG and MFG in the same hemisphere. Laterality indexes were calculated as t-values for the ‘left > right’ comparisons for each regional group of channels in each subject. In the right-handed group, the laterality indexes were relatively small and variable with still significantly higher connectivity in the right hemisphere in the majority of RH subjects. In contrast, the LH subjects had highly negative laterality indexes thus demonstrating a significantly greater hemispheric asymmetry with the dominance of the right hemisphere

compared to the RH group.
Our data further demonstrate the feasibility of using NIRS for the analysis of resting state functional networks. NIRS-based connectivity patterns correspond well to the anatomical and functional compartmentalization of the prefrontal cortex (e.g., inferior and middle frontal gyri). Furthermore, the results provide first evidence on the hemispheric asymmetry of the RS connectivity which is especially strong in the left-handed individuals. These data encourage further exploration of the NIRS connectivity and its application for the analysis of hemispheric relationships within the functional architecture of the brain.

Disclosures: A.V. Medvedev: Research Grant; National Institutes of Health.

Poster

909. Individual Differences II

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Program#/Poster#:909.02/BBB37

Topic: F.01. Human Cognition and Behavior

Support: Levy Edersheim Gitter Institute for Neuroimaging

Adams Super Center for Brain Studies

Title: Neural-dissection of motivational processes underlying personality traits

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Abstract: Motivation is a psycho-behavioral concept which refers to the drive to facilitate or inhibit behavior in response to environmental incentives and threats. The “Reinforcement Sensitivity Theory“ (RST) is an animal based neurobehavioral model comprising three distinct systems involved in motivational processes, which we have recently validated in humans via fMRI. These motivational tendencies were further conceptualized as core personality traits of Sensitivity to Reward (SR) and to Punishment (SP). However, since reinforcements (both appetitive and aversive) carry the hedonic value of pleasure (i.e. "liking"), along with motivational value of incentive (i.e. “wanting"), it is important to dissociate these two processes in order to elucidate the neural correlates underlying personality related motivational processes. We conducted an fMRI study in 50 healthy participants using a novel competitive computer game which was constructed to manipulate approach and avoidance tendencies. The goal of the

game is to catch rewarding coins, and avoid punishing balls. Additionally, uncontrolled events of random rewards and punishments allow for separation of the intentional (incentive) from unintentional (hedonic) rewarding or punishing events. Following the brain scan, subjects completed the Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ). We used whole brain GLM analysis including four regressors derived from intentional and unintentional, reward and punishment events, to functionally identify the neural correlates of motivational processes related to wanting and liking. We further used the hippocampus, a key node of the RST motivation system, as a seed region for functional connectivity (FC) analysis, in order to assess the mutual operation of different motivational systems throughout the game and its relation to SR/SP personality traits.
We established dissociation between an incentive-action related network and hedonic DMN-like evaluative network. Furthermore, FC from the hippocampus revealed several key regions of the RST motivational systems such as the ventro-medial PFC (vmPFC) and amygdala. Moreover, differences in FC patterns were found to be related to the individual differences in SR/SP traits: stronger FC of the vmPFC with hippocampus was associated with higher RS but lower PS; whereas for the amygdala, FC with hippocampus was stronger for higher RS and PS.
Our findings suggest that personality traits related to motivation manifest as differential connectivity patterns between the sub-systems of the RST, rather than relating to a single sub-system's regional activation.

Disclosures: T. Gonen: None. **E. Eldar:** None. **T. Hendler:** None.

Poster

909. Individual Differences II

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Program#/Poster#:909.03/BBB38

Topic: F.01. Human Cognition and Behavior

Title: A case of isoniazid-induced delirium

Authors: *B. DEMARTINI, O. GAMBINI
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Abstract: ABSTRACT:
Objective: to report a case of isoniazid induced delirium
Case summary:
A 56-year-old man affected by alcoholism and hepatic cirrhosis was admitted to hospital for a suspected pneumonia with pleural effusion and thickenings. A Mantoux skin test was positive. A

core biopsy of the pleural nodules revealed a tubercular granulomatous pleuritis. An isoniazid, ethambutol and rifampicin therapy was then started. Subsequently the patient developed a state of delirium. A diagnostic work-up was carried out considering different hypothesis: hepatic encephalopathy was ruled out because of lack of response to appropriate treatment; negative encephalic CT scan, liquor examination and EEG excluded a tubercular involvement of the central nervous system or other neurologic etiologies. Haloperidol and delorazepam were administered under the suspicion of Wernicke-Korsakoff syndrome, without any benefit. Finally a toxic etiology was considered. Among isoniazid side effects psychosis is described, though rare. Isoniazid therapy was then stopped and substituted by moxifloxacin, with progressive improvement of the state of consciousness.
Discussion:
Cases of isoniazid-related psychiatric disorders reported include psychosis, obsessive-compulsive disorder and mood alterations. Two mechanisms are generally considered as responsible: pyridoxin deficiency and isoniazid toxicity, a molecule chemically near to iproniazid, a powerful monoamineoxidase inhibitors. This is the first report of a case of isoniazid-induced pure delirium, with all clinical features according to DSM-IV criteria. We also confirmed successful resolutions of symptoms only by discontinuation of therapy.
Conclusions: in the work-up of a state of delirium, when investigating the toxic hypothesis, clinicians should consider neuro-psychiatric side effects of isoniazid.

Disclosures: B. Demartini: None. **O. Gambini:** None.

Poster

909. Individual Differences II

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NIH Grant P41RR14074

HHMI

Title: Intrinsic hemispheric autonomy and its relation to functional lateralization

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Abstract: Certain functional systems of the human brain are strongly lateralized. Here we explored the asymmetric brain organization by quantifying the hemispheric interaction using intrinsic functional connectivity and investigated its relation to functional lateralization. We first introduce a novel concept, termed hemispheric autonomy, as the difference between within-hemisphere and cross-hemispheric connectivity. Hemispheric autonomy is computed on each voxel by subtracting the degree of cross-hemisphere connectivity from within-hemisphere connectivity. Both can be estimated based on the spontaneous activity measured by fMRI. Hemispheric autonomy was estimated for 1000 young adults. Strong autonomy was observed in left inferior prefrontal and temporal regions involved in language processing. Strong autonomy was also observed in right lateral frontal, insula and angular gyrus regions that associate with attentional control. Previous studies have suggested that these regions are among the most lateralized regions. Minimal autonomy was found in the motor, somatosensory, auditory and visual cortices. We next investigated the relation between hemispheric autonomy and language lateralization. 56 subjects participated in a semantic decision task. Autonomy in a left hemisphere mask, derived from the 1000 subjects, was estimated for each of the 56 subjects. We found that autonomy at rest correlated with language lateralization during the task ($r = 0.52$), indicating that the balance between within- and cross-hemispheric connectivity is associated with functional lateralization. Finally, a prefontal region that is involved in the frontoparietal control network showed strong autonomy in both hemispheres, implying that this region in different hemispheres may play different functional roles. To test this hypothesis, we used the brain parcellation recently reported by our group (Yeo et al., 2011) and computed autonomy for each of 7 functional networks across the 1000 subjects. In the left hemisphere, a significant correlation was found between the autonomy in the frontoparietal control network and the language/default network ($r = 0.49$). However, in the right hemisphere, the autonomy in the frontoparietal control network showed its strongest correlation with the attention network ($r = 0.51$). These results tentatively support the hypothesis that the frontoparietal network may be involved in the control of different functional elements in the two hemispheres.

Disclosures: D. Wang: None. **R.L. Buckner:** None. **H. Liu:** None.

Poster

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Title: Daytime sleepiness is associated with a loss of default mode network connectivity in cognitively intact elderly

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Abstract: Background: A set of cortical brain regions, collectively known as the default mode network (DMN) exhibit coherent activity during rest. DMN coherence can be quantified using functional connectivity magnetic resonance imaging (fcMRI) and has been suggested as a candidate biomarker for detecting early changes related to Alzheimer's Disease (AD). Specifically, decreases in DMN connectivity have been associated with mnemonic deficits and amyloid-β plaque pathology, but also "normal aging". The sleep-wake cycle is disrupted in AD, and these relationships may be detectable before the onset of symptoms. However, the relationship between chronic sleepiness and DMN connectivity in non-demented elderly individuals remains unclear.
Methods: Eighty-four elderly subjects (age 73.2±5.7 years, 44 male) were administered the Epworth Sleepiness Scale (ESS) questionnaire, a subjective rating of sleepiness, and underwent a resting-state fcMRI scan. A subset of subjects (n=75, age 72.6±5.6 years, 39 male) was assessed with a battery of neuropsychological (NP) examinations. A confirmatory factor analysis was applied to this NP data and factor loadings for 6 factors (Speed, Executive Function, Memory, Fluency, Switching, and Working Memory) were derived.
For the fcMRI analyses, we extracted timeseries data from a set of previously published seed regions (8mm diameter spherical) in the posterior cingulate cortex (MNI [0 -53 26]), medial prefrontal cortex ([0 52 -6]), and lateral parietal cortices (averaged between [-48 -62 36] and [46

-62 32]). The seed timecourses were correlated, normalized using Fisher's z, and averaged to produce a global measure of DMN integrity.
Results: We found that increasing daytime sleepiness was associated with decreased global DMN coherence. The correlations between nodes were also significantly related to ESS scores. We found no relationship with ESS and correlations between bilateral seeds in motor cortex, or between the DMN and three other cortical networks. We did not find a relationship with ESS and neuropsychological scores, except a trend-level relationship between increasing ESS scores and decreasing factor scores for the Speed factor, consistent with previous work. All analyses controlled for age.
Conclusion: A self-reported measure of chronic daytime sleepiness in cognitively intact elderly subjects is related to decreased DMN coherence during resting-state. Additionally, there is a trend-level relationship between NP speed of processing and sleepiness. These findings may have important implications for elucidating age-related and AD-related alterations in sleep and network dysfunction.

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Poster

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Title: Estimated cortical gray matter myelination of 1500 human brains using partial volume corrected T1- and T2-weighted MRI images.

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Abstract: The myelination pattern of the human cerebral gray matter has been investigated using MR techniques with results agreeing well with ex-vivo methods based on myelin staining. Recently Glasser & Van Essen (2011) presented their results for standard 1 mm isotropic 3T scanner protocols. At this resolution, a single voxel may contain both gray and white matter, as well as cerebrospinal fluid in the thinnest parts of the cortex (for example, V1). This motivated us to add partial volume correction to their technique. Our method estimates the intensities of different tissue classes at the boundaries, potentially allowing for a more precise myelin content measurement. We applied the method to a large cohort of subjects (N = 1500, 20.9 +/- 2.8 years, 610 males) to obtain group-averaged gray matter myelination maps from the ratio of raw T1- and T2-weighted images of each subject as well as bias-field corrected T1- and T2-weighted images independently. The Freesurfer pipeline was used to generate surfaces and estimate T1- and T2-weighted intensities in the gray matter layer. These data were then mapped to a common template using surface-based registration. Using 62 subjects that were scanned twice we investigated the reliability of the individual myelin maps. We found the primary sensory areas to be heavily myelinated and the association areas to be less myelinated, consistent with previous results. We further observed that partial volume corrections affect the gray matter myelination in the sulci more than in the gyri likely due to the interaction of voxel geometry and cortical geometry. Analysis of the test-retest data suggests improved reliability particularly in the prefrontal cortex when using partial volume correction. Since the cortical gray matter layer sends and receives projections from subcortical areas, next we applied our technique to investigate the covariation of cortical myelin map with subcortical volumes. After controlling for age, gender, handedness and intra-cranial volume, the most significant and reproducible correlation ($r \sim 0.15$) was found between visual cortex myelination and the volume of the thalamus. Additionally, we found a weaker correlation ($r \sim 0.11$) between amygdala volume and parahippocampal cortex myelination. These results are consistent with known anatomical projections.

Disclosures: R. Shafee: None. **R.L. Buckner:** None. **B. Fischl:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NICHD Grant HD29891

Title: Bilateral white matter microstructure accounts for individual differences in adult rapid naming and reading abilities

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Abstract: Mounting evidence from diffusion tensor imaging (DTI) studies suggests that the integrity of white matter tracts varies across individuals and may be related to specific cognitive skills. The current study sought to examine the relationship between adult reading ability and temporo-parietal white matter microstructure. Whole-brain DTI images were collected from 68 adults with varying reading ability, and white matter structures were selected on an individual basis using a semi-automatic segmenting tool. Consistent with findings from the developmental literature, fractional anisotropy in the superior corona radiata (SCR) was positively correlated with multiple measures of adult reading and language abilities, including rapid picture naming, timed single word reading, and passage comprehension. In contrast to the developmental literature, correlations were observed in both left and right SCR, suggesting that right hemisphere microstructure may play an increasingly important role in reading in adulthood and vary systematically with reading skill. Further, regression analyses revealed that after accounting for rapid picture naming scores, SCR microstructure no longer explained a significant proportion of the variance in either reading measure. Results indicate that SCR may underlie a common variable like verbal processing speed, which is in line with the hypothesis that the integrity of white matter fibers that transfer information between cortical “nodes” of language processing, in part, determines the speed and efficiency with which an individual computes linguistic stimuli.

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Poster

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Title: Neural correlates of political ideology: Individual differences in liberalism-conservatism are reflected in the brain's intrinsic functional organization

Authors: *C. L. COX[1], J. T. JOST[2], F. CASTELLANOS[1,3], M. P. MILHAM[4,3], C. KELLY[1], J. J. VAN BAVEL[2]
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Abstract: Though long thought to arise from differences in beliefs about human nature and personal experiences, recent research suggests that individual differences in political ideology (PI; liberalism/conservatism) are associated with more basic cognitive and motivational orientations (Jost et al., 2003; Oxley et al., 2008). Here, we examined whether individual differences in PI are associated with the intrinsic functional architecture of the brain.
Previous research has shown that PI is associated with both the structure and function of the dorsal anterior cingulate cortex (dACC). Liberalism is associated with increased gray matter (Kanai et al., 2011) and increased conflict-related processing in dACC (Amodio et al., 2007). The dACC is part of a network of regions, including the insula and striatum, that processes emotionally significant stimuli (Seeley et al., 2007). Regions within this network exhibit correlated low-frequency fluctuations in the resting state fMRI (R-fMRI) signal, quantified using intrinsic functional connectivity (iFC) methods. We investigated the relationship between the iFC of the dACC and individual differences in self-reported PI.
Forty-seven adults (mean age=23.7 yrs; 33 male) completed a 6 min R-fMRI scan and a self-

report questionnaire assessing PI. After standard preprocessing, the timeseries from regions of interest in bilateral dACC (Kanai et al., 2011) was extracted for each individual and correlated with every other voxel in the brain. PI scores were entered as a covariate in group analyses, producing maps of significant PI-iFC relationships ($Z>2.3$, $p<0.05$, corrected).
PI was significantly negatively related to iFC between the dACC and the right insula and putamen. Greater liberalism was associated with increased, while greater conservatism was associated with decreased iFC between these regions. A similar pattern was observed with system justification scores ($r=-0.42$, $p<0.05$), the tendency to believe that existing social and economic institutions and arrangements are fair and legitimate (Kay & Jost, 2003).
Our results provide the first evidence that political ideology is related, not only to task-evoked responses in the dACC itself, but to the intrinsic communication among regions within a broader functional network. These findings support the idea that basic cognitive orientations, such as preference for social stability/order over change (system justification), and the need to manage uncertainty and sensitivity to threat, all strongly associated with PI, are also reflected in the strength of connection among brain regions implicated in processing this information, even in the absence of any explicit task.

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Poster

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Support: National Institute of Mental Health R01MH087510

Wellcome Trust WT096195

Title: Myelination differences in preterm infants and toddlers

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Abstract: Introduction: Nearly 12.7 percent (more than 500,000) of pregnancies within the United States result in premature birth, while the rate of premature childbirths has been on the rise since the early 1990's (Martin, J.A. et al., 2007). Fortunately, the survival of these children has dramatically improved with advances in medical care and technology, however, 'preterms' are still faced with a significant risk of having neurodevelopmental deficits (Panigrahy et al., 2012). The maturation of myelinated white matter is fundamental to brain development; establishing coordinated communication pathways among unified neural systems. It is unknown, however, if this process is disrupted or altered in children born premature. Deoni et al. have investigated myelin maturation in healthy, typically developing children using an MRI-based myelin-specific imaging technique, multi-component single pulse observation of T1 and T2 (mcDESPOT; Deoni et al., 2011). This data provides a salient baseline for identifying myelination differences in children born preterm.
Objective: To investigate myelination differences between children born premature and those born at full term, 8 preterm (mean age=778.125 days, corrected to 40 week gestation [age range=187-1716 days], mean gestation=34 weeks) and 32 full term (mean age=794.125 days, [age range=179-1552 days], mean gestation=39.25 weeks) children were scanned and compared against data from children born at full term.
Methods: mcDESPOT-derived myelin water fraction (MWF) maps were acquired during natural, non-sedated sleep (Deoni et. al., 2011) and aligned to a common study template using the Advanced Normalization Tools (ANTs; Avants et al., 2008) package. Significant myelin content differences over time ($p<0.025$) between the preterm and term children were examined by calculating cluster-corrected voxel-wise t-statistics using FMRIB Software Library's (FSL) randomise tool.
Results: Widespread myelination deficiencies in the preterm group were found in areas that included bilateral frontal and parietal lobes, cingulate gyrus, right lingual gyrus, and left amygdala. These findings are consistent with previous imaging studies that have investigated differences between preterm and term children (Liu et al., 2012).
Conclusions: Presented results illustrate significant myelin-content differences between preterm and full term children. Although preliminary, these results suggest that myelination is in fact altered in preterm children and possibly underlies neurodevelopmental deficits associated with premature birth.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant 491746

Title: ADRA2b polymorphism influences attentional capture by emotional material and subsequent emotionally enhanced memory

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Abstract: Recent research has revealed that genetic variation associated with altered neurotransmission is linked to individual differences in the capacity for emotional memory. Specifically, a deletion variant of the ADRA2b gene, which influences norepinephrine (NE) activity in the amygdala, has been associated with emotional enhancement of memory as well as susceptibility to intrusive memory following trauma. Yet the distinct processes of emotional memory formation influenced by ADRA2b are not yet known. The goal of the present study was to test whether ADRA2b influences rapid encoding or more extended post-encoding processes, and to measure interactions with other genes at distinct phases of memory formation.
Saliva samples were collected from 224 young Caucasian adults (151 female) with no history of psychopathology. Effects of emotional salience on rapid attentional capture at encoding were measured with an attentional blink task. Participants also viewed arousing and neutral pictures, and recognition memory was measured one week later. Control measures of sex, neuroticism, working memory, and history of childhood abuse were also included. Polymorphisms of interest were ADRA2b, as well as the 5HTTLPR serotonin transporter gene (short vs. long) and a valine to methionine single-nucleotide polymorphism (SNP) in the COMT gene coding the catechol-O-methyl transferase enzyme that metabolizes prefrontal dopamine (DA).
Results showed that ADRA2b genotype predicted the influence of emotional salience on rapid encoding processes measured by the attentional blink after controlling for the influence of other genes and control variables (e.g., APOE, KIBRA). ADRA2b also influenced emotional enhancement of memory one week later in interaction with COMT genotype, with deletion carriers who had two copies of the COMT met allele showing greatest emotional enhancement of memory. History of childhood abuse also influenced emotionally enhanced memory but its contribution was unique, revealing no evidence of a gene-environment interaction. Overall, results show novel evidence for the influence of ADRA2b on the effects of emotion on rapid attentional capture. Effects on later recognition memory were more complex, suggesting that over longer-term consolidation processes, genetic variation linked to NE and DA processes interact to influence emotional memory.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: The retention rate and nature of Highly Superior Autobiographical Memories over time.

Authors: *A. K. LEPORT, J. L. MCGAUGH, C. E. L. STARK
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Abstract: We have recently documented a new memory ability known as Highly Superior Autobiographical Memory (HSAM). HSAM subjects have a remarkable ability to recall accurately a vast quantity of autobiographical details from autobiographical events that span across their lifetime. While it is clear their behavior is exceptional, there are many open questions concerning the qualities of their memories. The current study takes a step further by quantifying the number of details an HSAM participant can recall from very recent to very remote autobiographical memories and qualifying the elements that compose these memories. Both HSAM and age- and sex-matched controls were instructed to recall as many details as could be remembered from each of the past seven days as well as from each of the same seven days one and ten years ago (21 specific days probed). Comparisons between the two groups indicated overall similar levels of details recalled from one-day and one-week-old memories, but HSAM participants significantly outperformed controls at the one-year and ten-year-old time periods. This finding suggests that at least part of their ability stems from enhanced retention mechanisms. In addition, we will discuss results based on the Autobiographical Interview (Levine et al. 2002) scoring technique to analyze qualitative differences in the kinds of details retrieved.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Impact of the KIBRA polymorphism on autobiographical memory

Authors: *D. J. PALOMBO, R. S. C. AMARAL, B. LEVINE
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Abstract: Episodic memory refers to the recollection of temporally and spatially bound contextual details of an experience, which could include autobiographical episodes or information learned in a laboratory setting. Recently, the KIBRA gene has been associated with episodic memory performance in a replicated genome-wide association study (e.g, Papassotiropoulos et al., 2006). This gene is highly expressed in the hippocampus and has been linked to synaptic plasticity. Carriers of a (T to C) SNP in this gene show enhanced episodic memory performance in comparison to non-carriers. Although this association has been replicated in subsequent studies, conflicting results have also been found. Further, this research has focused on laboratory material to probe episodic memory. Employing more naturalistic autobiographical stimuli, with a greater reliance on memory vividness, may provide a more ecologically valid measure of this phenotype. In the present study, we examined episodic memory for laboratory and autobiographical stimuli in a large sample of healthy adults in relation to KIBRA genotype. Next, in a subset of these participants, magnetic resonance imaging (MRI) was employed to explore genotype-dependent differences in brain volume within the subfields of the hippocampus and in BOLD response associated with episodic and autobiographical memory tasks.

Disclosures: D.J. Palombo: None. **R.S.C. Amaral:** None. **B. Levine:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Title: Individual threat bias behavior is mediated by Limbic coupling with either lateral or medial PFC

Authors: T. LIN[1], S. VAISVASER[1], R. ADMON[1], D. PINE[2], D. CHARNEY[3], I. WALD[1], Y. BAR-HAIM[1], *T. HENDLER[4]
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Abstract: Background
Individuals differ in the extent to which they selectively attend to threat. Some exhibit high threat vigilance and others show threat avoidance, perhaps in an attempt to down regulate emotional responses to threats. Thus, allocation of attention to potential threats in the environment might have a central role in emotion regulation and through that in effective coping with threat. Assuming that lateral and medial PFC are involved in emotional regulation via attention allocation and/or appraisal, we hypothesized that functional connectivity of limbic areas with PFC regions would characterize different bias tendencies as measured during performance on a classic threat-attention task.
Methods
54 healthy males (mean age = 19) participated in an event related fMRI study using the dot-probe attention task with angry faces as the threat signals and neutral faces as the safe signals. Participants were allocated to one of two groups based on their attentional threat bias scores: threat avoidance (AVO, N=25) threat vigilance (VIG, N=29).
Results
Whole brain contrast to the threat signal revealed activation in key limbic structures involved in the reaction to threat stimuli: the periaqueductal gray matter (PAG) and left hippocampus. This regional threat activation though was more pronounced in the AVO group. Between-groups comparisons of functional connectivity revealed greater coupling of the PAG and the hippocampus with the dorso- and ventro-LPFC in the AVO group, and greater coupling of the hippocampus with the ventro-MPFC in the VIG group. Further network analysis using Dynamic Causal Modeling (DCM) indicated that these group differences may be attributable to a lack of PAG regulation by the ventro-LPFC resulting in higher medial-PFC connectivity with the hippocampus in the VIG group.
Conclusions
Our results show that threat bias patterns of avoidance and vigilance involve co-activation of major limbic areas with the Lateral or Medial PFC, respectively. Together with pervious evidence of associations between threat-related biases and psychiatric symptoms of anxiety and trauma, our finding point to the possibility of individually-tailored therapeutic interventions that take into account the brain markers of these personal biased behaviors.

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Poster

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Program#/Poster#:910.01/CCC2

Topic: F.01. Human Cognition and Behavior

Title: Pulse induction in pseudorandom sequences measured with pulse attribution and tapping rate

Authors: *M. J. VELASCO, E. LARGE
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Abstract: The question of pulse perception involves asking, for any given rhythmic sequence, whether the sequence has a pulse, and if so what is its frequency. Experimentally, these issues are usually addressed separately, by asking participants to provide judgments of isochrony for jittered sequences (pulse attribution) or by asking participants to tap at a perceived pulse rate for rhythmic patterns (pulse finding). In this experiment, the stimuli were isochronous sequences modified with varying levels of Kolakoski jitter. Levels of jitter ranged from small to large and were roved randomly. In separate blocks, participants were asked to provide pulse attribution judgments and to tap at the pulse rate. For small levels of jitter, pulse attribution ratings were high and participants tapped periodically at the mean sequence rate. For intermediate levels of jitter, pulse attribution ratings dropped and tapping variance increased. At certain high levels of jitter, pulse attribution ratings increased and participants entrained at a new tapping rate. Spectral energy peaks predicted mean tapping rate and stronger peaks corresponded to higher pulse attribution ratings, consistent with a neural resonance model of pulse perception. These results may lead to a better understanding of pulse perception in irregular or randomized sequences.

Disclosures: M.J. Velasco: None. **E. Large:** None.

Poster

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Title: Duration tuning in the human striatum

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Abstract: Temporal processing is crucial in many aspects of perception and behavior. Several theoretical models of timing, such as labeled-line models and population clock models, assume that elapsed time is represented by a population of neurons that fires selectively to their preferred duration (see Buonomano and Laje, 2010). Moreover, a recent behavioral study in humans suggested a channel-based processing of time intervals ("duration channels") based on the finding that sensory adaptation to a duration induces distortions in the perceived durations across a limited range of stimulus durations centered on the adapted duration (Heron et al., 2012). Despite these theoretical and behavioral studies, however, neural representations of duration tuning have not been identified to date. Here, we present evidence of temporal tuning in the human striatum by an fMRI-adaptation paradigm. In the experiment, participants (n=18) were asked to perform a duration discrimination task or a shape discrimination task (same or different judgments) depending on the instruction given at the beginning of each block consisted of five trials. On each trial, a reference (adaptation) stimulus was presented for 400 ms followed by a test stimulus, presented for a variable duration (either 160 (far-), 280 (close-), 400 (same), 520 (close+), and 640 (far+) ms). The combinations of stimulus shapes (a rectangle / triangle) for the reference and test stimuli were also varied to equate the stimuli for the duration and shape tasks. In a whole brain analysis, we examined which brain regions showed graded adaptation effects according to the duration difference from the reference stimulus (i.e., same < close+, close- < far+, far-). Only the left striatum exhibited suppression of the BOLD signal to the repetition of the same duration ($p < .05$ FWE corrected at cluster level, $p < .001$ uncorrected at voxel level). Moreover, the values of regression coefficients (beta) of the duration-adaptation in the left striatum correlated significantly with the individual differences in the duration discrimination performance (d') ($r = .58$, $p < .05$). The control condition (the shape discrimination task) confirmed that the adaptation effect observed in the striatum was specific to the duration task, and did not generalize to repetition of shape. These results indicate that the human left striatum is tuned to duration, and plays a significant role in human time perception. Our study demonstrates, for the first time, the neural representations of duration in the human brain.

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Poster

910. Timing and Temporal Processing II

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Program#/Poster#:910.03/CCC4

Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 21670004

Title: Distinction between neural correlates for temporal order and simultaneity judgments

Authors: *M. MIYAZAKI[1], H. KADOTA[2], K. S. MATSUZAKI[2], S. TAKEUCHI[3], H. SEKIGUCHI[3], T. KOCHIYAMA[4]
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Abstract: Perceiving temporal relations between external events is one of the key processes for coordinating human cognition and behavior. Temporal order judgment (TOJ) and simultaneity judgment (SJ) have been widely used in psychophysical studies on this perceptual process. TOJ and SJ provide two identical psychometric parameters: a point of subjective simultaneity and temporal resolution for judgment. Experimental results for both tasks are often not distinguished from each other in research discussions. However, a theoretical model suggests that TOJ and SJ are two distinct systems (Jakowski 1991). In fact, there are psychophysical distinctions between TOJ and SJ. For example, the cross-hand deficit of tactile TOJ (Yamamoto & Kitazawa 2001, Shore et al. 2002) has not been observed in SJ (Axelrod 1968). In this study, we used fMRI to identify the differences in neural correlates between TOJ and SJ. Sixteen healthy subjects (8 male, 8 female, all right-handed) participated in our experiment. During fMRI recording, the subjects performed TOJ and SJ with two tactile stimuli across both hands. The stimuli and the stimulus intervals were identical in both tasks. The response ratios were even between earlier right and left in TOJ and between simultaneous and non-simultaneous in SJ, and no difference was found between the tasks. Brain imaging data showed that SJ activated the left anterior insula and surrounding areas more than TOJ activation. Compared to SJ, greater activation by TOJ was found in the left ventral premotor cortex and the superior parietal cortex extending to the inferior parietal cortex, as well as in the bilateral dorsal premotor cortexes and thalamus. Additionally, the right cerebellum was activated more strongly in TOJ than in SJ at the uncorrected significance level. The activated areas specific to TOJ are known as motor-control-related areas. Our findings suggest that somatosensory TOJ shares neural correlates with motor control, which can account for recent psychophysical findings regarding temporal judgment [e.g., a cross-hand deficit in TOJ occurs in uncrossed hands when subjects are planning to cross their hands (Hermosillo et al. 2011)].

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: BMBF FKZ 01GQ1002

DFG CIN

Title: Predictable visual stimuli are perceived earlier than unpredictable events

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Abstract: How is it possible that we timely react to visual events despite the significant processing delays within the visual system? This delay is estimated to be already about 100ms in higher visual areas, a delay which is relevant if one needs to initiate fast reactions, such as catching a ball in flight or initiating escape. To compensate delays the brain employs predictive mechanisms. An example is the internal forward model which allows the motor system to predict an upcoming sensory state and thus to react even before sensory feedback is available. It is quite likely that - apart from this and other predictive mechanisms on the motor level - purely perceptual predictions could also contribute to delay compensation. Yet, to our knowledge, there is no compelling evidence for delay compensation on a strictly perceptual level. Hence, we asked whether predictability of a visual stimulus affects the time of perceived stimulus onset. Specifically, we hypothesized that predictable visual stimuli have an earlier perceived onset compared to non-predictable stimuli.
We tested our hypothesis in a psychophysical experiment, in which we initially screened 23 subjects in a dual task that engaged temporal interval judgements. In the middle of a black screen streams of individual letters were presented. Each letter was presented for 300ms with a 1000ms default interval between letters. The sequence of letters was either in alphabetic order and thus predictable or, alternatively, the last letter of a sequence was chosen at random and thus not predictable. In each trial subjects had to indicate whether or not the last letter agreed with the alphabetic order. Moreover, subjects had to estimate whether the duration of the last interval, which was of varying length, was either longer or shorter as compared to the preceding intervals of 1000ms duration. Varying the length of the last interval allowed us to estimate the point of subjective equivalence (PSE) between intervals. As we expected predictable letters to be perceived earlier, the PSE should be larger in predictable sequences than the PSE in non-predictable ones. Since our experiment required precise timing we applied rather strict exclusion criteria to both trials and subjects. For the six subjects that survived these criteria there was a significant shift of the PSE towards larger values for predictable sequences (median shift: 54.99 ms; $p=0.0313$). Hence, our findings show that predictable visual stimuli are in fact perceived earlier than non-predictable ones. This suggests that even the perceptual system is compensating

for delays in sensory information processing, allowing us to establish a timely percept of our environment.

Disclosures: B. Wirxel: None. **A. Lindner:** None.

Poster

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Support: This research was supported by WCU program through the National Research Foundation of Korea funded by the Ministry of Education, Science, and Technology (R31-10089).

Title: Neural substrates of interval timing in the human brain

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Abstract: A vast volume of psychophysical studies on interval timing (IT) point to its omnibus involvement in an extensive repertoire of sensory, executive, and motor behaviors, without any sensory channels dedicated to encoding of time intervals. This ubiquitous association of IT with other cognitive events makes it difficult to elucidate neural underpinnings of IT because neural responses pertaining to IT and those non-IT events are intimately intermingled. Recently, we developed an implicit IT task, in which subjects watched an object move at a constant speed and become invisible unpredictably, and guessed the arrival time of the invisible motion at a specified position. Exploiting the fact that stimuli are absent during the period of interval estimation, we adapted this task to fMRI experiments to explore cortical mechanisms that substantiate IT in humans. Subjects performed the task on three reference time intervals (7, 10, 16 s), which were defined (T=Dim/Vvm) on a trial-by-trial basis by variable combinations of a speed of visible motion (Vvm) and a distance of invisible motion (Dim). Trials of seamless visible motion, where interval timing was thus not required, were also included to control for cortical responses due to motor planning and execution. The distributions of reaction times exhibited the scalar property - the constant coefficient of variation across time intervals, confirming that they provided measurements of interval timing. The areas MT and superior parietal lobule (SPL), likely a human homologue of the macaque LIP, were both activated during the period visible motion, consistent with their well-known responsitivity to moving stimuli. Unlike MT, however, SPL remained active during the ensuing period of invisible motion, along

with other motor-associated regions including putamen and cerebellum. The SPL's sustained activity overarching the visible and invisible motion periods may be construed as (i) the cortical activity of accessing the spatiotemporal information available in sensory areas, (ii) the neural accumulation of elapsed time at timescale of seconds as suggested by the pacemaker-accumulator model, or, alternatively, (iii) a neural correlate of the hazard rate of elapsed time, in line with previous findings in the primate brain.

Disclosures: H. Sohn: None. **S. Lee:** None.

Poster

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Program#/Poster#:910.06/CCC7

Topic: F.01. Human Cognition and Behavior

Title: Directional flow of TMS-induced phase perturbation across low frequency neural synchrony networks in the resting state

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Abstract: Even in a resting state without any input of sensory stimuli, our brain is active and constructs a default mode network (Raichle, et al., 2001). Such networks connect many brain regions including sensory areas, motor areas and frontal areas. However there is no explicit consensus about causal and directional information flow (i.e. bottom-up, top-down) within the default mode network. To address the issue, it is particularly useful to elucidate the causal perturbation of electroencephalograph (EEG) oscillations among specific brain regions by a transcranial magnetic stimulation (TMS). TMS is able to reset the phase of ongoing cortical oscillations and the phase resetting propagates from the stimulated area to other brain regions. Here, we applied single-pulse TMS to resting-state brains during 67-channel scalp EEG measurements and evaluated dynamic patterns of changes in EEG oscillations. Ten right-handed participants were required to rest with their eyes closed and completed 20 sessions; 4 TMS intensities (95, 80, 65, and 50%; 100% means motor thresholds) X 5 TMS-target locations (frontal (Fz), left motor (C3), right motor (C4), visual (Oz) and sham). To estitame the time-frequency patterns, current source density analysis was first applied and wavelet analysis was then performed for the EEG data which was preprocessed to extract the TMS and ocular artifacts

by a linear interpolation and an independent component analysis, respectively.
The TMS-induced increase in phase-locking values, which estimates phase resetting of the low-frequency EEG oscillations around the TMS target locations. In the visual-area targeted TMS, phase resetting of the theta (about 3Hz) and alpha (about 10Hz) oscillations transmitted from the visual areas to the motor areas (especially left motor area) via the frontal areas. Moreover, in the TMS to the frontal and right motor areas, the theta and alpha phase resetting converged to the left motor areas. Such phase reset transmissions decreased and disappeared as the TMS intensities decreased and the decrements were also found under the sham conditions. These results suggested the existence of potential bottom-up networks from the sensory regions to the output regions (i.e. motor area) in the resting state.

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Poster

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Program#/Poster#:910.07/CCC8

Topic: F.01. Human Cognition and Behavior

Title: The role of the basal ganglia in rhythmic entrainment and musical emotions

Authors: *W. J. TROST[1,2], D. SCHÖN[3], C. LABBÉ[1], S. FRÜHHOLZ[1], D. GRANDJEAN[1], S. PICHON[2], P. VUILLEUMIER[2]
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Abstract: Rhythmic entrainment is thought to contribute to emotion induction in response to music and may impact on motor as well as attentional performance. Furthermore, the basal ganglia (BG) are known to be both sensitive to musical rhythms and to be recruited during the processing of positive musical emotions. We investigated the neural mechanisms of rhythmic entrainment by musical meter and its modulation by aesthetic appreciation with the use of functional magnetic resonance imaging (fMRI). In particular we tested whether pleasant music would lead to stronger entrainment effects and asked whether such effects might involve BG. We designed an fMRI study where participants (n = 18) listened to piano music while performing a visual speeded response task. The task consisted of detecting visual cues that appeared time locked to either a strong or a weak beat of the musical meter. Each musical piece was presented in both a consonant and a dissonant version. Behavioral results showed a main effect of meter and a main effect of consonance on reaction times but no interaction. fMRI results showed a

main effect of meter in bilateral caudate nucleus, whereas a main effect of consonance was found in an attentional networks including parietal regions as well as motor areas. A selective interaction between meter and consonance was found in the caudate body and right anterior insula. These results show that the caudate nucleus is sensitive to subtle differences of rhythmic expectancies induced by the musical meter, an effect that is predominant during dissonant music. Behavioral and fMRI results together suggest that consonant music leads to a global broadening of attentional resources, whereas dissonant music may restrict attention on the rhythmical structure and make the temporal organisation of the music more salient, leading to stronger involvement of BG.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NSF Grant SBE-0542013

The Elizabeth H. Solomon Center for Neurodevelopmental Research

Title: Spectro-temporal analysis of auditory processing in young infants: Relationships between event related oscillations (EROs) and evoked potentials

Authors: *G. MUSACCHIA[1], N. CHOUDHURY[2,1], S. ORTIZ-MANTILLA[1], T. REALPE-BONILLA[1], A. A. BENASICH[1]
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Abstract: The averaged event-related potential (ERP) has been successfully used to track auditory processing development from early infancy to adulthood. New and ongoing studies suggest that analysis of both time and frequency components of single trial ERPs can add new knowledge about underlying neural mechanisms. The development of age-appropriate magnetic resonance imaging (MRI) models also facilitates more accurate source localization of ERPs at this early age. Thus, our aim is to localize and characterize the spectro-temporal dynamics of ERPs recorded to complex tone pairs in typically developing infants. A group of typical 4 month olds were fitted with a 128 scalp electrode net while seated in the parent's lap and silently

entertained. Free field 800 Hz tones with a duration of 70 ms were presented in pairs with either a 70 or 300 ms inter-stimulus interval (ISI), and 915 or 1140 ms stimulus onset asynchrony between pairs, respectively. After visual inspection and artifact removal, individual ERPs were averaged (EEGLAB, ERPLAB). Co-registered, age-appropriate MRIs were used to model a two-dipole source solution for the grand average ERP and each individual (BESA, Inc.). Within subject tests showed that the latency of the first positive deflection was higher (transaxial) and faster in the right hemisphere dipole than the left for the 300 ms ISI condition. This asymmetry lessened in the 70 ms ISI condition. Spectro-temporal analyses were conducted on the source waveform single trials using a 1-90 Hz frequency band range. EROs were calculated for each individual over time, relative to pre-stimulus baseline (BESA, Inc.), and subsequently pooled (MATLAB). Grand average and individual ERO spectra exhibited a 1/f characteristic, with peaks of activity in ranges of 2-8, 15-30, and 38-55 Hz. Temporal modulation of the EROs coincided with stimulus onset timing in both the 70 and 300 ms ISI conditions. Overall, an oscillatory hierarchy was observed with the EROs. In particular, desynchronization in the 17-30 Hz range was strongly anti-correlated with synchronization in the 38-53 Hz range. Linear regression showed that ERO timing and strength in these two bands strongly predicted the slope of the first positive-to-negative deflection of the ERP. These data show that the interplay of oscillations contribute to the initial stages of auditory processing in the infant brain. Because the timing of these EROs precede stimulus onset, we suggest that they reflect neural mechanisms of phase-coupled entrainment that facilitate sensory processing. Future data are needed to determine developmental changes in this mechanism and whether auditory training impacts this relationship.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Hand movements induce time compression for tactile stimuli

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Abstract: Saccadic eye movements cause compression of visual space towards the saccadic target, and also a compression of time [Morrone et al., 2005; Binda et al., 2009], both phenomena thought to be related to the problem of maintaining saccadic stability. Interestingly, similar phenomena occur around the time of hand movements, when brief tactile stimuli are systematically mislocalized in the direction of the movement [Dassonville, 1995; Watanabe et al., 2009; Maij et al., 2011]. In this study we measured whether hand movements also cause an alteration of the perceived timing of the touch signals. Participants compared the temporal separation between two pairs of tactile taps delivered to the right index fingertip while moving their hand. Each trial began with the presentation of the first pair of taps (the probe) separated by a variable time interval. After a variable pause an auditory tone cued participants to move their right hand as fast as possible along a track. At variable delays from the sound presentation, the second pair of taps (the test) was delivered with a fixed onset asynchrony of 150 ms. When the tactile stimuli were presented close to the onset of hand movement perceived temporal separation was strongly reduced. The time compression began approximately 200 ms before movement onset, it increased up to 30 % until just before the hand started moving and continued until the end of the movement. The results suggest that, as for vision, tactile time intervals are strongly compressed around the time of hand movements. As for vision, the mislocalizations of time and space of touch stimuli may be consequences of a mechanism attempting to achieve perceptual stability.

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Poster

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Title: Functional connectivity dynamics across the entire brain space in health and disease: Conceptual perspective

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Abstract: Recently, there has been increased interest in understanding brain networks and their connectivity , especially regarding structural and functional connectivity at the cortical level. Functional connectivity dynamics and particularly thalamo-cortical interactions are as yet insufficiently integrated with our knowledge of large-scale connectivity and regional function. An important question, yet to be answered in detail, is how typical and atypical cognitive functions and consciousness are temporally generated across the entire brain space. The challenge in current dynamic brain imaging is to analyze the detailed communication or connectivity dynamics across five dimensions, within 3D space across oscillatory frequency and time, and to quantify in detail the relation of these dynamics to typical and atypical cognition and consciousness.
Over the past two decades, MEG/EEG work has been reported on the dynamics of functional connectivity, particularly emphasizing that synchronization of neuronal oscillations both within and between brain regions is an important mechanism mediating formation and communication within distributed neural networks. In particular, there is a large and accumulating body of evidence that task-dependent gamma-band activations and connectivity correlated across frequency and time play a vital role in most cognitive brain functions and represent a critical component to the biological basis of consciousness.
We present a conceptual overview of oscillatory network dynamics and its implications for how brain imaging technologies can be further used to probe fully intact structural, functional and temporal connectivity in thalamo-cortical, cortico-cortical, and cortico-thalamic circuits to better understand typical and atypical cognition and consciousness.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Time-dilation and time-contraction with accelerating and decelerating dynamic visual stimuli.

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Abstract: Several studies show that visual stimuli traveling at higher velocities are overestimated with respect to slower, or stationary, stimuli of equivalent physical duration. This effect - "time dilation" (Brown, 1995; Kanai et al., 2006; Kaneko & Murakami, 2009)- relates more in general to several accounts highlighting a quantitative relationship between the number of changes a stimulus undergoes in a given time frame and its perceived duration. "Change-based" models of timing predict that faster stimuli, subject to a greater number of changes in space, lead to overestimated durations of displacement. Here we provide evidence of a new illusory effect, in which the apparent duration of a sensory event is affected by the way a constant number of changes are delivered in time, or in time and space. Participants judged accelerating and decelerating sequences of stationary flickering stimuli (exp 1 & 3), and accelerating and decelerating horizontally drifting visual stimuli (exp 2) on the fronto-parallel plane. Acceleration and deceleration were achieved by irregular sequencing of events in time (anisochronous flicker rate), or irregular sequencing of events in time and space (anisochronous and/or anisometric drift). Accelerating and decelerating sequences lead to opposite duration biases (under-estimation and over-estimation errors, respectively). This bias was observed in both subsecond (760 ms) and suprasecond ranges (1900 ms). Throughout all experiments, the crucial manipulation was that the mean number of changes in time (mean Hz of flickering stimuli), and mean number of changes in space/time (mean velocity of horizontally drifting stimuli) was always constant: subjects could not rely on a change based criterion to estimate stimulus duration. Change-based models therefore cannot account for such results. Overall, these data highlight how the spatio-temporal evolution of dynamic visual events, asides the total number of changes they are subject to, affect the perceived amount of time they require to unfold.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: ERC-2010-StG 263584

DGA-CEA PhD fellowship

Title: Phase shifts of low frequency oscillations in sensory cortices correlate with perceived shifts in temporal order

Authors: *A. KÖSEM[1,2,3], A. GRAMFORT[4,5], V. VAN WASSENHOVE[1,2,3]
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Abstract: Many ecologically relevant events (e.g. speech, danse/ music…) are multisensory and present temporal regularities that have been shown to influence the detection and perceived timing of events. Recent neurophysiological findings provide strong support for the role of oscillatory entrainment in the modulation of visual and auditory stimulus detection after rhythmic stimulation. Neural entrainment has thus been hypothesized as a mechanistic support for the modulations of perception through time. However the role of low frequency entrainment in time perception remains underexplored. Here, we asked whether the phase of entrained oscillations could be indicative of perceived relative temporal order between auditory and visual events. For this, we used a temporal recalibration paradigm in which adaptation to desynchronized audiovisual events lead to a shift of perceived temporal order towards the preceding stimulus.
Participants underwent temporal recalibration while being recorded with magnetoencephalography (MEG). Three types of adaptations were tested: synchronized audiovisual, auditory leading visual and auditory lagging visual by 200 ms. Eight adaptation blocks per condition were recorded, each followed by an assessment of participant's temporal order threshold. Stimuli were presented at an average rate of 1 Hz leading to a prominent tagging in both sensory cortices. Analyses were carried out in source space (MNE, dSPM, cortically constrained source orientations) and restricted to auditory and visual sensory cortices. Phase and power of 1 Hz oscillations in the first and last 15 trials of the adaptation blocks were compared. The preferential phase of 1 Hz oscillations significantly shifted between the beginning and the end of the adaptation period. Consistent with our hypothesis, phase shifts in each sensory cortex correlated with perceptual shifts in temporal order. Crucially, no significant power changes were observed in 1Hz. All together, our results provide a novel index of subjective simultaneity implemented as phase shift across sensory modalities. These results indicate that the relative phase of oscillations across sensory cortices can serve as an automatic "temporal marker" for the structuring of events in time.

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Poster

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Topic: F.01. Human Cognition and Behavior

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CONACYT 129337

Title: Visual attention to intermediate durations on a bisection timing task.

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Abstract: Time estimation is a fundamental processes underlying behavior organization. Participants were trained in a bisection task to emit differential responses to short (200 msec) or long (800 msec) visual stimulus presented at 5 different possible locations (Areas of Interest: AOI). After attaining more than 80% correct responses out of a training block of 30 trials, subjects were confronted with stimuli of intermediate durations. Cognitive models assume that pacemaker speed is related to the bisection point while attention may affect discrimination accuracy represented by Limen or Weber Fraction (WF). Subjects were instructed to fixate their gaze at a central AOI in a screen before the presentation of the stimulus to be timed; as an independent estimate of attention the time to fixation, the pupil diameter and duration of each gaze were recorded. The bisection point and Weber Fraction (derived from the behavioral responses correlated to the long stimulus) were in accordance to previous studies; longer latencies to respond were observed at intermediate durations representing the difficulty for categorizing the duration of the stimulus. Fixation times or pupil diameter of trained or intermediate durations were not related to location, color or intertrial interval. Despite that the stimulus to be timed appeared at several locations, fixations tended to be recurrent at the central AOI, suggesting that peripheral receptors (rods) in the retina may be involved in timing the stimulus. When subjects were confronted with a short stimuli, the cumulated gaze time to the long stimuli decreased, while as the duration of the stimuli increased the number of gaze visits to the central o peripheral AOIs increased. Pupil diameter increased at durations close to the bisection point or when subjects emitted ÒwrongÓ responses (response to ÒshortÓ key when confronted with a long stimulus or to the ÒlongÓ key when confronted with a short stimulus). This study raises the possibility that gaze visits may be a mediating response to time a visual stimuli.

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Poster

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Program#/Poster#:910.14/CCC15

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant SBE-0542013

The Elizabeth H. Solomon Center for Neurodevelopmental Research

Title: Characterization of spontaneous brain oscillations in 4-month old infants

Authors: *S. PETERS[1], G. MUSACCHIA[1], S. C. ORTIZ-MANTILLA[1], N. CHOUDHURY[2,1], A. A. BENASICH[1]
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Abstract: The characterization of infant brain oscillations is an ongoing process, and is historically based on the extrapolation of frequency bands of oscillations identified in adults using electrophysiology (EEG). Spontaneous EEG oscillations, which are collected in the absence of repeated stimuli, provide a unique opportunity to examine ongoing synchronized activity in the infant brain and are strong predictors of cognitive and linguistic function. For example, higher gamma power at 16, 24 and 36 months has been related to better language abilities at 3, 4 and 5 years of age. Although cross sectional EEG spectra data exist at several infant ages, longitudinal data tracking individual development is sparse. In addition, the extent to which infant EEG corresponds to that observed in adults is not entirely clear. The present study aims to examine and characterize the spectral composition of spontaneous EEG activity in 4-month old infants as a prerequisite for tracking group and individual developmental trajectories. Participants were a subset of a larger prospective, longitudinal study. The infants were typically developing and had no family history of language impairment or abnormal perinatal circumstances. EEG signals were recorded using a 128 electrode net while the child was awake, seated in the parent's lap and silently entertained. Five minutes of spontaneous activity were recorded following 15 minutes of auditory stimulation. Using EEGLAB, data were visually inspected, artifact laden channels were removed and interpolated according to channel statistics, eyeblink artifacts were identified and removed with independent component analysis. Clean

segments of data were selected from each individual's recording for further analysis. A Morlet wavelet decomposition was applied to all of the channels in each clean segment for frequency spectrum analysis and individual averages were compiled across segments. Peaks of activity in each individual average spectrum were picked and averaged, and the topography of peak activity for each band was examined. Topographical maps showing higher frequency ranges (e.g. ~25 Hz and ~55 Hz) were stronger at central and midline regions. A leftward asymmetry was observed in a frequency band in the 4-7 Hz range and the lowest peak frequency band was most evenly distributed across channels. This is one of the few studies that have characterized oscillations describing ongoing synchronized activity in the infant brain, and provides a base from which to study developmental effects. Results of this study underscore the importance of distinguishing developmental oscillations from mature adult-like patterns.

Disclosures: S. Peters: None. **G. Musacchia:** None. **S.C. Ortiz-Mantilla:** None. **N. Choudhury:** None. **A.A. Benasich:** None.

Poster

910. Timing and Temporal Processing II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:910.15/CCC16

Topic: F.01. Human Cognition and Behavior

Support: ANR-10-JCJC-1904

Title: Oscillatory mechanisms for subjective temporal representations: The role of entrainment and phase resetting of ongoing neural oscillations

Authors: *R. GUEVARA, V. VAN WASSENHOVE
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Abstract: Duration perception of very fast events (at the millisecond range) is characterized by illusory distortions. Subjective duration is prone to distortions in two different ways. First, outstanding, new or surprising events tend to be perceived as longer than repetitive, or easily predicted ones. The neural correlate of this effect has been proposed to be associated with the amplitude of evoked responses, whereby the amplitude of the evoked response is suppressed by repetition and predictability. Second, and crucial for this work, events that are presented at unexpected times induce temporal distortions. The degree of this illusion might be modulated by the latency of the target event.
In this work, we propose that the neural correlates of temporal distortions are due to a change in the phase of ongoing neural oscillations. It has long been recognized that oscillatory activity is

crucial for brain information transfer and encoding. The oscillatory nature of neural rhythmical activity matches well with theoretical proposals of timing mechanisms that make use of internal clocks.
To investigate this issue we performed an MEG experiment in human subjects in which two consecutive trains of regularly spaced standardized acoustic events were repeatedly presented. To explore subjective temporal distortions, participants were asked to evaluate if the duration of a variable sound sequence was longer or shorter that the duration of a standard one. The first train was an isochronous stream and the second was variable in both tempo and phase onset (or onset latency). The use of MEG techniques was motivated by its powerful temporal resolution (as compared to fMRI) and source-reconstruction algorithms (as compared to EEG).
Psychophysical results showed that subjective duration of the non-standard acoustic train was modulated by the phase of its presentation, in accordance with previous work (McAuley & Kidd, 1998). Time frequency-analysis and phase locking analysis was performed on source-reconstructed MEG signals with a particular focus on auditory cortex. Ongoing oscillatory neural activity showed entrainment to the external (periodic) stimulation in a non-trivial way. The phase resetting of ongoing oscillations at the onset of the non-standard presentation correlated with psychophysical results, indicating that duration perception was modulated by the resetting of ongoing oscillations.
In conclusion, our results confirm the role of oscillatory activity in time perception at the millisecond scale and establish the mechanistic nature of the interaction between ongoing oscillations and external stimulation to generate perceptual illusions of temporal duration.

Disclosures: R. Guevara: None. **V. van Wassenhove:** None.

Poster

910. Timing and Temporal Processing II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:910.16/CCC17

Topic: F.01. Human Cognition and Behavior

Support: ERC-YStg-263584

Title: How do cognitive mechanisms react and adjust after a disruption of the internal clock? A MEG-EEG combined study

Authors: *C. ROGER, M. BUIATTI, V. VAN WASSENHOVE
Neurospin, INSERM U992 - Cognitive Neuroimaging Unit, Gif-sur-Yvette Cedex, France

Abstract: In everyday life, the correct selection and programming of actions is a requirement of adaptation to the environment. This is made possible by cognitive control mechanisms which are engaged during perception and action. To better understand these mechanisms, researchers have investigated an EEG component, called the ERN ("Error related negativity"), which typically occurs a few milliseconds after an error is made. This component, likely generated by the anterior cingulate cortex, has been interpreted as the physiological manifestation of internal signal of error. Interestingly, the FRN ("Feedback Related Negativity") is considered the equivalent of the ERN in those situations where the error is signaled by external feedback. Both components have largely been studied in situations where errors pertain to the selection of a motor program. However, less is known about the extent to which these mechanisms are involved in situations when the required response is a judgment of when to act (at what moment) as opposed to how to act (which key to press). We hypothesized that as in the case of the selection of a motor program, cognitive control mechanisms can also make use of an internal clock to allow precise timing judgments.
To test this prediction, as well as the extent to which the internal clock is calibrated by externally generated information, we ran a study in which participants were required to estimate a duration (1.45 sec) while MEG and EEG were recorded. In each of three sessions, an initial block in which feedback on accuracy was given (too short, correct, too long) was followed by another without feedback. Feedback provided to the participants was either valid in that it was based on the actual duration to be estimated (Session 1) or not valid in that it was based on how participants responded relative to a new duration that was consistently shorter (Session 2; 1.2 sec) or longer (Session 3; 1.7 sec). Participants were not made aware of this manipulation. Preliminary ERFs results showed that the FRN first is sensitive to the information carried by the feedback, and second is reacting strongly in blocks where the feedback was invalid, i.e. in case of a mismatch between external information and internal representation of time. In a second step, we'll investigate how other brain regions get adapted to this mismatch to adapt to the new context.

Disclosures: C. Roger: None. **M. Buiatti:** None. **V. van Wassenhove:** None.

Poster

910. Timing and Temporal Processing II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:910.17/CCC18

Topic: F.01. Human Cognition and Behavior

Support: Grant to AAB NSF#SBE-0542013 to the

Grant to JAH # 127277 from the Academy of Finland

Santa Fe Institute Consortium

The Elizabeth H. Solomon Center for Neurodevelopmental Research

Title: Enhancement of induced gamma and evoked theta oscillations underlie language-specific processing in infancy: perception of native versus non-native syllable contrasts.

Authors: *S. C. ORTIZ-MANTILLA[1], J. A. HÄMÄLÄINEN[2], A. A. BENASICH[1]
[1]Rutgers The State Univ. of New Jersey, NEWARK, NJ; [2]Univ. of Jyväskylä, Jyväskylä, Finland

Abstract: Brain electrical signals recorded at the scalp reflect variations on spontaneous neuronal activity as well as time-locked activity after stimulus presentation (event-related potentials, ERPs). During sensory stimulation, event-related oscillations (EROs) are enhanced and generate evoked (phase-locked) and induced (nonphase-locked) rhythmic activity. In adults, stimulus-induced modulation of cortical rhythms has been found in the theta (3-7 Hz) and gamma (30-80 Hz) bands during speech perception. Oscillatory activity in the slow theta range is hypothesized to resolve information at the syllable level while fast gamma activity may resolve acoustic information at the segment level. However, very little is known about the spectral composition of infant ERPs during perception of syllables in the infant's native or non-native language. A group of normally developing 6-month-olds, born in English monolingual families, were presented with a two-deviant passive oddball paradigm using 3 stimuli: 80% of the time a standard consonant-vowel syllable (phonetically relevant in both English and Spanish), 10% a native deviant (phonetically relevant in English) and 10% a non-native deviant (phonetically relevant in Spanish). Dense-array EEG/ERPs were collected using a 62-channel EGI net and mapped into age-appropriate brain templates. Source modeling placed dipoles in auditory cortices and frontally, at anterior cingulate cortex. Temporal-spectral analyses of EROs were conducted in source space using a 2-80 Hz frequency band range, from -300 to 930 ms, with 1 Hz wide frequency bins and time resolution of 50 ms. Dynamic changes in frequency band amplitude, as a function of time relative to stimulus presentation, were evaluated by temporal spectral evolution (TSE) measures. Inter-trial phase locking (ITPL) values measured how consistently the phase at different frequency bands locked to cross-trial stimulation. Induced activity was calculated by subtracting the evoked activity from the TSE signal. Permutation testing in combination with data clustering analysis showed significant increases in theta band ITPL for native deviant as compared to standard processing in left auditory cortex. Enhancement of frontal oscillatory activity in the gamma band was seen during processing of the native deviant but not for standard or non-native deviant. Our results suggest that in a period critical for setting-up language, at least part of the infant auditory change detection response to native signals can be attributed to alignment of brain oscillations in the theta band, particularly in left auditory cortex, and to an increase in induced gamma activity in frontal areas.

Disclosures: S.C. Ortiz-Mantilla: None. **J.A. Hämäläinen:** None. **A.A. Benasich:** None.

Poster

910. Timing and Temporal Processing II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:910.18/CCC19

Topic: F.01. Human Cognition and Behavior

Support: NSERC Discovery Grant

Title: Testing the predictions of a Bayesian model for tactile spatiotemporal perception

Authors: *J. TONG, D. GOLDREICH
Psychology, Dept. of Psychology, McMaster Univ., Hamilton, ON, Canada

Abstract: When brief stimuli contact the skin in rapid succession at two locations, tactile perception remarkably underestimates the intervening distance, and overestimates the elapsed time, between events. These two phenomena - perceptual length contraction and time dilation - become progressively more pronounced as the actual distance between stimulus sites, divided by the elapsed time between stimuli, increases. Goldreich (2007) proposed that these tactile spatiotemporal illusions result from a process of Bayesian inference in which the brain interprets noisy sensorineural inputs in light of a prior expectation for low-speed movement along the skin. Conceptually, this tactile perceptual model is similar to a low-speed-prior visual perceptual model (Weiss et al., 2002). In its simplest form, the model has a single free parameter reflecting the precision of the low-speed prior relative to the skin's spatial acuity. Here we report preliminary results from ongoing psychophysical experiments designed to test the model's predictions. We separately measured subjects' tactile spatial and temporal acuity, using stimuli that do not evoke illusory percepts. We then characterized subjects' illusory perception of spatiotemporal sequences of varying speeds. Preliminary results suggest that the illusory percepts experienced by individual subjects conform rather well to those predicted by the model
References:
Goldreich, D. (2007). A Bayesian perceptual model replicates the cutaneous rabbit and other tactile spatiotemporal illusions. PLoS ONE, 2(3), e333.
Weiss, Y., Simoncelli, E. P., & Adelson, E. H. (2002). Motion illusions as optimal percepts. Nat Neurosci, 5, 598-604.

Disclosures: J. Tong: None. **D. Goldreich:** None.

Poster

910. Timing and Temporal Processing II

Location: Hall F-J

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Program#/Poster#:910.19/CCC20

Topic: F.01. Human Cognition and Behavior

Support: Excellence Cluster 302 ‘Languages of Emotion’, FU Berlin, Germany

Title: The brain is sensitive to subjective rhythmization: Evidence on the role of alpha oscillations

Authors: *C. OBERMEIER[1], M. VON KOPPENFELS[2], W. MENNINGHAUS[3], S. A. KOTZ[1]
[1]Max Planck Inst. for Human Cognitive and Brain Sci., Leipzig, Germany; [2]Inst. of Comparative Literature, Ludwig Maximilians Univ., Munich, Germany; [3]Inst. of Comparative Literature/Cluster 'Languages of Emotion', Free Univ. of Berlin, Berlin, Germany

Abstract: When listening to identical tones in an isochronous sequence (equitone sequence), participants often perceive some tones as more salient than others (e.g. Brochard et al., 2003). This phenomenon is termed subjective accenting and has been related to auditory perceptual organization. According to the dynamic attending theory (e.g. Large & Jones, 1999), listeners establish rhythmic expectancies from the first few events of a sequence. This information is then used to direct attention to specific tones and to temporally predict future events. However, little is known about the underlying neural mechanisms. Here, we used a combined ERP/time-frequency(TF) approach to clarify a) how listeners subjectively establish rhythmic regularities in equitone sequences and b) how they use this information to predict upcoming events. In particular, alpha oscillations (8-12 Hz), often related to attention and memory processes (e.g. Klimesch et al., 2010), may play an important role in the formation of such rhythmic regularities and expectancies.
For this purpose, we presented participants with 96 sequences of 13 to 16 identical tones that either contained one or two deviants at odd- or even-numbered positions. Standard tones had a frequency of 440 Hz, a duration of 50 ms, and an intensity level of 70 dB. Deviant tones were attenuated by 4 dB. The interval between tones was 600 ms. Participants counted the number of loudness changes encountered in a sequence. We measured ERPs to deviant tones and performed TF analyses on the deviant and pre-deviant tones.
The ERP analysis revealed an increased P50 as well as P300 for deviant tones in odd positions in contrast to deviants in even positions extending previous ERP findings on equitone sequence processing (e.g. Brochard et al., 2003; Schmidt-Kassow et al., 2011). The TF analysis showed reduced alpha power for deviants in odd positions compared to deviants in even positions. In contrast, alpha power was enhanced for pre-deviant tones in odd positions compared to pre-deviant tones in even positions. Furthermore, alpha power increased up to tone 4 of a sequence, followed by a decrease in alpha power up to the first deviant.

Thus, the present study is the first to show that differences in alpha oscillations between odd- and even numbered tones of a sequence reflect the establishment of subjective rhythmic regularities and expectancies. In addition, the combined ERP and TF data on the deviant tones show that these subjective rhythmic expectancies are used to predict upcoming events in a sequence.

Disclosures: C. Obermeier: None. **M. von Koppenfels:** None. **W. Menninghaus:** None. **S.A. Kotz:** None.

Poster

910. Timing and Temporal Processing II

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Program#/Poster#:910.20/CCC21

Topic: F.01. Human Cognition and Behavior

Support: Associação Fundo de Incentivo à Pesquisa (AFIP)

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ)

Title: Effects of chronic video game use on timing: Differences between sub-second and multi-second intervals

Authors: *M. B. REYES[1], T. S. RIVERO[2], C. PRISCILA[2], O. F. A. BUENO[2]
[1]CMCC, Univ. Federal Do ABC, Santo André, Brazil; [2]Psicobiologia, Universidadade Federal de São Paulo, São Paulo, Brazil

Abstract: Video game is an everyday part of normal childhood and adolescence in today's society. It is therefore worth investigating the potential effects of its chronic use on cognition. Even though video game players frequently report losing track of time while playing, only a few studies have addressed whether there are long lasting effects of such activities on time perception. In order to investigate this question, we compared the performance of a group of chronic video game players, who reported playing more than 40 hours per week, with occasional players, who played less than 5 hours per week in regularly used time perception tasks. Multi-seconds time perception was assessed by time estimation (10, 30 and 60 seconds) and a time production (5 and 45 seconds) tasks. To investigate the time perception in the sub-second range, we utilized a time discrimination task, in which subjects compared intervals varying from 100 milliseconds up to 1000 milliseconds with a reference duration (500 ms), and a bisection interval task, with stimuli varying from 200 to 800 milliseconds. Results show that video game players

had significant superior performance on sub-second tasks. These results point out for a long lasting effect of video game chronic use on time perception.

Disclosures: M.B. Reyes: None. **T.S. Rivero:** None. **C. Priscila:** None. **O.F.A. Bueno:** None.

Poster

911. Timing and Temporal Processing III

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:911.01/CCC22

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant EY021389

Title: Temporal limit of face perception in children and adults

Authors: *F. FARZIN, A. NORCIA
Dept. of Psychology, Stanford Univ., Stanford, CA

Abstract: Visual processing has a limited temporal resolution, which varies according to complexity of information being processed and cortical area. Early stages can process basic luminance information at faster rates than later stages that are required to form a global object representation. The purpose of the current study was to determine the temporal processing capacity for face perception in children and in adults, in order to begin characterizing the functional development of temporal visual processing.
To measure the temporal resolution of face perception we recorded high-density steady-state visual evoked potentials (ssVEPs) in response to a phase-scrambled and an intact face image alternating at a fixed temporal frequency, ranging from a rate of 2-9 Hz. The ssVEP technique is ideal for measuring temporal processing because it directly assesses the time-locked cortical processing of visual information at different rates, from which we can estimate a temporal limit. By design, face-specific responses are elicited only at the odd harmonics of the alternation rate. Responses at the even harmonics are elicited after each image presentation and are thus non-specific to the face. Participants included 16 children (ages 8-12 years) and 11 adults (ages 18-35 years).
Individuals' response amplitude at the first (1F) and the second (2F) harmonic was calculated for electrodes over left, right, and medial occipital areas for each temporal frequency condition. All participants showed a bilateral face-specific response over occipitotemporal areas, and the magnitude of the response was dependent on temporal frequency. Adult 1F responses were maximal around 4 Hz and were not present at 9 Hz, while childrens' 1F responses were maximal at the lowest frequencies and were not present above 4 Hz. Both age groups showed a 2F

response restricted to medial occipital areas, which was present at all temporal frequencies. These results are consistent with the theory that visual information processed at a higher stage, in this case the natural image of a face, is limited to low temporal rates, and demonstrate that temporal resolution increases during development.

Disclosures: F. Farzin: None. **A. Norcia:** None.

Poster

911. Timing and Temporal Processing III

Location: Hall F-J

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Program#/Poster#:911.02/CCC23

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant RO1 NS053960

Title: How does the brain combine temporal information from multiple sources to judge duration?

Authors: *M. CAI, D. M. EAGLEMAN
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Recent studies have demonstrated that the perception of duration can be changed by particular properties of a sensory stimulus. For example, a visual stimulus with higher temporal frequency is perceived to be longer than one with lower temporal frequency of the same physical duration (Kanai et al., 2006). Together with other findings, this supports the idea that the brain can use stimulus properties to estimate duration, contrary to the traditional view of time perception based on an internal clock that is independent of environment. Johnston et al. (2006) have further shown that duration perception can be distorted in a local retinotopic area. Together, these findings lead to an important question: how does the brain form a single duration representation when different visual stimuli provide conflicting information about duration? Specifically, a high temporal frequency stimulus results in a longer perceived duration than a low temporal frequency stimulus when they are presented separately. How does the brain perceive their duration if they are presented simultaneously? To answer this specific question, we conducted experiments in which participants judged the durations of several types of visual stimuli: a static Gabor pattern, a single Gabor pattern drifting at high temporal frequency (6Hz), a single Gabor pattern drifting at low temporal frequency (1Hz), or two simultaneous Gabor patterns, one drifting at low (1Hz) and one at high (6Hz) temporal frequency. Our results confirmed previous finding that high temporal frequency stimuli are perceived to be longer than low temporal frequency stimulus, and both are perceived to be longer than a static stimulus.

When comparing each type of drifting stimulus against a static stimulus, the perceived duration is significantly longer for a single high temporal frequency stimulus than for two stimuli of high and low temporal frequencies presented simultaneously. However, when directly comparing the duration of one stimulus of high temporal frequency against two simultaneously presented stimuli of high and low temporal frequencies, they are judged as equally long. Taken together, we argue that the brain may integrate conflicting information of duration from multiple sensory stimuli in a Bayesian way similar to cue integration, but only when comparing stimuli within the same temporal frequency channel is impossible. When comparison within channel is possible, separate representations of duration from each channel can be kept for comparison to reduce bias.

Disclosures: M. Cai: None. **D.M. Eagleman:** None.

Poster

911. Timing and Temporal Processing III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:911.03/CCC24

Topic: F.01. Human Cognition and Behavior

Title: 'Priming' the brain to generate rapid upper-limb reactions

Authors: *B. LAKHANI[1,2], V. MIYASIKE-DASILVA[3], A. H. VETTE[3], W. E. MCILROY[1,2,3]
[1]Univ. of Toronto, Toronto, ON, Canada; [2]Toronto Rehabil. Inst., Toronto, ON, Canada; [3]Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Background: In situations where urgent responses are essential, individuals with neurological impairments tend to be slow to react. Given the lack of insight into the underlying causes, the present work aims to advance the fundamental understanding of mechanisms that determine rapid reactions. Our previous research demonstrated that balance recovery responses generate reaction times that are significantly faster than cued volitional movements. As studies have been unable to reproduce these rapid reaction times in the absence of a balance disturbance, the afferent input evoked by a whole-body perturbation may be an important modulator of reaction time. The primary objective of the current study is to temporally pair auditory stimuli with whole-body perturbations to determine if conditioning can 'prime' the central nervous system (CNS) to respond faster to an auditory stimulus alone.
Methods: Healthy young adults (n=19) were seated in a custom chair, which tilted backwards upon the release of an electromagnet, and were instructed to reach-to-grasp a handle located in front of their dominant arm as fast as possible following an auditory cue. Three conditions were

completed in the following order: 1) *Baseline* - Auditory cue alone (5 trials); 2) *Paired* - Auditory cue, followed by a chair tilt 110 ms later (20 trials); and 3) *Auditory (post-pairing)* - Auditory cue alone (1 trial). Participants were not informed that the final trial, following the series of paired stimuli, would be *Auditory* alone. Reaction time was measured using electromyography (anterior deltoid onset), and autonomic nervous system activity was monitored via the electrodermal response (EDR).
Results: In the *Auditory (post-pairing)* condition compared to *baseline*, the reaction time was significantly faster (Δ = 40 ms; $p<0.0001$) and the amplitude of the EDR was significantly greater ($p=0.0159$). At the same time, the amplitude of contraction and overall time to handle contact were not significantly different between the *Auditory – post-pairing* and *Baseline* conditions ($p=0.1078$ and $p=0.5436$, respectively).
Conclusions: This study demonstrates that the CNS can be primed to generate rapid reactions in the absence of whole-body instability. The ability to reduce reaction time and boost the phasic EDR in response to an auditory stimulus indicates that the volume of somatosensory afferent information following a whole body disturbance is not the critical determinant of rapid reactions, emphasizing the role of stimulus context on reaction time. Ongoing work is focused on characterizing the mechanisms responsible for augmentation of processing speed along the sensorimotor pathway.

Disclosures: B. Lakhani: None. **V. Miyasike-daSilva:** None. **A.H. Vette:** None. **W.E. McIlroy:** None.

Poster

911. Timing and Temporal Processing III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:911.04/CCC25

Topic: F.01. Human Cognition and Behavior

Title: Encoding sensory and temporal components of expected outcomes

Authors: *N. NADLER[1,2], A. DELAMATER[3]
[1]CUNY, New York, NY; [2]Grad. Center, CUNY, New York, NY; [3]Brooklyn College, CUNY, New York, NY

Abstract: In computer based reaction time (RT) tasks, exploring whether the sensory and temporal attributes of the unconditioned stimulus are separable, human participants learned the relationships between distinct cues and associated targets to which they were to respond or not. The tasks involved the presentation of specific cue-target relationships and subjects were asked

to respond with a single button press. Two cues (distinct geometric shapes), were initially trained to predict different colored boxes. Additional trials occurred in which both cues were associated with a third target stimulus to which they were not to respond. Each trial began with a 1500ms fixation cross, followed by the presentation of a cue for 300ms, and then, after a 500ms gap, the target stimulus was presented for 1000ms or until the subject responded. The interstimulus interval was 800ms in training and varied in test. Reaction time data revealed that overall people responded slower when the targets were presented earlier than usual during the test phase, F(2, 58) = 9.262, p < .001, but, more importantly, that they also responded faster to validly cued targets (i.e., A-T1, B-T2) than invalidly cued targets (i.e., A-T2, B-T1), F(1, 29) = 4.321, p < .05. Importantly, this validity effect did not interact with ISI. The task results support that these attributes are encoded separately as participants responded more slowly to invalidly cued than validly cued targets, and RT was affected by varying the temporal presentation between the cues and targets, however, there was no interaction between these sensory and temporal components. These data indicate that people learn to associate cues with both the identity and temporal qualities of the target stimulus.

Disclosures: N. Nadler: None. **A. Delamater:** None.

Poster

911. Timing and Temporal Processing III

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:911.05/CCC26

Topic: F.01. Human Cognition and Behavior

Title: On the relationship between neural coding and the perception of the present moment.

Authors: *L. S. SMITH
Univ. of Stirling, Stirling, United Kingdom

Abstract: Temporal processing takes place at many scales, from tens of microseconds to years. From a sensory perspective, although one might consider olfaction to be the momentary concentration of molecules, visual perception normally uses visual context, and sound perception is of changing sound pressures. The auditory sense is most sensitive to time: we can distinguish two clicks that are 10ms apart. Our perception of the present moment differs from both the physicist’s perspective, where the moment is infinitesimally short, and the minimum detectable time interval. As William James noted in1890, “the sensible present has duration”, and that duration, is not fixed, but is of the order of a few seconds, much longer than 10ms. What gives it that duration? At a much lower level, from a behavior perspective, even simple single celled

organisms do not appear to experience time as physicists describe it, but have a number of timescales. For them, there is again always context, implemented by the internal content of the cell, in turn modulating the pores (channels) of the cell, and the sensors and actuators on the cell surface, so that the cell's behavior depends not just on the instantaneous environment, but on the environment over some extended period. For animals with neural systems, whose behavior is expressed by the whole animal, and not by single cells, context is implemented primarily neurally. Neural coding itself takes time: a single spike is the shortest possible code, but both rate coding and codes formed from combinations of spikes (except absolutely co-incident spikes) all take time to form. Cognitively speaking, we live "in the present moment", but what does this mean? Is it a issue purely of neural coding, or are there signaling cycles involved? The duration of the "sensible present" suggests something more than simple rate coding, or single informational patterns of spikes: yet the rapidity of the effect of (particularly unexpected) sensory stimuli suggests that the "present moment's" percept can be altered by a very small number of spikes. The duration of the "sensible present" is affected by what the person is doing. For example, in meditative states, where attention is not given to stimuli, it can be short, and in concentrated states, like playing a piece of music on an instrument, or scoring a goal in football, it can be extended. This suggests that a number of collaborating neural effects cause the build-up of the "sensible present". There is clearly a subjective element in the concept, making it difficult to measure. We model the relationship between the physical present, the "present" for unicellular animals, and the "sensible present" for animals with neural systems.

Disclosures: L.S. Smith: None.

Poster

911. Timing and Temporal Processing III

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Program#/Poster#:911.06/CCC27

Topic: F.01. Human Cognition and Behavior

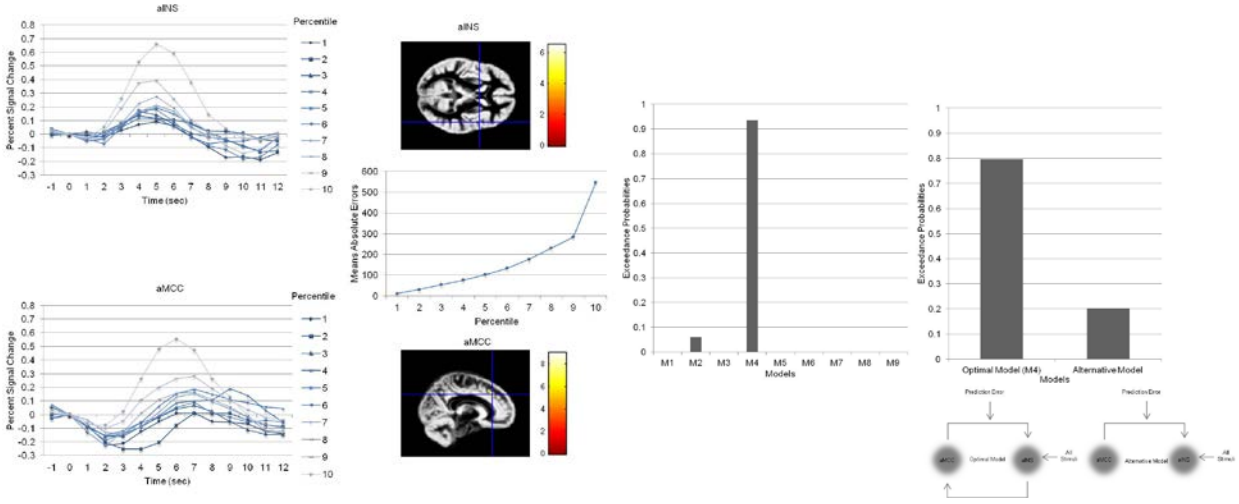
Support: Venezuelan Institute for Scientific Research

Southern Illinois University Carbondale Department of Psychology

Title: Temporal prediction errors modulate cingulate-insular coupling

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Abstract: Prediction error (i.e., the difference between the expected and actual event outcome) mediates adaptive behavior. Activity in the anterior midcingulate cortex (aMCC) and in the anterior insula (aINS) is associated with the commission of prediction errors under uncertainty. We propose a model of effective connectivity (neuronal coupling) between the aMCC and the aINS in which the task-context and the temporal prediction errors drives and modulate intrinsic and extrinsic connections respectively. With fMRI, 15 participants were scanned while performing a temporal prediction task. They observed visual animations and predicted when a stationary ball launched after being contacted by a moving ball. Three spatial gaps and three temporal delays between the balls introduced uncertainties in the launching time thus inducing prediction errors. The fMRI analysis provides evidence that the aMCC-aINS system not only responds when humans predict *whether* an event occurs but also *when* it is to occur. The dynamic causal model demonstrates that uncertainty-related tasks drive activity in the aINS and that aMCC-aINS projections are modulated as a linear function of the prediction errors. Our results inform current questions regarding the predictive functions of the insular cortex, the cingulate gyrus, and the conjoint aINS-aMCC system. Our model gives rise to the question of whether signals associated with temporal prediction errors originate in the ventral striatum or in the ventral tegmental area.



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Poster

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National Medical Research Council Singapore (StaR/0004/2008)

Title: Sleep deprivation slows visual processing in higher visual cortex

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Abstract: Sleep deprivation (SD) can impair attention and slow processing. An important underlying mechanism that has not been well characterized is its effect on temporal processing capacity, the rate at which visual information can be processed. Here we adapted McKeeff's (2007) rapid serial visual presentation (RSVP) paradigm to investigate if SD reduces temporal processing capacity.
Healthy young adults (n=13) underwent fMRI after a normal night of sleep during rested wakefulness (RW) and following 24h SD in counterbalanced order. Participants searched for targets in RSVP sequences of houses presented at different rates (4, 6, 8.5, 12, 15 and 20 items/s).
fMRI was used to measure visual cortex activation associated with the RSVP task. Early visual cortex ROI were determined using retinotopic mapping. The ROI used PPA analysis was based on an independent PPA localizer task. Temporal processing capacity was inferred from the presentation rate that maximized PPA activation. We predicted that SD would reduce the rate at which the maximum fMRI response was elicited.
Participants responded more quickly and accurately to targets during RW than following SD and when presentation rates were slow. fMRI responses in PPA mirrored these behavioral effects. Activity in PPA during RW increased from 4 to 6 items/s, and then dropped for all faster rates. Interestingly, during SD, PPA activation showed a leftward shift in peak activity toward 4 items/s, suggesting a reduction in temporal processing capacity. Contrastingly, activity in early visual cortex rose till 8.5 items/s and plateaued till 20 items/s. This activity showed a non-significant trend towards lower activation with the same temporal profile during SD.
These findings suggest that SD reduces the rate of object processing in higher order but not early visual cortex and may have implications when speeded recognition of objects is critical to performance.

Disclosures: D. Kong: None. **C.L. Asplund:** None. **M.W.L. Chee:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: IES Award R305A100389

Title: Visual and auditory timing interventions targeting magnocellular functioning in children significantly improve reading skills.

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Abstract: There is no greater educational problem facing our schools than students who have trouble reading. We hypothesize that a temporal processing deficit in both the auditory and visual pathways may be a key factor limiting reading performance. To investigate the efficacy of reading interventions designed to improve temporal processing speed, we compared two timing interventions, FastForWord (FFW) targeting the temporal dynamics of the auditory pathway, and PATHtoReading (PATH) targeting the temporal dynamics of the visual pathway. Both of these interventions, designed to improve temporal processing in the brain by increasing the sensitivity of magnocellular neurons in the motion or dorsal pathway, have been shown to be efficacious in randomized trials. This study examined whether FFW and PATH, which activate separate sensory modalities, can be combined into an intervention that results in an even greater benefit. We ran a randomized trial on dyslexic second graders in 7 public elementary schools in San Diego that includes a wide ethnic distribution of predominantly low socio-economic status students, comparing business-as-usual (control group), FFW, PATH, FFW and PATH combined, Orientation Discrimination (OD), and a group that did PATH followed by OD. The OD intervention was designed to train the pattern-sensitive ventral pathway composed of predominantly parvocellular neurons. FFW was done for 30 minutes, 5 days a week for 20 weeks. PATH and OD were done for 10-20 minutes, depending on child's ease of attending, 3 days a week for 20 weeks, doing silent reading in the remaining 30 minutes. Standardized tests of reading fluency and attention were the measures used to evaluate improvements in reading skills. ANCOVAs controlling for age, sex, ethnicity, English as second language status, and school enrolled were used to compare treatment response across groups. We found that PATH improved reading grade level significantly compared to business-as-usual, especially when combined with FFW, suggesting that visual and auditory temporal processing are fundamental for learning to read, contrary to claims that reading is only phonologically-based. Different models to explain these results will be discussed.

Disclosures: **T.A. Lawton:** Ownership Interest; Perception Dynamics Institute. **S. Rosenberg:** None. **K. Lawton:** Other Research Support; Perception Dynamics Institute. **J. Conway:** None. **S.D. Edland:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: DGAPA-PAPIIT IN 302512-3

Title: Evaluation of cognitive impairment in alcohol consumers using temporal Information

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Abstract: There is evidence that chronic consume of alcohol could cause impairment in concentration, executive functions and decision-making process. Several studies have shown that alcohol can cause cognitive deterioration that could decrease addict's performance in information processing tasks. Because of the previous statement it has been proposed that the models of timing can be an excellent tool to evaluate attention, concentration and memory on these subjects because they involved a series of cognitive areas, that is why the aim of the study was to evaluate this cognitive abilities in patients with chronic alcoholism using temporal estimation tasks to determine if exist impairment in processes such as memory and decision making. To evaluate these cognitive functions we took alcoholic patients from a "Celia Meneses Rehabilitation Center". We propose to made three bisection tasks with different interval times to observe if there may be differences in the in the participants information processing according to the speed of the stimuli, we hypothesized that the alcoholics will discriminate worse the short stimuli and better the long stimuli because they will have more time to process information. The first bisection task has an interval time between 100 and 400ms, the seconds one is between 200- and 800ms and the third one is between 400- 1600ms. The results showed that the chronic alcohol consumers present more difficulties in discriminating short stimuli than long stimuli, this was observed in an increase of the limen and the Weber fraction. This is corroborated by previous studies that have shown that the ability to discriminate between different interval times

correlates directly with the stimuli duration. This previous statements led us to think that there may be an impulsivity conduct related to the deterioration observed in alcoholics.

Disclosures: H. Sanchez-Castillo: None. **D. Ramos-Mastache:** None. **D.B. Paz-Trejo:** None.

Poster

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Title: Saccadic latency as a window on temporal processing

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Abstract: Anticipatory eye movements are often evoked by temporal expectation of an upcoming event. Temporal expectation builds up on the basis of an implicit estimate of the changing probability of occurrence of an event (hazard rate). Implicit timing relies on a sense of the passage of time that is not voluntarily controlled. However, anticipation about when an event could occur can also be based on an explicit estimate of time on the basis of information stored in memory. Explicit timing requires attention.
Explicit and implicit timing processes are thought to be under the control of different neural structures. We tested this hypothesis by the analysis of the latency of saccades in a paradigm where movement latency was used as a biomarker of temporal processing. Indeed, decisions based on different timing processes should lead to different saccadic latency distributions. Human subjects were required to make a saccadic eye movement towards a visual target appearing on a screen after a delay period that could take one of four different values randomly (400 ms, 900 ms, 1400 ms and 1900 ms). In the implicit timing experiment, no temporal information was provided about the duration of the delay. In the explicit timing experiment, subjects were informed of the duration of the delay period by the previous display of a visual cue at the center of the screen. The duration of the visual cue was equal to the duration of the

following delay period.
We found that during the implicit timing experience the average latency of saccades with respect to target onset decreased with increasing delay duration. Moreover, the fraction of anticipatory saccades also increased with delay duration. This effect was probably caused by a perception of the changing probability of target appearance as time elapsed during the delay. During the explicit timing experiments even more anticipatory saccades were observed. Importantly, the shape of saccadic latency distributions was different in the implicit and explicit timing experiments. These results support the hypothesis of the existence and relative independence of two different neural systems for timing.

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Poster

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Program#/Poster#:911.11/CCC32

Topic: F.01. Human Cognition and Behavior

Title: Capturing task-evoked functional brain connectivity using eeg

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Abstract: Functional brain connectivity refers to the statistical dependencies of the remote regions of the brain. Existing technologies used for monitoring brain activity have allowed researchers to explore functional connectivity from different aspects. Electroencephalography (EEG) system is one example that captures brain's neuronal activity by its electrodes distributed over the scalp.
A primary step toward understanding the brain's functional connectivity is employing an appropriate metric for investigating EEG data's proximity. These metrics could be built in the frequency domain or in the time domain, such as Euclidean distance, correlation, Dynamic Time Warping (DTW), and probabilistic model-based approaches like Hidden Markov Model.
In this paper, we will investigate brain's functional connectivity in the time domain. We propose an automatic and unsupervised paradigm for understating the brain's functional connectivity by employing cluster analysis which is mostly used in the machine learning field.

One auditory and one modified visual oddball task were tested for 5 subjects. In the auditory task, auditory stimuli were presented for 50 ms in. The stimuli were 1000 Hz (non-target) and 2000 Hz (target) in frequency, and 80 dBSL in intensity. Target stimuli were presented with the probability of 15%.
For the visual task, sequences of three stimuli were presented. Duration for broadcasting the stimulus was 50 ms. The target stimuli appeared with the probability of 15%, whereas the two non-target stimuli appeared frequently and infrequently with the probability of 58% and 25%, respectively. Overall, 50 target and 250 non-target stimuli were presented.
A proximity matrix was constructed by employing the DTW metric. On each pair of EEG signals, the DTW algorithm uses a dynamic programming to generate the most representative distance measure of their overall difference.
ERP signals from the two tasks were analyzed and the DTW-based clustering technique was applied for both cases. Clustering on the EEG data resulted in multiple clusters for each subject. The common cluster among all the five subjects was selected as the region that had shown similar behavior. Our proposed technique was able to capture very detailed similarities where a method such as correlation would fail.
A future direction of this work may be extending the proposed technique to be applied on at temporal-subset of the EEG's signal during the experiment. This approach has the advantage of revealing more number of potential functionally connected regions in smaller temporal-during an experiment.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH EUREKA

Title: Reactivation of visual-evoked activity using electrocorticography in humans

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Abstract: It is increasing being acknowledged that cortical networks exhibit replay of previously experienced sensory information in the absence of stimulation. Neuronal replay has been proposed as a possible mechanism for memory consolidation in cortical networks. In addition, neuronal replay has been associated with efficient encoding of information and improved perceptual performance. Whether and how replay occurs in human cortical areas is unclear. We have recorded from multiple cortical areas using electrocorticography (ECoG) in human patients undergoing treatment for epilepsy. We measured neuronal activity in four patients using ECoG and assessed replay across multiple frequency bands. The experiment consisted of multiple trials in which human observers were exposed to either short cartoon movies or blank screens (25 pre-stimulus trials, 50 interleaved movie and blank trials and 25 post-stimulus trials). During each trial (5.5 s) subjects were required to perform a letter identification task in the center of a computer screen (subjects maintained a 95% correct response rate across trials). To evaluate cortical replay we computed the similarity between neuronal responses in the movie, blank, and pre-stimulus conditions, by employing spectral analysis. Specifically, we computed the correlation distance between the binned Hilbert amplitude (BHA) of neuronal responses in each stimulus condition. If the degree of similarity was significantly higher in the movie/blank correlations than the pre-stimulus/movie correlations, the electrode was considered to exhibit cortical replay. We observed that, irrespective of cortical location, the highest percentage of replay electrodes (around 60%) was found in γ1 (32-64 Hz) and γ2 (64-128 Hz) bands. Using the correlation distance, we found the highest percentage of replay electrodes (around 44%) in γ1 and γ2 bands in frontal (N = 35 electrodes) and temporal lobes (N = 29 electrodes). In addition, replay electrodes exhibited significantly lower spectral differences between movie/blank than between pre-stimulus/movie. Finally, we measured the coherence between a pair of electrodes within and between cortical areas for each stimulus condition. We observed that the difference in coherence for the movie/blank condition was smaller than the difference for the pre-stimulus/movie condition irrespective of electrode location, specifically in the theta and gamma frequency bands. Our results indicate a surprising degree of plasticity in human cortical networks in the absence of visual stimulation that may constitute the neural basis of learning by stimulus exposure in the absence of attention.

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Poster

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Support: School of Psychology, M.Sc grants for SS & AD

Title: Can one keep to rhythm accurately? Depends on the underlying white matter structure.

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Abstract: A wide network of brain areas is involved in the control of timing. Control of force, and co-regulation of timing and force, have been primarily associated with basal ganglia structures; while general timing functions have been associated with cerebellar structures. These key structures in the brain are connected through white matter tracts
This study looks at the correlation of behavioral performance on timing tasks with fractional anisotropy (FA) of specific tracts derived from diffusion tensor imaging (DTI). In two behavioural experiments, we asked participants (N=11; mean age = 27.5 years) to either tap (a ballistic movement of the index finger) or pulse (the index finger continuously touches the surface) the finger in a specific rhythm. At onset of the block participants received aural and visual feedback to assist timing (synchronization phase). Feedback was then withdrawn and participants asked to continue to respond (continuation phase). Across the experiments we varied timing constraints (isochronous or rhythmic) and force constraints (constant peak force or varying force). Using the Wing-Kristofferson model we calculated the motor and clock variance of the inter-response intervals (IRIs). We also looked at the mean temporal drift in IRI for each condition. FA measures from the internal capsule (i.e. frontopontine fibers, corticospinal tract), the external capsule, and the superior cerebellar peduncle (SCP) were calculated from DTI obtained at 3T. We interpret higher FA values in a particular tract as being suggestive of greater myelination. Preliminary results show a positive correlation ($p < .01$, $r = .77$) between clock variance and FA of the internal capsule and SCP. A tract connecting the hand area in the primary motor cortex (M1) to the cerebellum and to the brainstem via the thalamus was identified, and had connections with the frontal lobe. Our results indicate that FA values for these tracts (referred to as timing pathways) can predict how well individuals can keep to time or rhythm. In future such analyses could be extended to study timing networks in patients with Parkinson's disease and cerebellar stroke.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Cortico-limbic interactions in social information processing

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Abstract: Research on social-cognitive functions of the human brain provides both, basic insights in the brains functional anatomy as well as promising endophenotypes of mental disorders (Walter et al. 2011). Social cognitions potentially involve cognition about social context and affective experience. It is of great interest, if these functions are linked to separate or overlapping functional systems of the human brain.
In two fMRI (3T) experiments we explored, if the i.) affective vs. non affective focus of cognition and ii.) the presence vs. absence of affective sensory stimuli elicits disparate or simultaneous activations of limbic structures involved in emotion processing (amygdala, hippocampus (HC)) and cortical networks associated with theory of mind (ToM: Minferring on mental content of others') generation - (dorsomedial PFC, superior temporal sulcus (STS) and temporoperietal junction).
First (Schnell et al. 2011) we explored the functional effects of affective/nonaffective content and cognitive perspective (1st person/3rd person) in social cognition in 28 participants. To control effects of stimulus features, 32 false-belief cartoon stories (3 pictures each) were randomly assigned to the 4 conditions. Within the 3rd person perspective, affective focus of cognition (vs. non affective focus) was linked to coactivation of the anterior ToM network (dmPFC, STS) and limbic system (left amygdale,HC). In general, anterior ToM-network activation was driven by affective content. In contrast, activation of the temporoparietal junction was associated with adaption of the 3rd person perspective.
In the second, yet unpublished, study 22 healthy participants constantly judged affective states of the story protagonist (3rd person affective perspective), while 10 cartoon stories contained facial expressions and 10 did not. The presence of affective stimuli elicited activation of both amygdalae and visual cortex, additionally modulated by the affective stimulus valence. Analysis of psychophysiological interaction revealed that the presence/absence of affective sensory signals modulated functional connectivity between right amydala and orbitofrontal cortex (OFC). Analysis of a structural equation model revealed direct interaction of amygdala and mPFC, which was, in turn, connected to OFC.
Our results indicate that co-activation of the cortical ToM-network and the limbic system depends on the affective focus of social cognitions. Within this cognitive focus, direct affective

signals resulted in additional amydala activation and modulated functional amydala-OFC connectivity, most likely relayed by the medial prefrontal cortex.

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Poster

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National Science Foundation Graduate Research Fellowship

Title: Valence modulates dorsomedial prefrontal cortex engagement during impression formation in healthy aging

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Abstract: Previous neuroimaging studies have demonstrated reliable engagement of the dorsomedial prefrontal cortex (dmPFC) during impression formation in both young and older adults. It is an open question, however, whether dmPFC is differentially recruited depending on the content (i.e., valence) of incoming social information. Extensive evidence suggests the existence of a negativity bias in young adults in many domains, including impression formation, memory, and moral judgment. Older adults, by contrast, show a positivity bias and tend to focus on more positive and emotionally meaningful material. This negativity bias reversal is apparent in the neural underpinnings of emotion processing, such that while young adults activate ventromedial prefrontal cortex (vmPFC) more when viewing negative over positive images, the reverse is evident in older adults. In this fMRI experiment, we investigated whether these reversals extended to impression formation. We explored dmPFC engagement in young and older adults when individuals spontaneously formed positive and negative impressions after learning about and reflecting on others' behaviors. Nineteen young and eighteen older adults viewed faces paired with behaviors varying in positive, negative, and neutral valence. Participants were instructed to focus on how each face-behavior pair made them feel. Consistent with prior evidence of a negativity bias in impression formation, young adults exhibited increased activation of dmPFC when focusing on how they felt about the negative relative to the positive face-behavior pairs. In contrast, older adults displayed increased recruitment of dmPFC

when focusing on how they felt about the positive relative to the negative face-behavior pairs. These findings lend credence to the idea that while young adults may place more weight on evaluating negative over positive person information when spontaneously forming impressions, older adults emphasize positive incoming person information, perhaps as a result of changing socioemotional goals. These data also show that age-related negativity bias reversals, seen in vmPFC activity during emotion processing, are also present in dmPFC engagement when forming impressions. Thus, while dmPFC function during impression formation may be spared with age, its recruitment may be influenced by the valence of incoming social material.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NSF GRFP

Title: Spatial and temporal distance decreases empathic responses to others' suffering

Authors: *D. I. TAMIR, E. M. TEMPLETON, M. D. FERREOL, J. P. MITCHELL
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Abstract: The human capacity for empathy allows an individual share in the experiences of others. Empathy for negative experiences, such as pain or suffering, allows one to respond to others with help and kindness, responses with significant positive societal consequences. Recent work in social neuroscience has linked empathy for pain and suffering to particular set of neural regions, including the dorsal anterior cingulate and bilateral anterior insula. However, people do not always respond to others in pain. In fact, people often ignore the suffering of individuals outside of their purview. Extant cognitive theories suggest that psychological distance may help explain when an individual might fail to empathize with others' suffering. Such theories suggest that individuals will respond more robustly to proximal suffering - pain that seems nearby or recent - than more distal suffering - pain that occurs on the other side of the world or in the distant past. Indeed, people donate more after natural disasters that occur close to home than further away, and those donations taper off as the disaster falls further into the past. To test the extent to which spatial and temporal distance diminishes empathy, we measured spontaneous neural responses using fMRI scanning while participants watched video clips of two target individuals suffering painful experiences. The two targets differed in their spatial and temporal

proximity to the participant: participants believed one set of videos was being recorded live and in a room down the hall from the scanner (proximal target); the other set of videos was previously recorded at a distant location (distal target). Both whole-brain and region-of-interest analyses revealed greater dACC and insula responses while watching suffering by the proximal target than by the more distal target. Additional analyses explored the extent to which individual differences in neural responses to the proximal and distal target correlated with implicit measures of psychological distance between the proximal and distal target. Together, such findings suggest that empathic responses diminish as individuals perceive the target of suffering to be further away in spatial and temporal distance.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Mu suppression, empathy and distraction

Authors: *C. C. WOODRUFF, S. KLEIN
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Abstract: Given the importance of perspective-taking, empathy and intention understanding to human sociality, it might be argued that the neural correlates of these would be engaged automatically. Indeed, some have suggested that social mirroring in the brain may be automatic and involuntary. Using a putative electroencephalographic measure of human mirror neuronal processing, mu rhythms, we tested the hypothesis that cognitive distraction would attenuate mu suppression. We also correlated mu suppression with the Empathy Quotient and the subscales of the Interpersonal Reactivity Index to determine whether previous research showing such correlations could be replicated. The first hypothesis was confirmed in that, while watching a video of hand movements, a secondary word generation distraction task eliminated evidence of mu suppression relative to a focused attention. These findings strongly indicate that mirroring processes as measured by mu suppression are not obligatory but require focused attention. Previous research indicating negative relationships between empathy and mu suppression were also replicated. A model to explain the seemingly counter-intuitive negative relationship is offered.

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Poster

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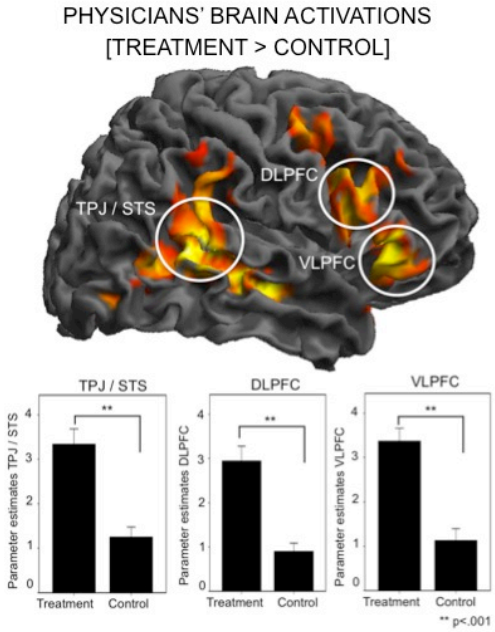
Support: Swedish Society for Medical Research

Title: Physicians activate their own brain regions for pain relief and reward while treating patients

Authors: *K. JENSEN[1], P. PETROVIC[2], C. KERR[3], I. KIRSCH[4], J. RAICEK[5], A. CHEETHAM[5], R. SPAETH[5], A. COOK[5], R. GOLLUB[5], J. KONG[5], T. KAPTCHUK[4]
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Abstract: Background: Patient-physician interactions significantly contribute to placebo effects and clinical outcomes. While the neurobiology of unspecific treatment effects have been studied in patients, the neural correlates of the *clinician* during treatment is unknown. Here, we investigated the neural correlates of physicians during treatment of patients, combined with ratings of treatment satisfaction and empathy-related measures.
Methods: Using functional magnetic resonance imaging (fMRI), we measured physicians' brain activations during analgesic treatment of patients in pain. The physician was placed in the brain scanner and equipped with a device that could activate patient analgesia. The patient was sitting next to the scanner, facing the physician.
Results: Physicians' reported significant levels of satisfaction during 'treatment' ($p<.05$) and strong dissatisfaction during a 'no-treatment' condition ($p<.001$) in which they passively watched the patient in pain. Brain regions implicated in expectancy for pain-relief and increased attention were activated in physicians during treatment, including the ventrolateral and dorsolateral prefrontal cortices. The physician's ability to take the patients' perspective correlated with brain representations of reward and subjective value, as reflected in the rostral anterior cingulate cortex. During the 'no-treatment' condition, physicians displayed empathy-for-pain related activations in the right anterior insula.
Conclusions: Our data suggests that physicians activate their own brain regions for pain relief and reward during administration of patient analgesia, paired with increased attention

activations. Our findings further the understanding of the neural representations associated with reciprocal interactions between clinicians and patients, which is a hallmark for successful placebo-responses.



Disclosures: K. Jensen: None. **P. Petrovic:** None. **C. Kerr:** None. **I. Kirsch:** None. **J. Raicek:** None. **A. Cheetham:** None. **R. Spaeth:** None. **A. Cook:** None. **R. Gollub:** None. **J. Kong:** None. **T. Kaptchuk:** None.

Poster

912. Social Cognition IV

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Topic: F.01. Human Cognition and Behavior

Support: NSF DGE IGERT 0549115 Interdisciplinary Perceptual Science

NSF Cyber Enabled Discovery Type I (Idea) #094158

NJ Governor's Council #10-403-SCH-E-0

Title: Congruent map between the kinesthetic and the visual perceptions of our physical movements, even with noise

Authors: *G. JOHNSON, P. YANOVICH, G. DIFEO, L. YANG, E. M. SANTOS, N. ROSS, E. B. TORRES
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Abstract: The information derived by perceiving and decoding gestures and movements underlies our social interactions, allowing us to infer intentionality, or to anticipate future actions from even subtle cues. Links between less intentional components of our movements-those occurring below our awareness-and social judgments are also important, but widely underexplored. We ask whether the movement percept emerging from our kinesthetic sensation of physical movements aligns in any way with the movement percept visually sensed when observing the motions of others. Here we designed experimental methods and objective metrics to gain insight into these issues. First, the physical movements of expert athletes with skilled timing in performing a tennis serve and a martial arts routine were recorded, then those of novices performing these routines as well. To have a broad range of typical noise, we manipulated sensory feedback during recording by performing in the dark, in front of a mirror, with glowing sticks in the dark reflected in a mirror, and others. For each individual, the patterns of variability of the time to reach their peak angular velocity for each accessible joint of the body, taken across hundreds of repetitions, were extracted. The parameters defining the personal stochastic signatures of each individual using the Gamma distribution family were then obtained. Using maximum likelihood estimation (MLE), the shape and scale parameters corresponding to the variability of the dynamics of each joint were estimated and plotted as points of the Gamma plane. We then built an avatar, and endowed the avatar with both the veridical motions of the subjects and also with noisy variants thereof. To create the avatar, we used the noise from the subjects' own stochastic signatures, the noise from those of others, and also we drew noise from the Exponential and the Gaussian distributions, which we had previously identified in the physical movements of autistic males and females. The experts and novices then performed a perceptual task where they decided, for each variant of the avatar, whether or not they were viewing themselves by pointing and touching a screen. We measured their movement decision time, and also extracted their stochastic signatures as above. The points were plotted on the Gamma plane and we found that, despite the noise, the timing of our visual motion perception aligns well to the timing of our kinesthetic perception of our actual physical movements. This relation, as it turns out, is well characterized by a power law.

Disclosures: G. Johnson: None. **P. Yanovich:** None. **G. DiFeo:** None. **L. Yang:** None. **E.M. Santos:** None. **N. Ross:** None. **E.B. Torres:** None.

Poster

912. Social Cognition IV

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Topic: F.01. Human Cognition and Behavior

Support: FPR-UCLA Center for Culture Brain and Development research grant

Title: Both physical and non-physical similarity modulate neural activity during imitation: Effects of political ideology and race

Authors: *E. A. REYNOLDS LOSIN[1], M. IACOBONI[2], M. DAPRETTO[2]
[1]Interdepartmental Neurosci. Program, Univ. of California, Los Angeles, CA; [2]Psychiatry and Biobehavioral Sci., UCLA, Los Angeles, CA

Abstract: Political ideology is an influential social category based on the way people *think*, yet it has many things in common with social categories like race, based on the way people *look*. One such commonality is that both types of social groupings influence whom people imitate. We have previously found that the neural systems underlying imitation are modulated by the physical characteristics of the person being imitated (the model), including his or her race. Here we tested whether political ideology, a category with no physical manifestation, modulates brain activity during imitation and whether this modulation is influenced by race, a social category accompanied by perceptual cues.

We used a functional MRI paradigm in which 17 highly liberal individuals imitated liberal and conservative actors performing novel hand gestures. In order to investigate the interaction of political ideology and race, actors were evenly divided among three racial groups [European American (EA), African American (AA) and Chinese American (CA)], while all participants were all EA. Critically, participants learned fictitious information about actors' political beliefs prior to scanning and no indication of an actor's political ideology was available during the imitation task.

The anterior cingulate cortex (ACC) and neighboring medial prefrontal cortex (MPFC) – regions not previously found to be modulated by a model's race – were more active during imitation of political outgroup compared to ingroup members. This result demonstrates that different characteristics of a model are encoded by different neural systems during imitation. We next examined the (imitate political outgroup > ingroup) contrast for actors of different races. We found evidence of a political ideology x race interaction such that increased activity for political outgroup members, in a more ventral part of the ACC/MPFC, was significantly greater when imitating racial ingroup members (EA) compared to racial outgroup members (AA or CH). These data suggest that information about the model's non-physical characteristics (i.e. political ideology) may be more influential for those most similar to the self in physical characteristics (i.e. race). Finally, this political ideology x race interaction was also observed in the lateral fronto-parietal and visual regions we previously found to be modulated by a model's race. Taken together, these findings suggest that social categories modulate neural activity during imitation even in absence of perceptual cues. Furthermore, they suggest that the neural encoding of social

categories associated with perceptual cues, such as race, may be altered by non-perceptual information.

Disclosures: E.A. Reynolds Losin: None. **M. Iacoboni:** None. **M. Dapretto:** None.

Poster

912. Social Cognition IV

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Program#/Poster#:912.08/CCC42

Topic: F.01. Human Cognition and Behavior

Support: AFOSR FA9550-07-1-0178

NIH MH091523

Wellcome Trust

Title: Behavioral and neural evidence for an associative account of stereotype formation

Authors: H. J. SPIERS[1], *B. C. LOVE[1], C. GIBB[1], M. E. LE PELLEY[2], R. A. MURPHY[3]
[1]Cognitive, Perceptual and Brain Sci., Univ. Col. London, London, United Kingdom; [2]UNSW, Sydney, Australia; [3]Univ. of Oxford, Oxford, United Kingdom

Abstract: There is strong evidence that aspects of human learning, like conditioning, involve error-driven learning. Can aspects of stereotype formation in which minority groups are viewed less favorably also be explained by such learning mechanisms? Our work on the illusory correlation in stereotype formation is consistent with predictions generated by models like that proposed by Rescorla-Wagner (1972). As with the original illusory correlation (IC) effect demonstrated by Hamilton and Gifford (1974), people learning about fictitious social groups form an illusory correlation between group membership and the affective value of behaviors attributed to the group members. Consistent with the associative model predictions, IC seems to be a pre-asymptotic learning effect (Murphy, Schmeer, Vallee-Tourangeau & Hilton, 2010). A further prediction of the associative account is that this learning is the result of an error correction principle guided by a negative evaluative response. Using recent work on the localization of these aspects of brain function we used functional magnetic resonance imaging (fMRI) to explore this prediction. Results support the characterization of IC as the product of an associative process. Eighteen subjects were scanned with fMRI with a 1.5T scanner. SPM8 GLM analysis revealed that anterior striatum (pallidum) was more active for minority groups than majority groups when all trials were compared. In an additional analysis, the Rescorla-Wagner

model was fit to subjects' behavioral data and quantities from this model were entered into the GLM as model-based regressors. Of primary interest was the model's trial-by-trial predicted affective response for an item. Bilateral anterior temporal pole activity was correlated with this measure that captures affective response to the group names. These results clarify the role that the anterior temporal pole plays in social and emotional processing (cf. Olson, Plotzker & Ezzyat, 2007).

Disclosures: H.J. Spiers: None. **B.C. Love:** None. **C. Gibb:** None. **M.E. Le Pelley:** None. **R.A. Murphy:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: National Eye Institute Grant R01EY021755

National Science Foundation Grant BCS-0823749

National Science Foundation Grant DGE 1148900

Title: Robust selectivity for faces in human amygdala

Authors: *P. MENDE-SIEDLECKI, S. C. VEROSKY, N. B. TURK-BROWNE, A. TODOROV
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Abstract: Single-unit recordings have identified neurons that respond selectively to faces in inferotemporal (IT) cortex and the amygdala. Functional magnetic resonance imaging (fMRI) has replicated these findings in cortical regions, showing elevated blood-oxygen level dependent (BOLD) responses to faces in both monkeys and humans. However, the face selectivity of the amygdala remains uncharacterized. Prior fMRI studies relied on localizer tasks in which participants are presented with photographs of faces and objects from other visual categories (e.g., houses) to identify face-selective BOLD responses. Here we show that the discrepancy in the face selectivity of neuronal and BOLD responses in the amygdala results from limited statistical power in the standard localizer approach. First, the amygdala's small size and location make it difficult to image because of a reduced signal-to-noise ratio relative to cortical regions. Second, group analyses of localizer data are not typically reported to avoid cortical

misalignment, which compounds the problem because of increased measurement error. To overcome these challenges, we performed group analyses of functional localizer data from more than 200 human participants. These data were collected as part of 10 studies, using various behavioral tasks and visual control categories. Beyond the posterior network of cortical regions observed in prior studies, we found robust face-selective responses in the amygdala. In addition, the pattern of responses to faces across amygdala voxels had high reliability over time. Finally, examining the covariance of these reliability scores across face-selective regions revealed separable cortical and subcortical face-processing networks. These results bridge an extant gap between single-unit recording and neuroimaging studies, suggesting that the amygdala has comparable face selectivity to putative "face" areas in posterior visual cortex. This provides some of the first evidence that subcortical regions can be specialized for high-level cognitive processes.

Disclosures: P. Mende-Siedlecki: None. **S.C. Verosky:** None. **N.B. Turk-Browne:** None. **A. Todorov:** None.

Poster

912. Social Cognition IV

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Program#/Poster#:912.10/CCC44

Topic: F.01. Human Cognition and Behavior

Support: ESRC Grant (to JW)

Donation from the Mortimer and Teresa Sackler Foundation

Title: The social contagion of itch

Authors: *J. WARD[1], A. SETH[1], H. CRITCHLEY[1], K. WARNE[1], H. HOLLE[2]
[1]Univ. of Sussex, Brighton, United Kingdom; [2]Univ. of Hull, Hull, United Kingdom

Abstract: Watching someone scratching themselves can elicit subjective sensations of itchiness in the perceiver - this is a form of sensori-motor contagion. In this fMRI study participants watched movies of someone itching themselves versus control movies of tapping the same body part and participants also rated their subjective itchiness. Observing itching (relative to tapping) movements activated most major areas implicated in the veridical perception of itch, including the thalamus, primary somatosensory cortex (BA2), premotor cortex (BA6) and the insula. This suggests a shared system between the veridical perception of itch and the social perception of itch - analogous to that reported for pain. Whereas the observer of pain does not typically report

painful sensations from observing pain, the same is not true for itch. Most people report subjective sensations of itchiness during the itch trials and the intensity of these sensations correlated with the BOLD response in many of the key regions. In addition, individual differences in 'itch contagion' were positively correlated with the personality dimension of agreeableness (but not empathy) and this dimension also correlated with the degree of activity within the primary somatosensory cortex.

Disclosures: J. Ward: None. **A. Seth:** None. **H. Critchley:** None. **K. Warne:** None. **H. Holle:** None.

Poster

912. Social Cognition IV

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Program#/Poster#:912.11/CCC45

Topic: F.01. Human Cognition and Behavior

Title: A dyadic fMRI (dfMRI) study on quantifying the default-mode network's response to social interaction

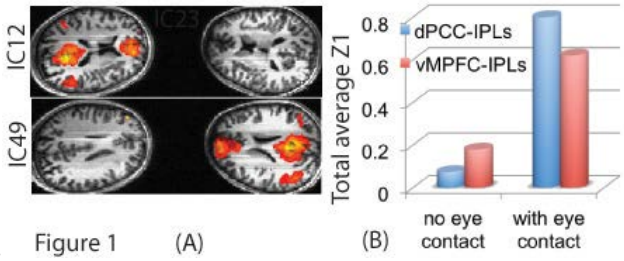
Authors: *R. LEE
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: INTRODUCTION: Dyadic fMRI (dfMRI) (1) can directly quantify the social interaction at the brain network level. In the presence of the same sampling environment, the dependency among the activated regions in dyadic brains can be mathematically defined by ICA which provides a platform to simultaneously observe multiple brain networks' responses during social interaction. In this study, the dyadic default-mode network (DMN)'s response to eye contact is quantitatively characterized.
METHODS: The dfMRI hardware, protocols, and the eye-contact experiment design, are detailed in the Ref. (1). The dyadic data is first preprocessed with in-house Matlab codes. Then using ICA (FSL's MELODIC) generates a set of independent components (IC). The ICs containing DMN are selected as masks to extract the time series from four DMN nodes: PCC, MPFC, left and right IPL. The correlations between the four nodes are calculated; and their correlation differences are tested by Steiger's Z1 and Z2 (3).
RESULTS: 20 pairs of subjects were studied. Three of the major findings regarding DMN are: (i) Any two subjects' DMNs are always independent, as in Fig. 1A. The DMN of one can be coupled with other's non-DMN networks, but no DMN-DMN coupling was observed. (ii) Eye contact causes left-right asymmetry in a subset of DMN (dosal PCC, ventral MPFC, left and

right IPL). Using Steiger's Z1 to test both differences in correlation r(dPCC, lIPL) vs. r(dPCC, rIPL) and in correlation r(vMPFC, lIPL) vs. r(vMPFC, rIPL), the average asymmetry scale (Z1) due to eye contact is shown in Fig. 1B. (iii) If Steiger's Z2 is used to describe the DMN modulations due to eye contact, and the coupling between the DMN of one brain and the other brain is defined as the sum of square of all crossing brain correlations, then the coupling is a function of both Z1 and Z2.

CONCLUSION: Certain aspects of social interaction can be directly quantified by measuring asymmetry, modulation and coupling of the DMN.

Ref.: (1) Lee, et al, Magnetic Resonance in Medicine 2011, DOI: 10.1002/mrm.23313; (2) Steiger, Psych, Bulletin, 1980, vol 87, No. 2, p.



245. Figure 1

Disclosures: R. Lee: None.

Poster

912. Social Cognition IV

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Topic: F.01. Human Cognition and Behavior

Support: NIMH Grant K99MH087813

Title: Medial prefrontal cortex and the emergence of self-conscious emotion in adolescence

Authors: *L. H. SOMERVILLE[1], R. M. JONES[2], B. J. CASEY[2]
[1]Psychology, Harvard Univ., Cambridge, MA; [2]Sackler Inst., Weill Cornell Med. Col., New York, NY

Abstract: Adolescence is a time of life associated with heightened self-consciousness and arousal in social contexts. The current study sought to evaluate whether developmental differences in the sensitivity of social-cognitive brain circuitry parallel this behavioral trend. Participants ranging from 8 to 22 years of age underwent a simulated social evaluation task during fMRI and skin conductance recording. During certain blocks, participants believed a

hidden video camera was projecting their image to be viewed by a peer in real-time (evaluation condition), and during other blocks participants waited for the camera to turn on (anticipation condition). Analyses focused on a) identifying adolescent-specific effects paralleling behavioral findings using age as a continuous variable and b) testing such effects for specificity to evaluation versus anticipation conditions. Adolescents showed heightened embarrassment and skin conductance to anticipation and evaluation periods compared to children and adults. This was paralleled by adolescent-selective engagement of the medial prefrontal cortex (MPFC) both while anticipating and being the target of evaluation, and stronger MPFC-striatal connectivity in adolescents. Taken together, these findings suggest that sensitization of social-cognitive brain circuitry and connectivity between social-cognitive and reward systems support the heightened propensity for adolescents to experience self-conscious emotion.

Disclosures: L.H. Somerville: Research Grant; K99MH087813. **R.M. Jones:** None. **B.J. Casey:** None.

Poster

912. Social Cognition IV

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Program#/Poster#:912.13/CCC47

Topic: F.01. Human Cognition and Behavior

Support: DFG, ESF

Title: Differential neural processing of guilt and regret

Authors: *U. WAGNER, H. WALTER
Dept. of Psychiatry and Psychotherapy, Div. of Mind and Brain Res., Charité - Univ. Med. Berlin, Berlin, Germany

Abstract: Human decision making critically relies on the ability to weigh expected outcomes of choices. In addition, humans use counterfactual thinking in order to evaluate their choices in hindsight, comparing the actual outcome of a choice with what would have happened if they had chosen another action option. Two prototypical emotions resulting from such counterfactual evaluations are guilt and regret, both of which are thought to play an important role in regulating human behavior. Empirical studies asking people to describe typical situations of guilt and regret found that guilt specifically occurs for choices in social (interpersonal) contexts, while regret occurs for individual (intrapersonal) choices more or to the same degree as for social choices. However, little is known how this psychological distinction is represented in the brain. Here, extending existing decision-making paradigms used for the neuroscientific investigation of

intrapersonal regret, we developed a method to distinguish guilt and regret and their neural underpinnings (using fMRI) by systematically inducing these emotions under controlled conditions. Healthy human subjects choose in each trial between one of two lotteries, with the outcome of the chosen lottery (win or loss) either assigned to the self (individual choice condition) or to another person (social choice condition). Behavioral results confirm that guilt but not regret specifically occurs in a social context (i.e. after losses for another person resulting from one's own choices). On the neural level, initial results point to a critical role for the dorsomedial prefrontal cortex and the subgenual anterior cingulate cortex in differentiating the two types of negative emotional experiences.

Disclosures: U. Wagner: None. **H. Walter:** None.

Poster

913. Functional and Neural Mechanisms of Attention

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Program#/Poster#:913.01/CCC48

Topic: F.02. Animal Cognition and Behavior

Support: NIMH Grant R01MH084906

Title: Prefrontal cortical neuronal activity during an automated operant set shifting task in rats

Authors: A. DEL ARCO[1,3], Y. KIM[1], J. WOOD[1], B. MOGHADDAM[1], *C. W. BRADBERRY[2]
[1]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Psychiatry, Univ. of Pittsburgh, PITTSBURGH, PA; [3]Physiol., Univ. Complutense, Madrid, Spain

Abstract: The activity of neurons in the prefrontal cortex (PFC) is critical for learning, representing, and maintaining rules which guide behavior. However, when rules change it is uncertain whether prefrontal neurons respond to transitions between rules or they encode rule shifting *per se*. We recorded single unit activity in the medial PFC (prelimbic cortex) during an operant set shifting task in which animals had to perform several shifts between two rules in the same session. The rules presented were based on two perceptual dimensions: illumination (light) and spatial location (side). Specifically, male Sprague Dawley rats were trained to poke in one of the three nose holes of an operant chamber according to a light rule (illuminated hole) or a side rule (right, center or left hole regardless illumination) to get the reward (sugar pellet). Incorrect trials still required the animal to poke the pellet trough, though the reward was not delivered (sham reward poke). The session finished when the animal successfully performed 3 shifts between 4 sets. A shift between sets occurred after 10 consecutive correct responses. Animals

had to adopt the current rule by trial and error based on the delivery or omission of reward after each trial. During the recording sessions, performance was stable and animals required 169 ±6 trials to complete the task, making 48 ±2 total errors and 28 ±1 perseverative errors per session. Different subpopulations of units encoded various task components. A majority of units increased discharge rate between 1500 and 500 ms before the instrumental poke (preparatory activity). The discharge of some of these units increased or decreased their firing rate after the set shifts. In addition, distinct subpopulations of units increased their activity after the instrumental poke during reward delivery or sham reward pokes, corresponding to correct or incorrect responses. Ongoing analyses are examining the trial-by-trial modulations of single units and population activity. Supported by National Institute of Health.

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Poster

913. Functional and Neural Mechanisms of Attention

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Program#/Poster#:913.02/CCC49

Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust Grant 084621/Z/08/Z

Title: Effects of NMDA, mGlu2/3, and mGlu7, receptor antagonism in the rat medial prefrontal cortex on performance in the 5 choice serial reaction time task

Authors: *A. BENN, E. S. J. ROBINSON
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Abstract: Disrupting glutamatergic function in the medial prefrontal cortex (mPFC) of rats has previously been shown to induce performance deficits in animal models of cognition and executive function including, the 5 choice serial reaction time task (5CSRTT). In this study we investigated the effects of blockade of NMDA receptors (NMDAR), and metabotropic glutamate receptors (mGluR) associated with regulating glutamate release, the mGluR2/3 and mGluR7 subtypes, in the mPFC. We also tested animals trained in a non-paced version of the 5CSRTT designed to increase attentional load.
Male Lister-Hooded rats (350-450g, n=6-10) were trained to a stable performance level before being implanted with mPFC guide cannula. Each animal received prelimbic (PL) then infralimbic (IL) infusions. Drug doses were MMPIP (a negative allosteric modulator at mGluR7 located within the active zone), 0.0, 0.1, 1.0 µg/µL, MK801 (non-competitive NMDAR

antagonist) 0.0, 3.0, 10.0 µg/µL, and LY341495 (orthosteric antagonist at mGluR2/3 expressed away from the active zone in pre-terminal axon regions) 0.0, 0.3, 1.0, 10.0 µg/µL.
Our results show that MMPIP impaired accuracy [F(2, 18)=4.75, p=0.022, 1.0µg/µL versus vehicle p=0.010] when infused into the PL cortex but not IL cortex, with no effect on omissions or premature responses in either brain region. MK801 increased premature responses when infused into the IL cortex [F(2, 10)=11.75, p=0.002, 3.0µg/µL p=0.038 and 10.0µg/µL p=0.002], and tended to increase premature responses in the PL cortex [F(1.1, 8.6)=3.45, p=0.096]. Accuracy and omissions remained unaffected in either brain region. LY341495 had no effect on performance variables in any brain region.
Our results show that disruption of mGluR7 but not mGluR2/3 selectively impairs accuracy when infused into the PL cortex, an area thought to mediate goal-directed behaviour. We surmise that accuracy in this task requires proper functioning of mGluR7 located in the active zone of neurotransmitter release, and not glutamate receptors located perisynaptically (mGluR2/3). Similar to previous studies, NMDAR antagonism in the IL cortex increased premature responding although accuracy was not affected by treatment targeted at the PL cortex. This difference may be due to the use of a non-paced version of the task. We conclude that disruptions to glutamate transmission cause differential effects on attention and impulse control depending on the receptor subtype and brain region involved.

Disclosures: A. Benn: None. **E.S.J. Robinson:** None.

Poster

913. Functional and Neural Mechanisms of Attention

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Program#/Poster#:913.03/CCC50

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant EY019179 (EIK)

Title: Shared neural mechanisms for bottom-up and top-down control of spatial attention

Authors: *S. P. MYSORE, E. I. KNUDSEN
Neurobio., Stanford Univ., STANFORD, CA

Abstract: Operating in complex environments, animals must select the most important among competing stimuli to pay attention to at any instant. Both the physical properties of stimuli (bottom-up signals) and their behavioral relevance (top-down signals) contribute to stimulus “priority” in this competitive selection. However, whether bottom-up and top-down signals operate via shared or distinct neural mechanisms to influence competition is unclear. Here, we

investigate this question in the barn owl using visual and auditory stimuli to invoke competing bottom-up signals, and electrical microstimulation of the forebrain gaze field to invoke space-specific top-down signals. We demonstrate that focal and reversible inactivation of a GABAergic midbrain nucleus, the Imc, abolishes both bottom-up and top-down mediated competitive suppression of responses in the optic tectum, a midbrain structure known to be essential for normal selection behavior in attentional tasks. Our results reveal novel insights into the neural mechanisms that control spatial attention.

Disclosures: S.P. Mysore: None. **E.I. Knudsen:** None.

Poster

913. Functional and Neural Mechanisms of Attention

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Program#/Poster#:913.04/CCC51

Topic: F.02. Animal Cognition and Behavior

Title: General anesthesia is reversed by electrical stimulation of midline areas of midbrain and thalamus

Authors: *K. D. ONOS, S. R. STEVENS, B. A. WORMWOOD, R. G. MAIR
Psychology, Univ. of New Hampshire, Durham, NH

Abstract: General anesthesia is associated with suppression of thalamic activity. Previously we reported that rats can be aroused from general anesthesia by electrical stimulation of midline thalamus, a result consistent with effects of pharmacological stimulation of this site (Alkire et al., 2009, Anesthesiology 110:766-773). Here we compared the effects of stimulation in midline thalamus to a control site located in the area of the rostral linear raphé nucleus. Bipolar stimulating electrodes (twisted pairs of 0.125 mm SS wires with polyamide insulation, PlasticsOne) were implanted in midline thalamus or the control site. Isoflurane anesthesia was induced until rats showed no toe-pinch response and were inactive during a 3-minute stabilization period (0.75 to 1.0 % in 5 L/min of O2). Rats were then placed on their backs in a sleep trough and anesthesia administered at constant rate and concentration through a nose cone. Constant current electrical stimulation (a 10 s train of 0.2 ms pulses @ 120 Hz) was delivered through a stimulus isolator (A 365, WPI) driven by a Pulsar stimulator (FHC). Current levels were increased from 1 microA to 3 mA with a 60 s observation after each stimulation. Each animal underwent 3 days of testing that were videotaped. Surprisingly, we found the control site was more effective reversing the effects of anesthesia. All controls (8/8) showed full arousal on at least one of the three testing days with full arousal (active attempt to escape from nose cone)

on 20/24 (83%) of test sessions and isolated movements of extremities on 24/24 (100%) sessions. By contrast only 6 of 11 rats in the thalamic group showed full arousal on at least one test day with full arousal on 7/32 (22%) of test sessions and isolated movement of extremities on 26/32 (81%). This confirmed our previous results in which 6 of 10 animals righted (from a flat surface) with comparable midline thalamic stimulation. There was no difference between the groups for the level of stimulation necessary for arousal. These results indicate that midline thalamus is not unique as a site where electrical stimulation can reverse general anesthesia. It seems more likely that anesthesia can be reversed by stimulation of a distributed neural network that includes midline areas of brainstem and thalamus.

Disclosures: K.D. Onos: None. **S.R. Stevens:** None. **B.A. Wormwood:** None. **R.G. Mair:** None.

Poster

913. Functional and Neural Mechanisms of Attention

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Program#/Poster#:913.05/CCC52

Topic: F.02. Animal Cognition and Behavior

Support: Canadian Institutes for Health Research (CIHR)

Natural Sciences and Engineering Research Council (NSERC)

Title: Response latencies of error detecting signals dissociate anterior cingulate and prefrontal contributions for performance monitoring and outcome predictions

Authors: *C. SHEN[1], D. KAPING[1], S. ARDID[1], S. EVERLING[2], T. WOMELSDORF[1]
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Abstract: Performance monitoring refers to the continuous process of outcome evaluation and becomes particularly apparent when errors or unexpected outcomes occur. Neuronal activities signaling errors and evaluating the outcomes of choices have most prominently been found in the anterior cingulate cortex (ACC), with similar signals reported within more lateral subdivisions of the prefrontal cortex (lPFC). It remains unclear, however, whether error detecting signals in ACC/lPFC are distinguishable.
We therefore examined error-related (ER) single neuron activities across a large extent of the lPFC/ACC of two rhesus monkeys during a selective attention task that contained errors across different task epochs (Kaping et al., PLoS Biol 2011). ER firing rate modulation was quantified in successive ± 0.15 sec. windows around the time of error commission. Among n=649 recorded

neurons, 26% (n = 170) showed significant ER firing rate modulation and were further analyzed. The proportion of ER neurons varied across time with separable populations of neurons showing early onset latency of error modulations ≤0.10 sec. (~29%), and late onset latency of error modulations around 0.20 and 0.45 sec. following error commission (~37% / ~34%, respectively). Early onset error signals were particularly prominent in the lPFC while error signals emerging ≥0.2 sec. clustered anatomically in the ACC. We next tested whether ER neurons distinguished errors committed (i) during sustained selective attention on a stimulus (indicative of motivational lapses), (ii) around the time when a salient distractor occurred (indicative of faulty attentional capture), or (iii) during a perceptual choice about the rotation direction of a stimulus. We found that the largest subset of neurons (>30%) coded for errors in at least two task epochs, with only few neurons signaling errors committed solely during focused attention or during the distractor period (both < 5%).
These findings reveal that the latency of error detection distinguishes ER neuronal signals within the ACC and lPFC, suggesting that the later onset ER signals in ACC reflect the integration of outcome signals from the lPFC. In summary, these findings support the unique role of the ACC to encode outcome predictions (Alexander & Brown Nat Neurosci 2012) and suggests that error coding in the ACC can be instrumental as learning signals to improve choices and attentional deployment in trials subsequent to error trials (Bryden et al. J Neurosci 2011).

Disclosures: C. Shen: None. **D. Kaping:** None. **S. Ardid:** None. **S. Everling:** None. **T. Womelsdorf:** None.

Poster

913. Functional and Neural Mechanisms of Attention

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH086530 (MS)

Whitehall Foundation and the University of Michigan (JB)

Title: Distinct behavioral and neurophysiological correlates of prefrontal acetylcholine and glutamate transients during attentional task performance.

Authors: *W. M. HOWE, H. GRITTON, N. LUSK, J. BERKE, M. SARTER
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Abstract: Transient increases in medial prefrontal (mPFC) acetylcholine (ACh) release aid the detection of behaviorally relevant cues during attentional performance (Parikh et al., 2007). These cholinergic transients are generated locally and require cue-evoked glutamate release from mediodorsal thalamic afferents (Parikh et al., 2008, 2010). Attentional performance is also associated with gamma oscillations within the mPFC. Here we investigate the relationships between each of these processes during attention task performance. Rats were trained to discriminate trials with a short, unpredictable visual cue from cue-absent trials by pressing one of two levers extended into the operant chamber. We monitored release of acetylcholine and glutamate using fixed-potential amperometry and enzyme-coated microelectrodes placed in the thalamic input layer of mPFC. Both glutamatergic and cholinergic transients were evoked by visual cues that were detected (hits), but not by cues that were missed. During misses or non-signal trials yielding correct rejections, glutamatergic transients were also evoked by lever extension, suggesting glutamate transients are associated with the processing of stimuli that evoke behavioral responses or decisions. Cue detection and lever extension were also associated with a corresponding increase in gamma-power. Cholinergic transients were evoked by cues in trials yielding hits but only if such signal trials were preceded by non-signal trials or misses ("incongruent hits"). Thus, cholinergic transients are hypothesized to mediate shifts between ongoing processing and cue-directed attention and behavior. Compared to successive hits, incongruent hits, which evoked both glutamatergic and cholinergic transients, were associated with greater gamma power coincident with cue detection. This augmentation may support shifts in attentional processing modes by optimizing interactions across widespread cortical and subcortical networks. Combining the recording of second-based neurotransmitter release with local field potentials reveals the contributions of individual afferent systems and local circuitry to fundamental cognitive operations.

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Poster

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Title: Effects of orexin A administered into the basal forebrain on attentional performance in rats

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Abstract: The neuropeptides, orexins, are released from neurons that are primarily localized in the lateral hypothalamus and contiguous perifornical area. The basal forebrain, among other regions, receives orexinergic projections. Normal attentional processing requires the integrity of the basal forebrain in rats. Our previous research demonstrated that orexin receptor blockade impairs attention and that infusions of orexin A into the lateral ventricle enhances attentional performance. The goal of the present experiment was to test whether the basal forebrain mediates the attention-enhancing effects of orexin A. Male FBNF rats were trained on a sustained attention task that required discrimination of visual signals (500, 100 or 25-ms illumination of a centrally located panel light) from trials when no signal was presented. After stable performance levels were established, rats received guide cannulae implanted bilaterally into the basal forebrain. Postsurgically, rats were trained in a version of the task in which attentional demands were augmented by presenting a visual distracter during the middle block of trials. Rats then received 0 (vehicle), 0.1, 1.0 or 10.0 pM orexin A prior to task performance. Each rat received each dose once in a counterbalanced order. Accuracy tended to be higher during the first block of trials following vehicle administration compared with orexin A administration. However, attentional performance decreased from block 1 to block 2, when the distracter was presented, following vehicle treatment. This distracter-induced impairment in accuracy was attenuated following administration of higher orexin A doses. These findings suggest that intrabasalis orexin A administration blocks sensitivity to the distracter during the second block of trials. These results provide additional support that orexin A can enhance attentional processing and suggest that the basal forebrain may be one important neural substrate that mediates these effects.

Disclosures: K.N. Zajo: None. **J.R. Fadel:** None. **J.A. Burk:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH MHO80426

NSF 0726285

NSF Graduate Research Fellowship

Title: Not all cues are equal: Neurochemical and functional-imaging measures reveal neuronal mechanism connecting cues to internal representations

Authors: *A. S. BERRY[1], W. M. HOWE[1], J. FRANCOIS[2], S. LOOMIS[2], G. GILMOUR[2], C. LUSTIG[1], M. SARTER[1]
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Abstract: Successful goal directed behavior involves shifts between ongoing activity and the processing of instructional external cues (or signals). We investigated the neural mechanisms underlying cue-directed processing shifts using a translational, cross-species sustained attention task (SAT). This task consists of long variable-duration monitoring periods during which a signal (visual stimulus) may or may not occur, followed by a prompt to report the prior presence (hit/false alarm) or absence (miss/correct rejection) of a signal. These long monitoring periods as well as the unpredictable distribution of non-signal and signal events lead to stable ongoing processing (monitoring and committing correct rejections) with intermittent periods of signal-directed behavior (hits). To examine the neural mechanisms of breaks from ongoing processing to signal-directed behavior, we examined transient changes in prefrontal acetylcholine levels in rats and transient fMRI BOLD activations in humans during “shift-hits,” that is, correct signal detections (hits) preceded by CRs or misses. Neural activity on shift-hit trials was contrasted with activity associated with non-signal responses (CRs and misses), and with hits that followed another hit. Cues in shift-hit trials, but not during successive hits, evoked transient increases in cholinergic activity. In humans, the same shift-hits were associated with transient activation in a region of right rostral prefrontal cortex (BA 10). To further link these findings, we measured prefrontal oxygen levels in task-performing rats, again finding increases specifically evoked by cues in shift-hit trials. Further, stimulation of cholinergic receptors was sufficient to evoke oxygen responses comparable to those seen during shift-hit trials. Together, these parallel and converging findings from human and animal experiments reveal a prefrontal mechanism involved in connecting cues to internal representations. Further, we demonstrate that the cholinergic system is integral to this operation and may underlie the BOLD response observed in humans.

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Poster

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Deutsche Forschungsgemeinschaft

Title: Learning expected stimulus values in a selective attentional foraging task by nonhuman primates

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Abstract: Goal-directed behavior depends on covert attentional selection of stimuli prior to overt choice behavior (Rangel & Hare, Cur Opin Neurob 2010). In order to optimize future choices to achieve maximal positive outcome it is necessary to assign experienced outcome values not only to the correct action, but also to the stimulus that was selected prior to the overt choice. Reinforcement learning frameworks and value based decision making theories suggest that humans are often near optimum in their credit assignment based not only on action values, but also based on more abstract stimulus values. Very little is known, however, about stimulus value coding in primates as commonly used paradigms typically do not dissociate stimulus from action valuations.

We therefore devised a selective attentional foraging task in which two rhesus monkeys were trained to flexibly learn the expected values of stimuli and discount action values that were linked to a two-alternative forced-choice decision performed on the previously selected stimulus. We analyzed choice behavior of 46 sessions entailing 396 blocks of trials with novel stimulus value assignments. Overall, the animals learned to guide covert attentional choices in > 90% of blocks based on the intrinsic compiled stimulus value, discounting action values and time delays beyond 1.5 sec. The learning rate of the expected correct stimulus value approached an avg. of 11-12 trials, revealing a rapid credit assignment of stimulus related outcomes, albeit slower than the corresponding assignments of action related outcome values. In one animal we tracked initial learning of the task across days, identifying a tendency to erroneously assign values to immediate actions early during learning and a smooth increase of learning rates after progressive experience in the task.

These findings demonstrate that nonhuman primates optimize choice behavior by an internal learning process that represents the expected value of a basic sensory feature. The rapid learning rates reveal that the selective attentional foraging task successfully tracks the “learning of attention” within few trials, allowing in the future to study the neuronal correlates of how top-down attentional control processes emerge during task performance.

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Poster

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Support: NIH Grant EY017921

Title: Neural mechanisms of visual selection in prefrontal cortex, FEF, and IT cortex during a free-viewing search task

Authors: N. P. BICHOT, M. T. HEARD, *R. DESIMONE
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Abstract: To investigate the neural systems mediating the ability to find relevant objects in crowded scenes based on their visual features, we trained two monkeys to perform a free-viewing visual search task. After fixating a central fixation spot, monkeys were presented with a central cue showing the target stimulus for that trial. The cue was extinguished after 1 s, and the fixation spot reappeared for 800 ms. Monkeys were required to hold fixation at the center of the screen during this period, after which time the search array was presented. Monkeys had 10 s from search array onset to find the target, and no constraints were placed on their eye movements so that they could conduct the search naturally. When the monkeys found the target, they fixated it for 800 ms continuously. We used a fixed set of eight natural object stimuli throughout the study. On each trial one stimulus was chosen as the target, and the remaining seven stimuli were distracters. Stimuli were positioned at eight of the twelve possible locations, and the target location and identity were chosen pseudo-randomly from trial to trial. Multi-unit spike activity and local field potentials were recorded in three areas: 1) IT cortex, which plays a central role in the recognition of natural objects such as those used in this study, 2) FEF, which plays a key role in visual spatial selection, and 3) area 45 of prefrontal cortex (PFC) which has been shown to play an important role in working memory and executive control.
The responses of IT neurons were enhanced shortly before a saccade to a preferred target object in their receptive field (RF). In contrast, while FEF neurons did not respond selectively to the different objects, their responses to targets in their RF were enhanced compared to distracters, with a latency of about 100 ms, which was earlier than the enhancement observed in IT. Most interestingly, neurons in PFC signaled both cue information and the location of the target in their

RF. PFC responses were enhanced prior to search array presentation when the neurons' preferred object was the cue, and this enhancement persisted throughout the trial whether the animals made a saccade to the target or a distracter. Furthermore, when the target was in their RF, the activity of PFC neurons increased to signal its location within 100 ms of search array presentation, similar to the time course of selection in FEF. These results suggest that PFC encodes target properties and locates the target stimulus and feeds this information to FEF. FEF, in turn, may feedback a spatial selection signal to IT cortex and earlier visual areas.

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Poster

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Title: Attentional modulation of spike-phase coupling in theta band of local field potentials in mt area of the macaque cortex

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Abstract: Visual attention decreases the interneuronal correlation in the visual cortex. Furthermore, switching attention between two locations changes both the amplitude and phase of local field potentials (LFPs) recorded from the corresponding area of the cortex in low frequency bands(Fries et al., 2001; Lakatos et al., 2008). We also know that in many areas of the brain, spike times are modulated by the phase of LFPs at low frequency bands (<10 Hz). However, any attentional modulation of the relationship between spike times and the phase of low frequency LFPs is still largely unexplored.
In the current study, we recorded both spikes and LFPs from area MT of three male macaque monkeys in two attentional conditions: attention inside the RF and attention outside of the RF. In each trial, a random dot pattern (RDP) moving in the neuron's preferred direction was presented

inside the RF and one or two RDPs with preferred or anti-preferred directions were shown outside the RF.
We used a five-channel Mini-Matrix system to record responses from 301 (monkey H), 101 (monkey C) and 73 (monkey T) neurons. We calculated the power of coupling between the spikes from each isolated neuron and the LFP recorded from the same recording site as the neuron.
We filtered LFPs in 1 Hz steps into frequency bands of 4 Hz width between 1 and 15 Hz. We calculated the spike-LFP coupling power in each frequency band for the time interval between 400 and 1000 ms after stimulus onset. We used the same formula used by (Siegel et al., 2009) to calculate spike-phase coupling (SPC).
We found that across the population of neurons recorded in the three monkeys, the power of SPC for the theta frequency band (3-8 Hz) was decreased by attention ($p < 0.001$, permutation test). The same analysis on each of the monkeys separately showed a significant decrease of SPC for bands of 4-8 Hz (monkey H, $p < 0.05$), 3-7 Hz (monkey C, $p < 0.001$), and 3-7 Hz (monkey T, $p < 0.01$). The size of the effect was 46% across the monkeys.
We conclude that phase coupling between spike occurrences and the large-scale ongoing activity of neuronal populations at theta frequency is decreased in area MT by visual attention. This suggests that attention decreases determinism in spike times across the neuronal population at low frequencies. This can be a possible mechanism for the decrease of interneuronal correlation by attention.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Title: Spike count correlations amongst dorsolateral prefrontal cortical neurons during target selection and allocation of attention

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Abstract: Visual attention allows us to give the processing of behaviorally relevant stimuli priority over irrelevant ones. An area in the primate brain known to be involved in visual attention is the dorsolateral prefrontal cortex (dlPFC). Neurons in the dlPFC of macaque monkeys can discriminate between relevant and irrelevant stimuli (targets and distractors) through their firing patterns. Interestingly, the degree of suppression of the neurons' response to a distractor is correlated to the animals' performance at filtering behaviorally relevant information. The mechanisms by which dlPFC neurons implement this selection process is unknown. One possibility is that neurons selective for the position of a given attended stimulus interact with other neurons. We investigated such neuronal interactions by measuring spike count correlations between dlPFC neurons in one macaque monkey during a cognitive task: Two visual stimuli were presented on a computer screen on both sides of a central fixation point. The animal had to select the target stimulus based on its color and sustain attention to it in order to indicate a change in one of its features while ignoring similar changes in the other pattern. We implanted a 96-electrode microarray (Utah array) in the monkey's left dlPFC to simultaneously record the activity of many neurons while the animal performed the task. We isolated responses of 74 single units. We divided the neurons into two groups, based on their spatial selectivity. The first group contained the neurons with no preference for the spatial location of the target (n=60), i.e., with similar firing rates for targets on either side of the fixation point. The second group contained those neurons that responded more strongly for targets in the hemifield ipsilateral to the recording site (n=11) (The number of neurons showing a preference of contralateral targets was too small (n=3) to allow a quantitative analysis.). We quantified spike count correlations within each group of neurons. We found that spatially selective neurons showed greater spike count correlations than non-selective neurons. Specifically, they showed an increase of ~80% in mean correlation in their response to an ipsilaterally presented target when compared to the non-selective neurons. When the target was presented on the contralateral hemifield, the spike count correlation increased by ~100% for spatially selective neurons compared to non-selective neurons. Both of these differences in correlation were statistically significant ($p<0.01$). These results suggest that dlPFC neurons that show a preference for the target location interact more strongly during the allocation of attention.

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Andrew Mellon Pre-Doctoral Fellowship

Title: Theta oscillations in the rat medial prefrontal cortex, but not the anterior cingulate or ventral tegmental area, are phase entrained to an attended but not unattended stimulus

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Abstract: The phase entrainment of local field potential (LFP) oscillations to the timing of a stimulus may improve attentional selection by increasing neuronal excitability at the time of stimulus onset. Studies in humans and monkeys have demonstrated phase entrainment to rhythmically presented stimuli, whereas other studies have demonstrated phase entrainment to the onset of non-rhythmic, but attended stimuli. These studies support the idea that the brain may intrinsically generate phase entrainment in expectation of a task-relevant stimulus in order to improve attentional selection. While this idea has been investigated using humans and monkeys as subjects, it has not been studied in the rat. Therefore, we recorded LFP in the rat from brain regions that have been implicated in expectancy and preparatory attention: the medial prefrontal cortex (mPFC), the anterior cingulate cortex (ACC) and the ventral tegmental area (VTA). We employed a well-characterized rodent preparatory attention task that requires the rat to anticipate a brief visual stimulus, which would appear at 1 of 3 randomly selected locations on each trial. Phase entrainment to the stimulus onset was measured by performing Rayleigh's Z test on the distribution of phase angles across all trials, for each subject (n=8 rats for PFC and ACC and n=14 rats for VTA). We assessed phase entrainment between 4 - 50 Hz and only found significant entrainment of ~ 5 Hz oscillations. Across subjects, the excitatory LFP troughs of 5 Hz oscillations were locked to the stimulus onset. Furthermore, this entrainment was only observed in the mPFC, not in the ACC or in the VTA. The 5 Hz entrainment in mPFC was not observed when the rat selected the incorrect stimulus location suggesting a critical linkage between phase entrainment and attention task behavior. At the time of stimulus onset, correct and incorrect trials involved similar motor preparation, motor execution, and reward expectation. Therefore, we suggest that low-frequency oscillations may be a cross-species neurophysiological mechanism for attentional selection of expected stimuli. Selection of an expected stimulus may be implemented by enhancing neuronal excitability at the time of stimulus onset.

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Poster

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VA BLR&D 1IO1BX000782

Title: Cortical excitation during stop signal task performance in the rhesus macaque.

Authors: *H. P. JEDEMA[1], E. BAEG[2], J. WOOD[3], C. W. BRADBERRY[2,3,4]
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Abstract: Impairments in inhibitory control are associated with addiction. The stop (signal response) task is used to assess impairments in speed or efficacy in inhibitory control. Imaging and lesion studies have outlined a network of areas involved in response inhibition, but studies examining inhibitory control at the neuronal level have been limited.
To obtain more insight in the neuronal activity in these regions, we made simultaneous recordings in the pre-supplementary motor area (pSMA), SMA, and BA44/45 in a rhesus macaque while she performed a stop task on a touch screen. The appearance of the stop signal was adjusted between trials to maintain successful inhibition in approximately 50% of trials. Go trial accuracy was ~90% and the time required to successfully respond to the stop signal (stop signal response time) was ~247±6msec with a mean go trial response time of ~466±9msec. Latencies are reported from the onset of the go stimulus.
Preliminary analysis indicates that the basal firing rate was 6.1±0.7, 3.5±1.0, and 5.3±1.1Hz in BA45 (n=78), pSMA (n=24), and SMA (n=44), respectively. The neuronal responses were classified in successful go trials (GoCorrect), and successful (StopCorrect) and non-successful stop trials (StopError).
The majority of neurons in BA45 and SMA exhibited activations time-locked to the behavioral response. BA45 and SMA neurons were activated shortly before the release of a hold stimulus was initiated on all trial types (mean release time 112±5msec; activations peaked around 80-90msec for BA45 and SMA).
SMA neurons exhibited a prominent second period of activation on GoCorrect and StopError trials (latency ~420msec). The period of activation on StopCorrect trials was blunted and had a shorter latency to peak (310msec). The second excitation on GoCorrect and StopError trials coincided with the touching of the screen.
BA45 neurons exhibited a second period of activation immediately following the appearance of

the stop signal on both StopCorrect and StopError trials that was absent in GoCorrect trials. The peak of the second excitation in BA45 preceded the peak in the SMA. The second period of activation on StopError trials preceded the commission of the actual error and lasted longer than on StopCorrect trials.
The present data demonstrate prominent activations related to inhibitory control in BA45 neurons and may further our understanding of the mechanisms involved in inhibitory control of prepotent responding.

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Poster

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Support: Emil Aaltonen foundation

Title: Theta and gamma oscillations in the rat hippocampus during attentive lever pressing

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Abstract: Several experiments have demonstrated the importance of hippocampal theta (4-12 Hz) and gamma rhythms (40-80 Hz), and their mutual coupling, for working memory. However, it is not known whether the reported correlations between these brain rhythms and performance in a memory task relate to attention to the to-be-remembered information or to the maintenance of that information in memory. The present study addressed the role of hippocampal theta-gamma rhythms in a simplified task setting that only required maintained attention to a visible cue.
To control for movement, the known confounding factor for theta, we devised a task in which the rat had to attend to a cue light while continuously pressing a lever for a random duration of 2.5 - 3.0 s. Once the central cue light turned off and a peripheral cue light turned on, the rat had a limited time to make a nose poke into a hole below the peripheral light to obtain a water reward. Under these conditions, theta power did not significantly vary during the entire lever-press period, but increased during the movements associated with the lever release and turning around. In contrast, increased gamma-power during the lever press indicated an erroneous premature

release of the lever.
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Poster

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the University of Michigan

Title: Bidirectional optogenetic control of cortical acetylcholine signaling demonstrates vital contributions to attentional performance

Authors: *H. GRITTON[1], C. S. MALLORY[2], V. L. HETRICK[1], J. D. BERKE[1], M. SARTER[1]
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Abstract: Photostimulation of cholinergic neurons expressing channelrhodopsin-2 (ChR2) produces transient increases in acetylcholine release that resemble those seen during cue detection in attention task-performing animals (Gritton et al., 2010). Cholinergic lesions of the prefrontal cortex also produce cue detection deficits in attentional tasks (Parikh et al., 2007). Here we use optogenetics to determine if cholinergic signaling plays a causal role in signal detection and if inhibition during cue presentation reproduces the deficits noted in animals with cholinergic lesions. To achieve specificity of expression, viruses for doublefloxed channelrhodopsin (ChR2) or halorhodopsin (eNpHR3.0) were infused into the basal forebrain (SI/nbM) of ChAT-Cre mice. Animals were later implanted with optic fibers (200µm) into the basal forebrain and the medial prefrontal cortex, thereby allowing illumination of either cholinergic cell bodies or select terminals for comparisons during task performance. We found that combining optogenetic cholinergic stimulation with cue presentation augmented cue detection, and cholinergic stimulation without cue presentation produced more false alarms. A comparison between stimulation sites revealed that photo-activation of cholinergic prefrontal

terminals alone was as effective as somatic stimulation in producing this effect and scaled with light intensity and expression levels. Importantly, increases in false detections were not observed in non-infusion controls. Conversely, inhibition of cholinergic cell bodies beginning before cue presentation and extending through the cue period significantly attenuated cue detection. Unlike the outcomes noted for ChR2, inactivation of prefrontal terminal fields did not impair cue detection, suggesting that inhibition across larger cortical fields is necessary to mimic the effects of cholinergic lesions. These results directly demonstrate the key contribution of timed cholinergic signaling to the detection of unpredictable cues in attentionally demanding contexts and provide insights into the impact of poorly orchestrated cholinergic signaling during cognitive performance.

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Poster

913. Functional and Neural Mechanisms of Attention

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:913.17/CCC64

Topic: F.02. Animal Cognition and Behavior

Support: RO1-NS34086

NS18787

F31-NS073309

Title: Relationship between spike-synchrony & 'noise' correlation mechanisms in animals performing feature selective attention tasks

Authors: *M. GOMEZ-RAMIREZ, N. TRZCINSKI, S. S. HSIAO
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Abstract: The prevalent model of feature selection indicates that attention increases a neuron's response when the attended-feature matches the feature-preference of that neuron. This mechanism has been described as the feature-gain similarity model of attention (FGSM), and it has been primarily observed in the firing-rate (FR) of visual cortical neurons. However, attention can also influence activity across neural ensembles by either increasing the spike-synchrony (SS) or reducing the interneuronal correlated noise (ICN) across neural pairs. Yet, whether these mechanisms play a role in sensory selection and how they interact with each other is unknown.

Here, we examined whether attention modulates the SS and/or ICN of neural pairs according to the FGSM, and whether they hold any bearings to perception by examining their relationship to behavior. Finally, we characterized the links between SS and ICN to ascertain whether they share common sources of top-down attention. To answer these questions, simultaneous recordings were made from multiple neurons in secondary somatosensory cortex (SII) while animals' performed three different selective attention tasks. On every trial, a visual cue instructed the animal to engage in a tactile-orientation, a tactile-frequency, or a visual task that was unrelated to the tactile stimulus. Following 1000 ms the cue onset, a vibrating-oriented bar was indented to the animals' hand, which was followed by a visual response cue consisting of a pair of circles. The animal made a saccade to the correct circle depending on the task. If the animal performed the visual task, it ignored the tactile stimulus and made a saccade to the circle with higher brightness. The data showed that attention increased the SS across neural-selective pairs when it matched the feature-preference of the neurons. This effect was also replicated in the FR of individual neurons, but with lower prevalence across the population. However, attention did not affect the ICN across neural pairs in a feature specific manner. Instead, the ICN of the entire cell population decreased when attention was deployed to touch compared to vision. Importantly, the data also revealed a relationship between behavior and SS, whereby greater SS was associated with higher performance during the tactile task, whereas the opposite was observed when attention was deployed to vision. Finally, the data revealed an inverse relationship between ICN and SS. Our findings are in agreement with the FGSM, but suggest that SS and ICN are affected by different sources of top-down attentional signals. Finally, these data highlight the importance of studying perceptual mechanisms at the population level.

Disclosures: M. Gomez-Ramirez: None. **N. Trzcinski:** None. **S.S. Hsiao:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: BMBF Grant 01GQ0705 (Bernstein Group for Computational Neuroscience Bremen)

Leibniz Graduate School for Primate Neurobiology (NEUROPRIM)

Title: Interareal gamma-band synchronization in primate ventral visual pathway underlies signal routing during selective attention

Authors: *I. GROTHE[1,2], S. D. NEITZEL[1,2], D. ROTERMUND[2,3], S. MANDON[1,2], M. LINKE[2,3], U. A. ERNST[2,3], K. R. PAWELZIK[2,3], A. K. KREITER[1,2]
[1]Theoretical Neurobio., [2]Bernstein Group for Computat. Neurosci., [3]Inst. for Theoretical Physics, Univ. of Bremen, Bremen, Germany

Abstract: When two stimuli are present in a visual receptive field (RF), the neuron can respond as if it only would receive the signal input from the attended stimulus, even if this stimulus is not preferred by the neuron. It has been proposed that selective gamma-band synchronization between downstream neurons and the subset of their inputs carrying the relevant signals may directly lead to such routing of the attended stimulus signals. However, a direct link between selective interareal synchronization and signal transfer remains to be demonstrated.
With this goal, we trained two macaque monkeys to attend to one of two sequences of morphing shapes and to respond to the reoccurrence of the initial shape in the cued sequence. While the monkeys were engaged in this spatial attention task, we recorded local field potentials (LFPs) from areas V1 and V4 simultaneously. The shapes were placed into two non-overlapping V1 RFs, encompassed by a V4 RF. Every 10ms, the luminance of the filled shapes was randomly changed, independently for each stimulus. We computed spectral coherence between stimulus luminance and the LFPs as a measure of the effective contribution (EC) of each stimulus to the neuronal activity in each area. The EC serves as a direct and unambiguous quantification of coupling in terms of linear causation.
We observed strong gamma-band LFP synchronization between the V4 population and the V1 input subpopulation representing the attended stimulus. As expected, this was accompanied by a high EC of the attended stimulus to the V4 LFP. Simultaneously, the EC of the non-attended stimulus was several times lower and also gamma-band synchrony between the same V4 population and the V1 input subpopulation representing the non-attended stimulus was only weak. A switch in attentional locus was accompanied by a switch in the interareal gamma-band synchronization pattern and a reversal of the stimuli's ECs to the V4 LFP. Importantly, this was not due to attentional modulation of the EC in the V1 LFPs. Lastly, the strength of the selective gamma-band synchrony and EC were found to be highly correlated. These observations were reproduced in a minimal model that uses interareal gamma-band synchronization as a selective gating mechanism, demonstrating that selective interareal gamma-band synchrony can indeed implement the observed selective information routing.
Altogether, our study shows convincingly that interareal gamma-band synchrony is directly related to the selective routing of attended stimulus signals through the visual cortex and thus supports the hypothesis that oscillatory synchrony between areas underlies the routing of information throughout neuronal processing systems.

Disclosures: I. Grothe: None. **S.D. Neitzel:** None. **D. Rotermund:** None. **S. Mandon:** None. **M. Linke:** None. **U.A. Ernst:** None. **K.R. Pawelzik:** None. **A.K. Kreiter:** None.

Poster

913. Functional and Neural Mechanisms of Attention

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Topic: F.02. Animal Cognition and Behavior

Support: SOMAS-URM Grant 490

Wellesley College Schiff Fellowship

Title: Long-range frontal-parietal gamma coherence is associated with sustained attention in rats

Authors: *M. C. WIEST, L. HERZOG, K. SALEHI
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Abstract: To test whether long-range neural coherence supports attention, local field potentials (LFPs) were recorded in frontal and parietal cortex while rats performed an auditory signal detection task. We observed significantly more frontal-parietal gamma coherence (30-80 Hz) before the signal on hit trials than miss trials. Gradual changes in behavioral responding over the course of a session failed to account for the hit-miss coherence difference. These results support that frontal-parietal gamma coherence underlies sustained attention to an external stimulus, and the rat as a model of primate attentional mechanisms.

Disclosures: M.C. Wiest: None. **L. Herzog:** None. **K. Salehi:** None.

Poster

913. Functional and Neural Mechanisms of Attention

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Topic: F.02. Animal Cognition and Behavior

Support: Canadian Institutes for Health Research (CIHR)

Natural Sciences and Engineering Research Council (NSERC)

Title: Switches of selective neuronal synchronization patterns segregate attentional and decision making processes in macaque prefrontal cortex

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Abstract: Multiple brain areas within the primate prefrontal cortex are implicated to subserve the control of attention as well as to guide decision making (Rushworth et al., 2011, Neuron). Covert attentional selection of an object or memory typically precedes the overt choice of the so selected representation. It has remained unresolved, however, how neurons ensuring focused attention, and neurons integrating decision variables to arrive at a choice are interacting during goal directed behavior. Previous studies suggest that the local integration and temporal coordination of distinct functions is subserved by rhythmic synchronization among the constituent neurons. We therefore set out to test whether selective synchronization patterns of single neurons across medial and lateral prefrontal cortex (PFC) subdivisions allow to identify the integration or segregation of attentional or choice related processes.
We recorded from >800 neurons in macaque monkeys trained on a selective attention task and quantified spike to LFP synchronization patterns during two distinct processing states: (1) A selective “attentional state” in which monkeys sustained focused attention towards one of two peripheral stimuli, and (2) during a “decision point” requiring a 2-discrimination forced choice about a transient clockwise/counterclockwise rotation of the attended target stimulus.
We found that a large proportion of single neurons reliably phase synchronized their spiking responses to the LFP across a broad range of frequencies during attentional states (~38%) and during the decision period (~19%).
During both, attention and decision epochs, about 25% of neurons with significant phase locking synchronized in the classical gamma range (40-70). The percentage of neurons synchronizing to theta/alpha and beta ranged from 10-15% in both epochs. Notably, the populations of neurons synchronizing at gamma / beta in both epochs was largely segregated. Common rhythmic modulation across epochs was restricted to neurons with theta/alpha synchronization.
These results reveal that large subsets of PFC neurons show statistically reliable synchronization and that transitioning between focused attentional and choice processes is evident in switches of synchronization. Apart from segregation of processing by switches of synchronization, the temporal integration of attentional and choice processes may be subserved by neurons with a common theta rhythmic firing.

Disclosures: S. Westendorff: None. **D. Kaping:** None. **M. Vinck:** None. **S. Everling:** None. **T. Womelsdorf:** None.

Poster

913. Functional and Neural Mechanisms of Attention

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Topic: F.02. Animal Cognition and Behavior

Support: NIH MH087978

NIH MH091372

Title: Attentional deficits in mouse offspring born small or large for gestational age are due to a double dissociation between distractibility and decreased motivation.

Authors: *T. M. REYES, N. M. GRISSOM, J. DESILETS
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Abstract: Overly small or large gestational size (SGA and LGA, respectively) and maternal obesity are frequently reported to increase in offspring the risk of mental disorders regulating cognition and attention, particularly Attention Deficit/Hyperactivity Disorder (ADHD), and in the case of maternal obesity, increase the risk of autism and cognitive delay. However, it is difficult in human work to disentangle gestational size and development from other variables that may promote offspring psychological dysfunction. Using mouse models of SGA (maternal low-protein diet) and and LGA/maternal obesity (maternal high-fat diet), we have examined adult male C57xDBA F1 offspring in an operant behavioral battery to determine if gestational nutrition plays a direct role in behavior and cognition related to reward. We first examined animals on a version of Pavlovian Conditioned Approach (PCA) developed for mice, as propensity to form Pavlovian approach associations with a “sign” that predicts reward has been associated with impulsivity and hyperactivity in rats. SGA offspring have a greater tendency to approach the sign relative to control and LGA offspring, suggesting SGA are particularly susceptible to distraction by environmental stimuli. After PCA, animals were trained on fixed ratio (FR1) nose poking followed by progressive ratio (PR) testing. LGA offspring were slightly delayed at learning FR1 over the first 5 days relative to SGA and control offspring, but performed equivalently after additional training. In PR, LGA offspring terminated responding slightly but significantly earlier than control and SGA, indicating deficient motivation to seek rewards as a specific deficit of LGA. Animals were then trained on a series of increasingly challenging 5-choice serial reaction tasks (5C) to assess their attentional abilities. Analysis early in 5C training indicates that both SGA and LGA offspring have similar performance deficits in an easy version of the 5C task, with fewer correct trials and fewer trials overall, driven by trends towards increased numbers of premature and omitted trials in both groups. We are continuing with 5C training, with animals progressing towards a 5-choice continuous performance test to overtly assess ability to inhibit prepotent responding during no-go trials. Overall, current data

collected indicate that both maternal overnutrition and undernutrition promote increased risk of inattention, supporting the human observations, but do so via dissociable mechanisms related to innate distractibility in SGA offspring versus decreased motivation to perform in LGA offspring.

Disclosures: T.M. Reyes: None. **N.M. Grissom:** None. **J. Desilets:** None.

Poster

914. Reward: Behavior and Drug

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Program#/Poster#:914.01/CCC69

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant EY16187

Title: Monkey math: Linear symbolic addition by macaques.

Authors: *W. W. PETTINE[1], B. MOORE[2], M. LIVINGSTONE[1]
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Neurobio., Vanderbilt Univ., Nashville, TN

Abstract: Three macaques were given an addition task using dots and then the same task with a learned symbol set. They had previously been trained on a pairwise comparison task to associate 26 distinct symbols with 0 to 25 drops of fluid reward. For the addition task, the macaques chose between a sum of value 0 to 25 represented by two addend symbols vertically separated on one side of the screen, and a single symbol representing a value between 0 and 25 on the other side of the screen. They were rewarded with the corresponding magnitude of whichever side they chose. After a period of several months on this addition task, their choice behavior indicated that they were combining the two addends to obtain an approximate value equal to their sum. To eliminate the possibility that they had memorized the value of all 351 possible sum combinations, we taught the monkeys a second symbol set with the same pairwise comparison task used for the first symbol set. Their performance, even from the first few days, on the task using the novel symbol set indicated that they had successfully generalized the addition task from the first symbol set to the second.
We then asked whether their valuation of the sum was consistent with a logarithmic or linear underlying scale for the symbols. If the underlying scale was logarithmic, their valuation of the sum would increase with the product of the addends. A linear underlying scale would result in the addends linearly combining to form the subjective sum. Their addition behavior was best

accounted for by a linear scale and showed no evidence of an underlying logarithmic scale.  

Disclosures: W.W. Pettine: None. **B. Moore:** None. **M. Livingstone:** None.

Poster

914. Reward: Behavior and Drug

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Program#/Poster#:914.02/CCC70

Topic: F.02. Animal Cognition and Behavior

Support: NIH R01-MH080318

NIH R01-DA030672

NIH T32-DA007234

Title: Restaurant row: Identifying revealed preferences in the rat

Authors: *A. D. REDISH[1], A. P. STEINER[2]
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Abstract: Valuation of reward is subjective and depends on personal preferences. How long one is willing to wait for a reward depends on the subjective valuation of that reward and the set of other options available. In order to directly measure the subjective valuation economically, one needs a task in which an animal can reveal its preferences.
Rats were trained on a new task (Restaurant Row), in which they had to make skip/stay decisions at a sequence of choices. The maze consisted of an octagonal loop with four spokes. At the end of each spoke, a feeder provided a unique flavor (white/non-flavored, banana, fruit, and chocolate). The rat ran clockwise around the loop; zones were only activated in sequence. As the rat passed each spoke, a tone sounded indicating the delay it would have to wait before receiving food. The rat could either stay on the spoke and wait out the delay (as the tone decreased in

pitch, counting down the remaining seconds until food delivery) or leave the zone and proceed on to the next spoke. Delays ranged from 0 to 45 sec and were generated pseudo-randomly, so the rat did not know what the delay of the next option would be. By examining which delays the rat would wait out and which it would skip, we were able to determine the flavor preferences for each rat. Each rat displayed a different array of preferences, but preferences remained generally consistent within rat. On occasions where the rat decided not to stay, it left after only a couple of seconds, independent of the delay, indicating that the decision was made based on an unwillingness to wait. Changing the size of the reward (from 1 to 3 pellets) increased the delay the rat was willing to wait, suggesting an economic valuation process.
Rats occasionally paused at the entry to each zone, and oriented towards the two options (down the spoke or around the loop), a behavior known as vicarious trial and error (VTE). VTE occurred maximally at the threshold between short delays (stay) and long delays (skip), even though the threshold differed between options. When rats encountered a long delay to a less-preferred food after skipping a long delay to a more-preferred food, they occasionally looked backward towards the previous spoke, but did not actually proceed backwards towards the previous spoke.
Preliminary neural data in OFC identified many neurons that showed responses for specific rewards, in agreement with prior research. Data from vStr also demonstrated reward responsive activity as well as showing increased firing rates prior to feeder arm entry. These results illustrate revealed preferences in rats and offer the potential to describe the time course of interaction between OFC and ventral striatum during decision making.

Disclosures: A.D. Redish: None. **A.P. Steiner:** None.

Poster

914. Reward: Behavior and Drug

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Topic: F.02. Animal Cognition and Behavior

Support: HFSP 2010/RGP0039

NIH R01 MH080318

NIH R01-DA030672

NIH T32 HD007151

Title: Vicarious trial and error (VTE) behaviors on a spatial delay-discounting task

Authors: *A. PAPALE[1], P. S. REGIER[2], J. J. STOTT[2], N. J. POWELL[2], A. D. REDISH[3]
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Abstract: Delay-discounting experiments measure choices made between taking a smaller reward sooner versus waiting for a larger one later.Discounting behavior can be inferred from an adjusting delay procedure in which the delay to the larger reward is increased when the larger reward is chosen and decreased when the smaller reward is chosen. The adjusting delay procedure is designed to allow the animal to titrate the delay until both options are preferred equally (the indifference point).
We present a spatial T-maze version of the adjusting delay-discounting task. Because rats naturally prefer to alternate between options on a T-maze (spontaneous spatial alternation), the spatial delay-discounting task does not need forced choice trials to ensure that rats try both options. Delay was increased or decreased by 1s on each lap depending on the choice of the rat on that lap; both sides had a minimum delay of 1s and no maximum delay. Each session, rats investigated the options for a given day, adjusted the delay to a preferred indifference point, and then alternated at that indifference point.
11 rats were trained to run 100 laps per session at a reward ratio of 3 to 1. The initial delay for the larger reward began each day at a pseudo-randomly selected delay from 1s to 30s . Rats reliably titrated the adjusting delay to approximately 6-8 seconds.
4 rats were trained to run 100 laps per sessions for larger rewards of 1 to 5 pellets. The size of the larger reward remained constant on each day but changed from day to day. An additional rat was not given a lap constraint but was allowed to run each session until he received 200 pellets (9g). All 5 rats reliably titrated to higher delays for larger rewards, with delay by reward size showing significant linear slope, consistent with hyperbolic discounting.
Rats sometimes pause and orient toward potential options before making a choice, a process termed vicarious trial and error (VTE). VTE has been hypothesized to reflect perceptual exploration of uncertain alternatives, mediation of conflict through conditioned orienting, discrimination among fixed-value alternatives, and activation of a deliberative, search-based decision process. On the spatial delay-discounting task, VTE primarily occurred during adjustment laps. VTE continued to occur long after the alternatives were known (rats reliably titrated to the indifference point, indicating knowledge of task structure). VTE did not occur during the final alternation phase of behavior (during spatial alternation when the discounted values were equal). VTE was most likely to occur when an adjustment lap interrupted a series of alternation laps, suggesting a role in flexible decision-making processes.

Disclosures: A. Papale: None. **P.S. Regier:** None. **J.J. Stott:** None. **N.J. Powell:** None. **A.D. Redish:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R01 MH-080318

UCRF-443349

Title: Vicarious trial and error (VTE) behaviors on a task that puts place and response strategies in conflict

Authors: *B. SCHMIDT[1], A. E. PAPALE[2], E. J. MARKUS[4], A. D. REDISH[3]
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Abstract: Spatial tasks can be accomplished by multiple decision-making strategies, using different information-processing computations. A well-studied dichotomy in these decision-making strategies on spatial tasks compares “place” and “response” strategies. Place strategies tend to depend on hippocampal integrity, while response strategies tend to depend on dorsolateral striatal integrity. Place strategies are needed when rats approach a single location from multiple starting positions, while response strategies are needed when rats need to take the same action from multiple starting positions.

When rats reach decision points, they sometimes pause and orient towards their routes, a process termed vicarious trial and error (VTE). VTE co-occurs with neurophysiological information processing in structures associated with place strategies, including sweeps of representation ahead of the animal in hippocampus and transient representations of reward in ventral striatum and orbitofrontal cortex. However, these effects are not present in dorsolateral striatum.

In order to examine the relationship between VTE and the place/response strategy dichotomy, rats were trained to continuously switch between place (“go east”) and motor-response (“turn right”) navigation strategies on a modified plus maze for food reward. Place trials were cued by the presence of a flashing light. (The light did not indicate which arm was correct, only that the trial was a place-trial.) The configuration of the maze allowed for place and response strategies to work competitively (each strategy indicating a different goal arm) or cooperatively (both strategies indicating the same goal arm).

VTE was quantitatively measured as the integrated absolute angular velocity across the pass through the central choice-point. VTE was preferentially exhibited in rats when cued to utilize a place strategy over a response strategy. VTE was preferentially exhibited on incorrect trials and on the more difficult trials that exhibited greater competition between the navigational systems. Interestingly, when individual trial types were examined, the use of place strategies was found to

exert a greater modulating factor than competition between the navigation systems. Upon further examination we noted that VTE was seen on trials when rats were cued to use the response strategy but incorrectly utilized the place strategy, further supporting a preferential role of VTE in place strategy performance.

Disclosures: B. Schmidt: None. **A.E. Papale:** None. **E.J. Markus:** None. **A.D. Redish:** None.

Poster

914. Reward: Behavior and Drug

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R01-DA024080

Title: Implications of the multiple-decision making systems theory for Contingency Management

Authors: *P. REGIER[1], A. D. REDISH[2]
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Abstract: Contingency Management (CM) consists of rewarding a subject for behavioral modification such as abstinence from drug-use (Petry 2011). In voucher-based CM, subjects earn points that are worth money for each proof of abstinence. These points accumulate towards the purchase of specific merchandise. Prize-based CM provides a chance to win a prize for each proof of abstinence. Clinical studies of Contingency Management have shown consistent and significant reductions of drug use in addicts for a number of different addictive drugs.
Although this treatment method has its basis in alternative reinforcement theory, providing monetary rewards contingent upon the proof of abstinence and extra incentive for continuous abstinence, the size of rewards is small compared to the elasticity of drug-use. We hypothesize that CM does not work by alternative reinforcement alone.
Abstinence is an abstract concept that typically includes delayed rewards while drug use includes concrete immediate rewards. Because temporally-distant rewards are discounted relative to more proximal rewards, one potential source of CM's success is that it provides concrete rewards for abstinence. Recent experimental data suggest that making a reward more concrete decreases the amount it is discounted for a given temporal delay (Peters and Buchel 2010). Recent modeling work suggests that this may occur because concrete rewards are easier for search processes to find (Kurth-Nelson, Bickel, Redish 2012).
The Multiple Decision-Making Systems theory (van der Meer, Kurth-Nelson, Redish 2012)

suggests that CM may also encourage the use of one decision-making system over another. An addict's desire to abstain can be undermined by Pavlovian action-selection systems which preferentially approach (and use) concrete, immediately available options that have been learned to have high Pavlovian value. Similarly, an addict's desire to abstain can be undermined by Habitual action-selection systems, which respond to learned cues. We hypothesize that a concrete choice between using a drug vs. receiving a voucher or prize for abstinence engages the Deliberation system. By providing concrete actions that Deliberative search systems can find allows the Deliberative search to outcompete other components and suggests that deliberative system training (such as through working memory training (Bickel et al 2011) may increase CM's success even further.

Disclosures: P. Regier: None. **A.D. Redish:** None.

Poster

914. Reward: Behavior and Drug

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC Grant RGPIN 6303

Title: Delay discounting of oral morphine and sweetened juice rewards in dependent and non-dependent rats.

Authors: *C. HARVEY-LEWIS, J. PERDRIZET, K. B. J. FRANKLIN
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Abstract: Opiate dependent individuals are frequently reported to be more impulsive than non-dependent controls. Opiate dependent rats exhibit similar tendencies; we previously reported that inducing morphine dependence resulted in a transient increase in impulsive choice of sucrose rewards. Interestingly, opiate dependent humans are more impulsive towards opiate rewards than monetary rewards. This indicates that when drugs are involved, dependent individuals make even more impulsive decisions than usual. To test this finding in rats, we first validated the use of a cocktail of 10% grapefruit juice, 20% sucrose and 1mg/mL morphine as an oral morphine reward. In a two-lever operant choice paradigm rats preferred the morphine cocktail over a similar drug-free solution (10% grapefruit juice, 20% sucrose solution) but preferentially selected the drug-free solution over a cocktail where quinine was substituted for morphine. Rats consumed sufficient morphine to induce analgesia. We examined impulsive choice of morphine and drug-free cocktails in dependent and non-dependent rats using a delay discounting (DD)

paradigm. Rats were trained to press one of 2 levers for the drug-free solution. Pressing one lever yielded a small immediate reward (50µl of solution) and the other yielded a larger delayed reward (150µl of solution). DD sessions consisted of 5 blocks of 14 forced trials. On the first 4 trials of each block rats were forced to sample each reward twice. On the following 10 trials rats could choose either lever. Length of the delay was systematically increased (0s, 5s, 9s, 16s, 30s) between successive blocks within session. A DD curve was determined by plotting mean proportion choice for the larger reward against delay. Once baseline DD curves were determined, rats were randomly assigned to either a dependent group that received a nightly 30mg/kg subcutaneous dose of morphine, or a non-dependent group that received a nightly saline injection. Test DD curves were then determined 22hr after nightly injections. In each group, half of the rats received morphine cocktail as reward for test DD and half received the drug-free solution. When compared to baseline and the non-dependent group, dependent rats showed a significantly decreased preference for the large reward at 5s, 9s, 16s delays. However, both reward types were discounted similarly. These results show that morphine dependence can cause a general increase in impulsivity that is not enhanced by drug reward.

Disclosures: C. Harvey-Lewis: None. **J. Perdrizet:** None. **K.B.J. Franklin:** None.

Poster

914. Reward: Behavior and Drug

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA027718

Title: Outcome expectancy signals in macaque OFC in a curiosity task

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Abstract: A recent theory holds that the chief function of the OFC (especially the lateral portion, sometimes known as lOFC) is to signal outcome expectancies - rewards predicted from specific stimuli (Schoenbaum, Roesch, Stalnaker, and Takahashi, 2009). This theory holds that OFC signals serve to predict the rewards that would be obtained following the choice of a given option and also track the reward obtained following the choice. This primitive reward signal is distinct from other reward signals, especially the reward prediction error, which signals the difference in reward (cued or obtained) and prediction. In order to test the idea that OFC signals outcome expectancies, we recorded responses in a task that offered a choice between two 50/50 gambles

with delayed (2.25 sec) resolutions. One gamble offered information about the upcoming reward immediately while the other offered no such information (Bromberg-Martin and Hikosaka, 2009). Extending earlier work by Bromberg-Martin and colleagues, we found that monkeys are highly information-seeking, preferring earlier resolution to delayed resolution by approximately 20% of average reward size. We recorded single unit activity in area 13m, a centrally located region in the lateral network of the OFC (Ongur and Price, 2000). We found that neural activity signalled rewards expected following valid reward-predicting cues, but not during matched invalid cues. We also found that neural activity signalled reward obtained following the resolution of the gamble and did not depend strongly on expectation. These results endorse the outcome expectancy hypothesis, and thus help carve out a unique role for OFC in reward-based cognition.

Disclosures: M. Mancarella: None. **T.C. Blanchard:** None. **B.Y. Hayden:** None.

Poster

914. Reward: Behavior and Drug

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:914.08/CCC76

Topic: F.02. Animal Cognition and Behavior

Support: Champalimaud Neuroscience Programme

Fundação Ciência e Tecnologia

Title: How to get there?: Action sequence strategies for solving navigation problems

Authors: *A. R. FONSECA, M. MURAKAMI, Z. F. MAINEN
Champalimaud Neurosci. Programme, Lisboa, Portugal

Abstract: Navigation is a fundamental behavioral problem solved by animals. Nevertheless, its neural bases are poorly understood. Computationally, defining the path to a goal requires integrating information about current self-location and heading with information about goal location. Several kinds of neural representations of self-location and heading have been described in the rodent brain, including place cells, head direction cells and grid cells. In comparison, much less is known about how goals or goal paths are represented neurally. Ultimately, this navigational information must be used to select which action sequence will be executed to reach the goal. To explore these problems, we developed a behavioral paradigm where a rat has to solve a navigation problem by selecting which action sequence will lead to a specific goal on a trial-by-trial basis. We designed a squared grid maze with 9 water ports and

1.5m side. In each trial, water availability was assigned to a given port location and cued by a constant light co-localized with the assigned port. The light was on until the rat poked in breaking a light beam which simultaneously turned the light off and activated the release of water followed by a 2 s inter-trial interval. Our training strategy was to offer the rat the possibility of harvesting reward in several cued locations and then decrease slowly this number until rat could only get water from one port. After 5 days of training, rats performed more than 250 correct trials in one hour session. Because we could control indirectly the current position of the rat and the goal, manipulations of navigational behavior were very simple to introduce. For instance, we could force the rat to change its action sequence plan by changing the goal port after the animal had started its action execution. This is equivalent to introduce a barrier in a familiar path.Our aim is to gain a better understanding about the neural representations underlying the use of navigational information to select motor sequences and this task due to characteristics both as a navigational paradigm and an action sequence paradigm seems to us as good behavioral framework to look for these neural correlates.

Disclosures: A.R. Fonseca: None. **M. Murakami:** None. **Z.F. Mainen:** None.

Poster

914. Reward: Behavior and Drug

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Program#/Poster#:914.09/CCC77

Topic: F.02. Animal Cognition and Behavior

Support: FCT SFRH/ BD/ 32947/ 2006

HFSPO - RGP0027/2010

Title: Interplay of confidence and value in the transformation of olfaction to action

Authors: *G. M. COSTA[1,2], Z. F. MAINEN[1]
[1]Champalimaud Neurosci. Programme, Lisboa, Portugal; [2]Ctr. For Neurosci. and Cell Biol., Coimbra, Portugal, Coimbra, Portugal

Abstract: Difficult decisions can occur because stimuli are hard to perceive or because the rules of what should be done given a certain stimulus are uncertain to the decision maker. We would like to understand how this second form of uncertainty is represented by the brain and may be assessed and used for adaptive behavior. In addition, we are interested in the relationship between uncertainty and value in such decisions. To study these issues we developed a perceptual decision task in which rats are trained to perform a binary categorization of an odor

mixture and report their decision confidence by reward waiting time (Kepecs at al., 2008) in a post-decision wager paradigm. From trial-to-trial odour mixtures were presented at one of three different mixture ratios. The distance of the mixture ratio from 50/50 determined the uncertainty or difficulty. Rats indicated their choice by moving from the odor port to a left or the right choice port. For correct trials, a reward was delivered after a random exponentially distributed delay (2 s mean). In error trials and catch trials (15% of correct trials) no reward was delivered and rats had to make a decision when to exit the choice port and reinitiate a new trial. Decision confidence was indexed by waiting time in those trials. In order to dissociate neuronal representations of confidence and value we introduced an additional manipulation of reward magnitude: in alternating blocks of trials one of the two choice sides was rewarded with 3 times more water than the opposite side. As expected, this reward manipulation biased the choice function of the animals towards the more rewarded side. In contrast, the confidence report was unaltered by the value manipulation: leaving times for the high and low reward ports were not different. We are currently investigating the neural substrates of this behavior. Recordings from the anterior piriform cortex (aPCx), together with previous behavioral results, suggest that rats have a high fidelity representation of the odor cue, but that variability is introduced downstream of this sensory representation. We hypothesize that the olfactory tubercle (OT, an olfactory striatum) is the site where odor representations are linked to action values and therefore the site at which uncertainty in the olfactory categorization task originates. To test this we are conducting electrophysiological recordings from multiple single units in aPCx and OT while rats (n=6) performed the categorization task and we have been exploring this dataset.

Disclosures: G.M. Costa: None. **Z.F. Mainen:** None.

Poster

914. Reward: Behavior and Drug

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Program#/Poster#:914.10/CCC78

Topic: F.02. Animal Cognition and Behavior

Support: SFRH / BD / 33937 / 2009

Title: Coordination in rats: The use of game theory to study the proximal mechanisms of cooperation.

Authors: *S. M. RENNIE, M. MOITA
Champalimaud Fndn., Lisbon, Portugal

Abstract: Animals must survive in an ambiguous world governed by shifting probability landscapes where the likelihood of available outcomes is reciprocally intertwined with their own behavioural choices. This is most
evident in social interactions in which the outcome of one's decision can be influenced by the decisions of other animals. An automated double T-Maze for testing behaviour in 2x2 social dilemma games has been developed to examine how rats make adaptive behavioural choices during social interactions. We have employed a simple coordination task with pellet rewards corresponding to a Stag Hunt (SH) game that are both discernible and desirable to rodents. By fixing a pseudo random strategy for one rat (stooge) and observing the choice of a freely behaving rat (agent) it was found that rats rapidly engage in coordination. This suggests that rats are able to understand the economic terms of the SH game and optimize their behaviour accordingly.We will then observe the behaviour of two agents in a SH game. Importantly in this game there are two Nash equilibria: mutual cooperation which yields the highest reward but entails the highest risk and mutual defection which yields a constant intermediate reward. In this case rats must choose whether to risk cooperating first, defecting, or following the other. We propose that choosing to cooperate first may be used as a measure of 'trust'.

Disclosures: S.M. Rennie: None. **M. Moita:** None.

Poster

914. Reward: Behavior and Drug

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:914.11/CCC79

Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI 21240024

Title: Behavioral study of fictive outcome in a monkey using a value-based binary choice task

Authors: *H. TAKAHASHI[1], S. FUNAHASHI[2]
[1]Grad. Sch. of Human and Envrn. Studies, [2]Kokoro Res. Ctr., Kyoto Univ., Kyoto, Japan

Abstract: Fictive outcome refers to the outcome which is not directly experienced. We refer to both actual outcome, outcome of our own past choice, and fictive outcome to make a choice. Although many studies have focused on the actual outcome, much less is known about the fictive outcome and its contribution to choice. To examine how the fictive outcome modulates animal's choice, we developed the following value-based binary choice task. Two visual cues which were associated with different reward probability and amount were presented and a monkey freely chose one of them. Once the monkey chose a cue, outcomes (either hit or miss) for both chosen

and unchosen cues were presented. The outcome of the chosen cue represented whether the monkey could receive reward. The outcome of the unchosen cue represented whether the monkey would receive reward if the monkey chose the unchosen cue. Therefore, regardless of the outcome, the unchosen outcome did not affect giving of reward.
We examined whether the outcome of the unchosen cue influence monkey's choice behavior. We predicted that the monkey would be more likely to change his choice in the next trial under the condition in which the outcome of the unchosen cue was hit and the outcome of the chosen cue was miss, compared to the condition in which the outcomes of both the chosen cue and the unchosen cues were miss. However the monkey did not show such a choice pattern. We also examined how the outcome of the unchosen cue affected the length of trial start time (an interval between presentation of a fixation point and the time when the monkey starts gazing at it). Trial start time is considered to reflect animal's motivational state, such that it tends to be longer as animal's motivation decreases. We predicted that the trial start time would be longer if the outcome of the chosen cue was miss. Furthermore, the trial start time would be longer under the condition in which the outcomes of both the chosen and unchosen cues were miss compared to the condition in which the outcomes of the unchosen cue was hit.. However, significant difference in the trial start time was not observed between these conditions. Thus, the fictive outcome tends not to affect the monkey's choice, if the monkey has learned association learning between the cue and its reward probability/amount. To examine the effect of the fictive outcome on animal's choice, it is appropriate to analyze choice behavior during the process of the association learning.

Disclosures: H. Takahashi: None. **S. Funahashi:** None.

Poster

914. Reward: Behavior and Drug

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Program#/Poster#:914.12/CCC80

Topic: F.02. Animal Cognition and Behavior

Support: NSERC 1152030000386210

Title: Dissociable effects of amphetamine on stopping and performance monitoring for rats in the countermanding task

Authors: *J. BEUK[1], R. J. BENINGER[2], M. PARÉ[3]
[1]Dept. of Physiol., [2]Psychology, [3]Physiol., Queen's Univ., Kingston, ON, Canada

Abstract: The countermanding paradigm has been developed to examine a subject's ability to withhold responses to a go stimulus when a stop signal is presented occasionally, as a measure of executive function (Logan & Cowan, 1984). Deficits in countermanding task stopping, which are indicative of impairments in behavioural inhibition, have been ameliorated with amphetamine treatment in humans and rats (de Wit et al 2002, Eagle et al 2003, Feola et al 2000). The countermanding task provides a separate measure of executive function through the analysis of performance monitoring by tracking trial-to-trial changes in task performance produced by differences in trial and performance history. The aim of our experiment was to examine performance monitoring and changes produced by amphetamine treatment in rats. Male Wistar rats were trained to respond to a visual stimulus (go signal) by pressing a lever below an illuminated light for food reward, but to countermand the lever press (25% of trials) subsequent to an auditory tone (stop signal) presented after a variable delay. Rats displayed go response time (goRT) speeding after consecutive go trials and goRT slowing on trials following errors. Amphetamine administration improved countermanding task performance by speeding goRTs and producing baseline-dependant alterations in stop signal response time. Preliminary results suggest that amphetamine administration impaired performance monitoring by decreasing goRT speeding and post-error slowing. Together, these results suggest that amphetamine administration in rats improves stopping, but may impair performance monitoring. These results may reveal dissociable measures of executive function for rats in the countermanding task. (Funded by NSERC)

Disclosures: J. Beuk: None. **R.J. Beninger:** None. **M. Paré:** None.

Poster

914. Reward: Behavior and Drug

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:914.13/CCC81

Topic: F.02. Animal Cognition and Behavior

Title: Behaviour and decision making: Neuroeconomics, corruption in higher education and shock waves

Authors: *M. ORKODASHVILI
Vanderbilt Univ., Tbilisi, Georgia

Abstract: The paper discusses the issue of corruption in higher education through neurocognitive prism. Mental causation, human behavior, cognitive processes taking place in the brain, risk-taking endeavors, all lend to *neurocognitive* field of research. The paper refers to the

examples of institutional and departmental mergers in higher education sphere that are usually accompanied by staff dismissals and shock effects that spread shock waves.
Departmental and institutional mergers at Tbilisi State University, Ilia State University and Tbilisi Free University present further instances that are useful for *neuroeconomic* and *neurocognitive* analysis of profit-maximization considerations, coercive pressures coming from governmental agents and brain's manipulative capacities that allowed for quality evaluation distortions in all those instances.
By explicitly modeling physiological properties, it is possible to provide *foundations for some elements of preferences traditionally considered exogenous*, such as risk aversion, ambiguity aversion, or time-preference rates. Choices involving risk, uncertainty, or time delays may require complex trade-offs. Measures of brain activity allow us to determine if the evaluation process is centralized or if different brain systems compete to influence the final decision. *Neuroeconomic* theory proposes to model the actual brain organization, determine the behavior that emerges from it, and evaluate which theory fits best.
Research uses neurobiological evidence to model inter-temporal choices as the result of a conflict between two brain systems, one interested in immediate gratification and one that can form a mental representation of future rewards.
The following important points have been singled out in the paper: • The amount of information available • Risk aversion strategies - increasing confidence level • How choices are made in the brain of an agent During staff and faculty dismissals at Tbilisi State University the linguistic means of communication, expressed through ambiguous syntactic structures, metaphors with dubious meanings and multiple neurolinguistic techniques engendered and deepened cognitive perceptions and feelings of insecurity, instability and intimidation among the dismissed individuals. The result was the 'production' of two main types of actors: those who were shocked, confused insulted, and intimidated, and those who got grips with the ongoing developments and joined in the manipulative and coercive circles of individuals who used the created shock waves, ambiguity and confusion to their advantage.1

Disclosures: M. Orkodashvili: Employment; Vanderbilt University. Research Grant; n/a. Other Research Support; n/a. Speakers Bureau/Honoraria; n/a. Ownership Interest; n/a. Consultant/Advisory Board; n/a. Other; n/a.

Poster

914. Reward: Behavior and Drug

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:914.14/CCC82

Topic: F.02. Animal Cognition and Behavior

Support: NIH AA201013

Title: Impulsive choice is driven by perception of time in male, but not female, adolescent rats

Authors: *J. MCCLURE[1], F. JACKSON[2], H. N. RICHARDSON[3]
[1]Univ. of Massachusetts Amherst, South Hadley, MA; [2]Univ. of Massachusetts Amherst, Amherst, MA; [3]Univ. of Massachusetts, Amherst, MA

Abstract: Previous findings in our laboratory suggest that a measure of early adolescent impulsive choice can predict late adolescent alcohol consumption selectively in female rats while no relationship has been detected for males.
This lead to the question of whether different mechanisms underly the measured impulsive choice in the two sexes.
Impulsive choice can have both perceptual and cognitive components; variation in impulsivity may then be explained either by individual differences in the perception of time, or how individuals assign value to delayed rewards.
To explore whether male and female impulsive choice was driven by different mechanisms, we used a peak interval task to assess the accuracy and precision of each rat's perception of time after they had been scored on an impulsive choice task.
Overall sex differences in time perception were subtle, but there were notable differences in how this perception related to impulsivity.
Accuracy of timing was correlated with impulsivity in males, but not in females.
Males less accurate in timing were more impulsive.
Overall, this suggests that much of the observed variation in male impulsive choice can be explained by variation in temporal perception.
Variation in impulsive choice in females, however, is likely due to cognitive components of how values are assigned to rewards.
This may contribute to the observation of impulsivity relating to alcohol consumption selectively in female rats.

Disclosures: J. McClure: None. **F. Jackson:** None. **H.N. Richardson:** None.

Poster

915. Learning and Memory Systems

Location: Hall F-J

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Program#/Poster#:915.01/DDD1

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01DA027688

Title: Nicotinic acetylcholine receptors are necessary for learning and response inhibition during negative occasion setting

Authors: *D. J. BUCCI, R. B. PUTNEY
Dartmouth Col., HANOVER, NH

Abstract: Our laboratory has carried out a series of studies in rats investigating the neural basis of a form of learning known as negative occasion setting. In a typical negative occasion setting procedure, a subset of trials consists of presentation of a tone followed immediately by food reward. On other trials, a light precedes the tone and no food is delivered. Normal adults learn to discriminate between these two trial types after ~8 training sessions, indicated by approaching the food cup in anticipation of receiving a reward when the tone is presented by itself, but inhibiting that response when the light precedes the tone. We have previously shown that nicotine administration reduces the number of sessions needed to learn the discrimination and also enhances the magnitude of discrimination between trial types specifically by reducing responding during the non-reinforced trials (MacLeod et al., 2006; 2010). In the present study, we tested whether acetylcholine, acting through nicotinic acetylcholine receptors, is necessary for negative occasion setting. Adult male Long-Evans rats were maintained at 85% free feeding body weight and treated with mecamylamine (2.0 mg/kg), a broad acting nicotinic acetylcholine receptor antagonist, 20 minutes before each daily training session. Compared to vehicle-treated control rats, those treated with mecamylamine needed more sessions to learn the discrimination and also did not exhibit as robust discrimination between the trail types. Together with the finding that rats with lesions of the prefrontal cortex are impaired in negative occasion setting (MacLeod and Bucci, 2010), these data indicate that acetylcholine, acting through cortical nicotinic receptors, may be necessary for learning to inhibit behavioral responses. Moreover, these findings may inform our understanding of inhibition deficits associated with disorders such as ADHD and schizophrenia; two conditions characterized by very high levels of nicotine use.

Disclosures: D.J. Bucci: None. **R.B. Putney:** None.

Poster

915. Learning and Memory Systems

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:915.02/DDD2

Topic: F.02. Animal Cognition and Behavior

Title: Spinal nerve ligation (L5) leads to neuropathic pain and memory (Episodic, Spatial and Emotional) deficits in rats.

Authors: *P. JAYARAJAN, V. GOURA, R. ABRAHAM, R. MEDAPATI, R. YARAMASU, R. NIROGI
Suven Life Sci. Ltd, Hyderabad, India

Abstract: The most common form of chronic pain is neuropathic pain. Clinical evidence has revealed that chronic pain patients usually suffer from memory deficits. The aim of this experiment was to evaluate the cognitive performance of neuropathy rats using the various methods of preclinical memory testing. In the present study, Male wistar rats underwent tight ligation of the left spinal (L5) nerve and in sham control, nerves were exposed but not ligated. We assessed mechanical allodynia using manually held Von Frey monofilaments, the animals which developed allodynia condition were selected and tested for episodic memory functioning using novel-object-recognition task. Spatial learning was assessed using T-maze and emotional memory was assessed using passive avoidance paradigm. In this study SNL rats with allodynia condition showed episodic, spatial and emotional memory deficits in novel-object-recognition task, T-maze and passive avoidance respectively in comparison with sham control and naive rats. The memory deficit in neuropathic pain rats is similar to the clinical condition of patients suffering with chronic pain. Spinal nerve ligation (L5) can serve as a model to assess pain associated memory deficits.

Disclosures: P. Jayarajan: Employment; Suven Life Sciences Ltd. **V. Goura:** Employment; Suven Life Sciences Ltd. **R. Abraham:** Employment; Suven Life Sciences Ltd. **R. Medapati:** Employment; Suven Life Sciences Ltd. **R. Yaramasu:** Employment; Suven Life Sciences Ltd. **R. Nirogi:** Employment; Suven Life Sciences Ltd.

Poster

915. Learning and Memory Systems

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:915.03/DDD3

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: The effects of parity and estrogen on learning bias in female rats.

Authors: *D. HUSSAIN[1,2], B. WOODSIDE[2], W. G. BRAKE[2]
[1]Montreal, QC, Canada; [2]Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: Previous studies have shown that estrogen (E) affects whether a hippocampus-mediated place (allocentric) or a striatum-mediated response (egocentric) memory system is

employed when searching for a food reward in a maze (Korol, 2004; Quinlan, Hussain, & Brake, 2008). It has been suggested that reproductive experience alters the response to E in a variety of systems. To investigate whether reproductive experience would alter the effect of E on response and place learning in a maze, in this experiment, 35 ovariectomized nulliparous (*n*=16; no reproductive experience) and primiparous (*n*=19; having had one litter of pups) Wistar rats were trained on place and response plus-maze tasks. Rats were also exposed to two hormonal treatment conditions: they received low 17β-estradiol (E2) or high E2 replacement. Results showed a 3-way significant interaction between task type, E2 level, and parity. In congruence with previous findings, low E2 nulliparous rats took significantly fewer days to reach criterion on the response task than on the place task. However, high E2 nulliparous rats learned both tasks in the same amount of time. Interestingly, the facilitatory effect of low E2 on response task learning seen in nulliparous rats was not observed in low E2 primiparous rats. In conclusion, E2 levels do dictate the rate at which female rats learn a response task, but only in those with no reproductive experience.

Disclosures: D. Hussain: None. **B. Woodside:** None. **W.G. Brake:** None.

Poster

915. Learning and Memory Systems

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Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant IBN-03122212 (Mark G. Packard)

Title: Posttraining peripheral administration of cannabinoid 1 (CB1) receptor agonist WIN 55,212-2 impairs the consolidation of dorsolateral striatal-dependent habit memory

Authors: *J. GOODMAN[1,2], M. G. PACKARD[2]
[1]College Station, TX; [2]Dept. of Psychology, Texas A&M Univ., College Station, TX

Abstract: Extensive research indicates that the cannabinergic system plays a major role in modulating memory processes. Previous studies have shown that the peripheral administration of cannabinoid 1 (CB1) receptor agonists impair, whereas CB1 receptor antagonists enhance, learning in hippocampal-dependent memory tasks. In the present study, we examined the effects of posttraining peripheral administration of CB1 agonists and antagonists on the consolidation of a dorsolateral striatal-dependent habit memory task. Adult male Long-Evans rats were trained for 5 days (6 trials per day) in a stimulus-response version of the water plus-maze task, in which they were released from different start points and had to make the same body turn response (i.e.

turn right) at the choice point to reach the hidden escape platform. Immediately following training for the first two days, rats received peripheral (i.p.) injections of either the CB1 receptor agonist WIN 55,212-2 (3mg/kg), the CB1 receptor antagonist AM 251 (3mg/kg), WIN 55,212-2 (3mg/kg) and AM 251 (3mg/kg) concurrently, or the vehicle treatment alone. Relative to the vehicle-treated controls, WIN 55,212-2 significantly impaired memory consolidation. AM 251 did not affect task aquisition. However, the dose of AM 251 that was ineffective when given alone was sufficient to block the memory enhancing effects of WIN 55,212-2 when rats received concurrent drug injections. Taken together, the results indicate that peripheral administration of a CB1 receptor agonist impairs consolidation of dorsal striatal-dependent habit memory and that this effect can be reversed by co-administration of a CB1 receptor antagonist. Considering evidence that activity of the dorsal striatal habit memory system may underlie the persistence or development of some anxiety disorders, the findings are consistent with the hypothesis that pharmacological agents that target the cannabinergic system may prove useful in the treatment of human psychopathology

Disclosures: J. Goodman: None. **M.G. Packard:** None.

Poster

915. Learning and Memory Systems

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Program#/Poster#:915.05/DDD5

Topic: F.02. Animal Cognition and Behavior

Support: Georgia State University Brains and Behavior Seed Grant

Title: Voluntary consumption of a high-energy diet prevents the memory-enhancing effects of acute stress in male rats

Authors: *J. DARLING[1], A. P. ROSS[2], M. B. PARENT[2]
[1]Neurosci. Inst., Georgia State Univ., Atlanta, ; [2]Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Over the past 5 decades, caloric intake has increased by approximately 28% in the United States. High-energy diets (HEDs), which contain excessive calories, negatively impact the hippocampus and hippocampal-dependent memory by decreasing neuronal growth factors, neurogenesis, and synaptic communication. Previous studies from our lab suggest that HEDs impair memory in stressful tasks such as water maze but not during less stressful tasks such as spontaneous alternation. Moreover, HEDs increase secretion of the stress hormones epinephrine and corticosterone. As a result, we hypothesized that HEDs impair memory, in part, through diet-

induced increases in the acute stress response. To test this, adult male Sprague-Dawley rats were fed a high energy cafeteria-style diet containing choices of standard lab chow, animal lard, tap water, and a 32% sucrose solution for 4 weeks. Control rats were fed standard chow and tap water. The rats were then trained and tested in a low stress or high stress version of a spatial object recognition task. Surprisingly, the HED did not impair memory. Instead, the HED prevented the memory-enhancing effects of stress that were observed in control rats. These findings are significant because they raise the possibility that some previous results suggesting that HEDs impair memory may actually reflect deficits in stress-induced facilitation of memory. Future experiments should investigate the role of epinephrine and corticosterone in the interaction between HEDs and stress on hippocampal-dependent memory.

Disclosures: J. Darling: None. **A.P. Ross:** None. **M.B. Parent:** None.

Poster

915. Learning and Memory Systems

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Program#/Poster#:915.06/DDD6

Topic: F.02. Animal Cognition and Behavior

Support: NIH/NIAAA R21

Title: Effects of the phosphodiesterase (pde) inhibitor papaverine on alcohol consumption in mice.

Authors: *P. J. WEBSTER[1], Y. JIANG[1], L. LUEPTOW[1], S. DOPPALAPUDI[1], J. LU[2], H.-T. ZHANG[1]
[1]Behavioral Med., West Virginia Univ., Morgantown, WV; [2]401 Professional Drive, Suite 160, Codex Biosolutions Inc., Gaithersburg, MD

Abstract: Alcohol abuse and excessive alcohol consumption kill 79,000 people per year and cost the US more than $200 billion annually according to the CDC. Phosphodiesterase 4 (PDE4), one of the 11 PDE families (PDE1-11), has been implicated in alcoholism; however, it is not known whether other PDEs are also involved in alcohol drinking behavior. In the present study, we examined the role of PDE10, a dual substrate PDE enzyme that hydrolyzes both cyclic AMP (cAMP) and cyclic GMP (cGMP), in ethanol intake and preference utilizing papaverine, a PDE10 inhibitor. C57BL/6J mice, a strain known to drink alcohol excessively, were provided 24-h access to water and various levels of ethanol in a two-bottle choice test to gauge intake and preference for ethanol. In the drink-in-dark test, ethanol intake was measured during the time with the peak of ethanol drinking, followed by determination of the blood ethanol concentrations

(BECs). Treatment with the PDE4 inhibitor rolipram (0.5 mg/kg) decreased ethanol intake and preference in both two-bottle choice and drink-in-dark tests. Similarly, treatment with papaverine at doses of 10 and 30 mg/kg reduced ethanol consumption in the drink-in-dark test, although at lower doses (0.3-3.0 mg/kg) papaverine did not significantly affect ethanol consumption in either of the tests. Consistent with the reduced ethanol consumption, BECs were decreased by rolipram and papaverine (30 mg/kg). Given that pretreatment with papaverine or rolipram increases striatal cAMP levels, the results suggest that inhibition of PDE10 decreases ethanol consumption most likely via activation of cAMP signaling in the striatum. PDE10 inhibitors may be used for treatment of alcoholism and alcohol abuse [This work was supported by a research grant from NIH/NIAAA (AA020042)].

Disclosures: P.J. Webster: Research Grant; NIH/NIAAA R21. **Y. Jiang:** Research Grant; NIH/NIAAA R21. **L. Lueptow:** Research Grant; NIH/NIAAA R21. **S. Doppalapudi:** Research Grant; NIH/NIAAA R21. **J. Lu:** None. **H. Zhang:** Research Grant; NIH/NIAAA R21.

Poster

915. Learning and Memory Systems

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Program#/Poster#:915.07/DDD7

Topic: F.02. Animal Cognition and Behavior

Support: NSF Grant 0924674

Title: Modeling varieties of list learning: Surface, structural and observational learning effects in macaques

Authors: *B. GASSER, M. ARBIB
Neurosci. Grad. Program, USC, Los Angeles, CA

Abstract: Macaques have been shown to be able to reproduce simultaneously presented images in an arbitrary order through trial-and-error learning. Slowly, across many lists, they develop task expertise that increases learning rate and performance on novel list instances. Expert monkeys passively observing other expert monkeys executing novel lists subsequently perform at higher rates on those lists compared to other novel list instances in control conditions. Additional probe lists support the conclusion that the monkeys learn to use ordinal representations to organize their performance. Here, we present a computational model demonstrating these varieties of learning and the representations the monkeys use to organize their behavior. We provide explanations for the development of task expertise and show how distributed regions in parietal and frontal cortices contribute to the

performance of the animal.   Crucially, we explain the observational learning effects not as a form of 'cognitive imitation' as first proposed, but as an enhancement of attention to learned, task-relevant features which develop associations to the cognitive structures only an expert monkey would have developed. We consider data on gaze-following, and neural responses sensitive to others' gaze direction, such as recently discovered LIP 'mirror neurons', as supporting the notion that monkeys are sensitive to the attentional states of others. We hypothesize this sensitivity contributes to the observational learning effects demonstrated.

Disclosures: B. Gasser: None. **M. Arbib:** None.

Poster

915. Learning and Memory Systems

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:915.08/DDD8

Topic: F.02. Animal Cognition and Behavior

Title: Evoked neural activity following environmental enrichment in young rats: A initial c-fos study

Authors: W. C. WHITE, Jr[1], A. M. FRENCH[1], A. M. DOYLE[2], D. E. COBB[2], *M. C. ZRULL[1]
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Abstract: Environmental enrichment (EE), or exposure to a setting with opportunities for investing and interacting with objects and conspecifics, can have an impact on the brain and behavior. While many EE studies have focused on changes in neural morphology, trophic factors and/or neurogenesis, relatively fewer investigations have addressed how EE might evoke neural activity related to learning and memory in young rats. We used c-fos to examine changes in numbers active neurons at baseline and after EE in amygdalao-hippocampal transition area (AHA), hippocampus, and entorhinal cortex (EntCtx). Young Long-Evans rats (n=8) were exposed to EE in cages with ramps, platforms, inanimate objects, and other rats for 1.5 hr/day for two of every three days between postnatal days (PND) 34 and 64. Age-matched rats (n=8) served as not enriched controls. Between PND 77 and 79 and immediately prior to sacrifice, 4 EE and 4 control rats spent 120 minutes in the enrichment cage, and 4 EE and 4 control rats spent 120 minutes in the quiet and dark. Brains were processed using immunohistochemistry to visualize the c-fos protein. Light microscopy and stereology were used to examine neural activation in the AHA, EntCtx, subiculum (SUB), dentate gyrus (DG), and CA1 region by counting fos-positive neurons in multiple sample frames per section and structure. When compared to rats not

experiencing EE immediately prior to sacrifice, an EE session evoked activity across examined brain regions for rats with and without a history of EE exposures. The EE session increased the number of active neurons from a high of 233% in AHA (p<.01) to a low of 93% (p<.06) in EntCtx for rats with an EE history, and the session increased active neurons from 275% in CA1 (p<.01) to 101% in DG (p<.07) for rats without an EE history. For rats not experiencing a final EE session before sacrifice, a history of EE reduced (-42% in AHA, p<.02; -49% in DG, p<.09; -32% in SUB, p<.07) or did not appreciably alter (-15% in CA1, +5% EntCtx) numbers of fos-positive neurons relative to not enriched controls. While a series of enrichment experiences reduced or did not alter neural activity levels in the investigated structures, a single EE exposure produced neural activation in all examined regions: AHA, EntCtx, SUB, DG and CA1. However, the greatest increase in number of activated neurons over non-evoked baseline differed for rats with (AHA, DG) and without (EntCtx, CA1) an EE history. Although the results suggest a need for more research, the data do suggest that self-directed, not explicitly rewarded investigation of and interaction with objects can activate neurons in brain regions known to be important for learning and memory.

Disclosures: W.C. White: None. **A.M. French:** None. **A.M. Doyle:** None. **D.E. Cobb:** None. **M.C. Zrull:** None.

Poster

915. Learning and Memory Systems

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:915.09/DDD9

Topic: F.02. Animal Cognition and Behavior

Title: Observations on environmental enrichment and exploratory behavior in young rats

Authors: *T. J. HINES[1,2], R. E. EICHORST[2], A. M. DOYLE[3], S. G. HINNANT[2], D. E. COBB[3], B. R. MINTON[2], M. C. ZRULL[2]
[1]Fayetteville, NC; [2]Psychol, [3]Honors Col., Appalachian State Univ., Boone, NC

Abstract: The effects of environmental enrichment (EE) on recovery from brain injury, enhancing learning and memory in adults, furthering brain development of infant and very young animals, and aiding the aging brain are often researched. EE has a positive impact on both brain and behavior in these settings. In this study, we began examining how EE affects behavior in rats that might be classified as from a "juvenile through adolescent" age group. Young Long-Evans rats (n=16) were exposed to EE in cages with ramps, platforms, inanimate objects, and other rats for 1.5 hr/day for two of every three days between postnatal days (PND) 34 and 64. Age-

matched rats (n=16) served as not enriched controls. Animals experienced behavioral testing between PND 66 and 75. On PND 78, 8 EE and 8 control rats were sacrificed after 2 hr in the quiet and dark, and those brains were processed to visualize the c-fos protein. Light microscopy and stereology were used to examine neural activation in the amygdala. During the final 3 minutes of a 5 minutes open field trial (1-m x 1-m), EE rats spent 130% more time in the field interior (i.e., not along the walls) than control rats (p<.01). Similarly, EE rats moved through 140% more squares of the field interior than non-EE rats (p<.01). During Trial 2 of a two-trial object preference task (3 minutes per trial, 15 and 30 minute delays), little difference in exploration of a novel object was observed between EE and control groups after a 15 minute delay. However, with a 30 minute delay, EE rats spent 20% less time exploring the field area adjacent to a novel object than control rats (p<.02) and showed a 28% decrease in the proportion of time in contact with the novel object (p<.02). Behavioral data suggest that rats experiencing EE during adolescence show less fear and explore more in a new environment than not enriched controls; however, EE rats exhibit some generalized adaptation to a novel object in that environment. Histology revealed differences in the number of active neurons, as indicated by the presence of c-fos, between EE and control rats. In basolateral amygdala of EE brains, there was a 34% reduction in active neurons relative to controls (p<.01). Lateral amygdala of EE brains showed an 18% reduction in fos-positive neurons relative to controls. EE appears to reduce baseline activity in amygdala, which correlates with reduced fear observed in behavioral tasks. While our results are certainly preliminary, the data seem to suggest that in our rat model enrichment during "juvenile through adolescent" development enhances exploratory behavior in general and may also promote adaptation to novelty within new and familiar environments.

Disclosures: T.J. Hines: None. **R.E. Eichorst:** None. **A.M. Doyle:** None. **S.G. Hinnant:** None. **D.E. Cobb:** None. **B.R. Minton:** None. **M.C. Zrull:** None.

Poster

915. Learning and Memory Systems

Location: Hall F-J

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Program#/Poster#:915.10/DDD10

Topic: F.02. Animal Cognition and Behavior

Support: BFU2011-29089

P07-CVI-02487

BFU2011-29286

P07-CVI-02686

Title: Synaptic changes in strength at the hippocampal formation during the acquisition of classical eyeblink conditioning in behaving rabbits

Authors: *A. CARRETERO, SR[1], R. PACHECO-CALDERÓN[1], R. SÁNCHEZ-CAMPUSANO[1], M. T. HASAN[2,3], J. M. DELGADO-GARCÍA[1], A. GRUART[1]
[1]Div. De Neurociencias, Sevilla, Spain; [2]Dept. of Mol. Neurobio., Max Planck Inst. for Med. Res., Heidelberg, Germany; [3]NeuroCure Cluster of Excellence, Charité – Universitätsmedizin, Berlin, Germany

Abstract: Despite of the high number of studies focused in elucidating the role of the hippocampus in learning and memory processes, the precise contribution of hippocampal circuits to those processes is still a matter of discussion. In this presentation, we will show data of hippocampal recordings carried out in conscious rabbits during the classical conditioning of eyelid responses. Briefly, four groups of rabbits were used, one of them for baseline recordings, one for pseudoconditioning, and the other two for classical eyeblink conditioning using delay or trace conditioning paradigms. The conditioned stimulus (CS) consisted of a tone, whilst the unconditioned stimulus (US) consisted of an air puff. For the baseline group, electrophysiological recordings of hippocampal field excitatory post-synaptic potentials (fEPSPs) were carried out whitout applying any conditioning stimulus, to see the putative effects of enviromental (i.e., context) influences on activity-dependent changes at the different hippocampal synapses. In the case of pseudoconditioning, the CS and US were presented at random to check if uncoupled stimuli evoked the same synaptic changes than in their coupled presentation. A pair of electrical pulses were applied to the perforant path (PP), or CA3, during the CS and before the US in the case of the delay paradigm, or during the CS-US interval for trace paradigm, in the CS for pseudoconditioning, and in a fixed interval of time for baseline. The slope of evoked fEPSPs did not change across baseline sessions except for the CA3-CA1 synapse. similar results were observed in the pseudoconditioning case. In contrast, an increase, or a decrease, tendency was observed in conditioning sessions depending on the synapse and on the paradigm used. These results show that a constant context do not evoke significant changes in strength at the hippocampal synapses, in contrast with changes evoked by relevant but uncoupled stimuli, and to those evoked because of the associative learning proccess. To understand the role of dentate gyrus in memory acquisition and retrieval processes, we used recombinant adeno-associated viruses, which were equipped with doxycycline (Dox)-controlled genetic switches for reversible silencing of synaptic transmission. With this method, a single intraperitoneal injection of Dox induced synaptic silencing within two days and unsilencing after ten days. Preliminary results indicates the important role played by the dentate gyrus in the acquisition and retrieval processes.

Disclosures: A. Carretero: None. **R. Pacheco-Calderón:** None. **R. Sánchez-Campusano:** None. **M.T. Hasan:** None. **J.M. Delgado-García:** None. **A. Gruart:** None.

Poster

915. Learning and Memory Systems

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:915.11/DDD11

Topic: F.02. Animal Cognition and Behavior

Support: F. Hoffmann-La Roche

Title: Enhancing cognition across multiple domains in non-human primates

Authors: *C. GLAVIS-BLOOM[1], J. CEFALU[1], G. CHIU[1], T. M. BALLARD[2], J.-L. MOREAU[2], J. G. WETTSTEIN[2], T. L. WALLACE[1]
[1]SRI Intl., Menlo Park, CA; [2]F. Hoffmann-La Roche, Basel, Switzerland

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive impairment across several domains, with only modestly effective treatments currently available. For these reasons, continued drug discovery efforts are critical, and may be enhanced by establishing reliable preclinical animal models. Non-human primates (NHPs) are an ideal species for studying complex cognitive functions and can enhance the translatability of data into clinical applications. The objective of these studies was to establish pharmacologically sensitive cognitive tasks in NHPs that were relevant to AD. In these studies, we trained separate cohorts of animals (n=8/group) to perform a delayed match-to-sample (DMTS) test of working memory, a continuous performance test (CPT) of sustained attention, and a self-ordered spatial search (SOSS) test of spatial working memory and executive function using the CANTAB® system, and then we assessed the properties of several cognitive enhancing and impairing agents in these models. In DMTS, NHPs were presented with a sample stimulus and following a short (3-10 sec), medium (11-30 sec) or long (31-100 sec) delay period were presented with the sample and a distractor stimulus. A food reward was obtained following correct match to the original sample. DMTS performance accuracy decreased with increased delay times and acute administration of nicotine and donepezil produced dose-related improvements in the long delay of the test. In CPT, NHPs were presented with a target stimulus and two distractor stimuli and were trained to touch only the target stimulus until performance was near maximal levels (i.e., 90-100% accuracy). Administration of the muscarinic receptor antagonist, scopolamine, dose-dependently increased reaction times and impaired the performance accuracy, which was reversed by acute nicotine treatment. Finally, in the SOSS paradigm NHPs were presented with 2, 3, or 4 identical stimuli and trained to touch each of the stimuli once without repeating to receive a food reward. Performance accuracy decreased with increasing trial difficulty, an effect that was dose dependently worsened with scopolamine. Both donepezil and nicotine reversed the scopolamine impairments in the SOSS paradigm. These data demonstrate the pharmacological

sensitivity of using NHPs to assess cognitive function and support the utility of this approach for improving translatability of preclinical data into the clinic for novel therapeutic assessments.

Disclosures: C. Glavis-Bloom: Other Research Support; F. Hoffmann-La Roche. **J. Cefalu:** Other Research Support; F. Hoffmann-La Roche. **G. Chiu:** Other Research Support; F. Hoffmann-La Roche. **T.M. Ballard:** Employment; F. Hoffmann-La Roche. **J. Moreau:** Employment; F. Hoffmann-La Roche. **J.G. Wettstein:** Employment; F. Hoffmann-La Roche. **T.L. Wallace:** Other Research Support; F. Hoffmann-La Roche.

Poster

915. Learning and Memory Systems

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:915.12/DDD12

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH080052

Title: Source memory in the rat

Authors: *J. D. CRYSTAL, W. T. ALFORD, W. ZHOU, A. G. HOHMANN
Dept Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: An emerging body of evidence suggests that rats have a detailed representation of earlier episodes. This includes information about what-where-and-when an earlier episode occurred (Zhou & Crystal (2009) *PNAS* 106:9525-9529) and the ability to retrieve information that was incidentally encoded and unexpectedly requested (Zhou, Hohmann & Crystal (in press) *Current Biology*). Here we asked if rats remember the origin of encoded information. Source memory includes a representation of the origin (i.e. source) of information and is related to episodic memory. We developed an animal model of external source monitoring by asking if rats can discriminate self-generated (walking along a runway, encountering food) and experimenter-generated (placement at a food site without walking) events. Placement occurred at randomly selected chocolate- or chow-baited arms of a radial maze in a daily study phase. The source by which chocolate was obtained at a study location determined chocolate replenishment at the same location in a subsequent test. Other randomly selected locations in study and test phases were baited with chow, were encountered by walking along the runways, and did not replenish. Rats revisited the chocolate location at a higher rate in replenishment than non-replenishment conditions. A transfer test to a different room suggests that performance was not based on memorization of a series of spatial contingencies, documenting some degree of flexibility in source memory. Manipulation of the retention interval between study and test suggests that

source memory decayed during delays whereas flavor-location memory was intact, providing a dissociation between source and other memories. We next asked whether temporary inactivation of the hippocampus, a brain region critical for episodic memory, would preferentially impair source memory. Bilateral intra-hippocampal infusions of lidocaine before the study phase eliminated source memory whereas performance in control conditions was intact. Our studies suggest that rats remember source information about flavors and locations of previously encountered food.

Disclosures: J.D. Crystal: None. **W.T. Alford:** None. **W. Zhou:** None. **A.G. Hohmann:** None.

Poster

915. Learning and Memory Systems

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:915.13/DDD13

Topic: F.02. Animal Cognition and Behavior

Support: ISAO

Title: Deep brain stimulation of the fornix and entorhinal cortex in an experimental model of memory impairment

Authors: *S. HESCHAM[1], L. LIM[1], A. JAHANSHAHI[1], J. PRICKAERTS[1], H. STEINBUSCH[1], A. BLOKLAND[2], Y. TEMEL[1]
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Abstract: Deep brain stimulation (DBS) is a surgical treatment involving the implantation of electrodes which give electrical impulses to specific parts of the brain. Recently, DBS in the region of the fornix has been applied in Alzheimer's disease with the purpose of improving or reducing the progression of memory loss. When structures of the memory circuitry are stimulated, DBS is thought to enhance neural activity and thus improves performance on memory tasks. In the following study, we implanted bilateral electrodes at the site of the fornix and entorhinal cortex in order to detect which stimulation parameters provide beneficial effects in spatial memory. Rats were then tested in the Object Location Task with the following conditions: (i) with attachment of stimulation cable (off stimulation), and (ii) with DBS at various amplitudes (50 μA, 100 μA and 200 μA), 100 μs pulse width and 100 Hz or 10 Hz stimulation frequency. Intraperitoneal scopolamine injections 30 min before the first trial were given to imitate memory impairment. DBS of both regions reversed the scopolamine effects in high current densities and showed superior memory performance when compared to sham rats. With the most efficient stimulation parameter rats did not show anxiety-like behaviour in the

Open Field and Elevated Zero Maze, suggesting no potential side effects regarding anxiety levels or general motor activity.

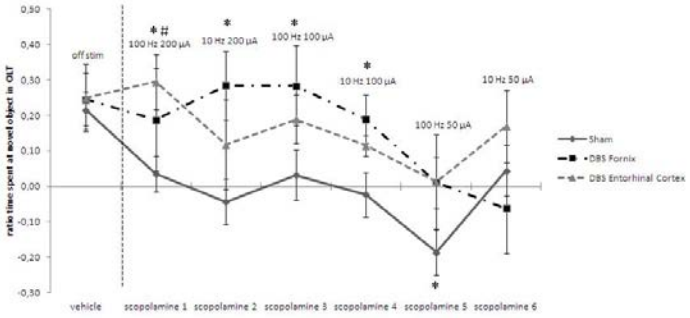


Figure: Ratio time spent at the new object for sham, DBS Fornix and DBS Entorhinal Cortex rats in the OLT. In the first session stimulation was off and saline was injected in all rats. In the following 6 sessions scopolamine was injected and DBS rats were stimulated with the parameters mentioned above. Sham rats only received scopolamine and were not stimulated. Significant effects were found for DBS Fornix rats (*) in high current densities (200 and 100µA) and independent of frequency (100Hz or 10Hz). DBS Entorhinal cortex rats (#) showed enhanced spatial memory at 100Hz and 200µA.

Disclosures: S. Hescham: None. **L. Lim:** None. **A. Jahanshahi:** None. **J. Prickaerts:** None. **H. Steinbusch:** None. **A. Blokland:** None. **Y. Temel:** None.

Poster

915. Learning and Memory Systems

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Program#/Poster#:915.14/DDD14

Topic: F.02. Animal Cognition and Behavior

Support: NASA Grant NNX08AM66G

Title: Sex differences in operant responding and survivability following xposure to space radiation.

Authors: K. L. CARRIHILL-KNOLL[1], *B. M. RABIN[1,2], B. SHUKITT-HALE[2]
[1]Univ. Maryland Baltimore County, BALTIMORE, MD; [2]Human Nutr. Res. Ctr. on Aging, USDA-ARS, Boston, MA

Abstract: On exploratory class missions, such as a mission to Mars, astronauts will be exposed to types and doses of radiation (galactic cosmic rays [GCR]) which are not experienced in low earth orbit where the space shuttle and International Space Station operate. Despite the fact that

the crew on such a mission is likely to be composed of both male and female astronauts, little research has been done on the effects of the space radiation environment on females.
As part of a series of experiments on the long-term effects of exposure to heavy particles on cognitive performance using a ground-based model for exposure to GCR, 2-month old male and female rats were exposed to 12C and 56Fe particles. The rats were maintained in the animal facility at UMBC for up to 24 months following irradiation. They were checked daily for the development of tumors or other health problems. The rats were euthanized if an evident tumor was bleeding or had grown to 5 cm in size, if the rat showed signs of distress or pain, or if the rats stopped eating or showed a general deterioration in health.
Both male rats and ovariectomized female rats with estradiol implants showed an equivalent disruption of operant responding following exposure to both particles. However, the female rats exposed to either particle survived for a significantly longer period of time than male rats. Although the mechanisms underlying these differences in the effects of exposure to heavy particles on survival have yet to be elucidated, the results of these experiments may have implications for astronauts on exploratory class missions.
Supported by NASA Grant: NNX08AM66G

Disclosures: K.L. Carrihill-Knoll: None. **B.M. Rabin:** None. **B. Shukitt-Hale:** None.

Poster

915. Learning and Memory Systems

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:915.15/DDD15

Topic: D.05. Visual Sensory-motor Processing

Title: Anterior vs. posterior lesions of the pedunculopontine tegmentum produce differential deficits in sensory gating and higher cognitive function

Authors: *J. ROBINSON, S. SCHMID
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Abstract: The pedunculopontine tegmentum (PPT) is part of the brainstem cholinergic system, composed of both cholinergic and non-cholinergic (Glu and GABA) cells. The posterior PPT receives sensory input whereas the anterior PPT receives forebrain (basal ganglia, amygdala) input, and there are both ascending and descending efferent connections. The proposed role of the PPT is two-fold: first, ascending connections regulate activity of thalamic and basal ganglia nuclei in relation to timing of sensory information, and second, descending connections provide forebrain machinery (i.e. basal ganglia) access to locomotor machinery in the brainstem and are

involved in sensory processing. Ascending cholinergic projections to the dopaminergic neurons of the substantia nigra (SNc) regulate timing of dopamine release in relation to sensory information, a process important for associative learning. Descending cholinergic projections to the brainstem are involved in prepulse inhibition (PPI), a basic form of sensory gating in which the startle response of a reflex-inducing startle pulse is attenuated when preceded by a sub-threshold stimulus, and potentially habituation. Disrupting ascending cholinergic projections should impair associative learning, and disrupting descending cholinergic projections should impair PPI and/or habituation. We lesioned the anterior or the posterior PPT bilaterally by microinjections of ibotenic acid, followed by testing of PPI and habituation in startle boxes, spatial memory in a Barnes maze, and locomotor behaviour in locomotor boxes. Preliminary results suggests that rats with posterior PPT lesions show impaired long-term habituation ($p = 0.033$) while short term habituation is unaffected. Interestingly, no effects on PPI were observed using 75dB or 85 dB prepulses and 10, 30, or 100 ms interstimulus intervals. In the Barnes maze test, animals with anterior and posterior lesions displayed a trend to slower learning than saline injected animals on day 1, and spatial memory was disrupted on day 12. Since the PPT degenerates significantly in Parkinson's disease and is currently a candidate for deep brain stimulation, understanding PPT function is of high clinical value. Future experiments will use a specific toxin for cholinergic neurons in order to separate cholinergic from non-cholinergic PPT function.

Disclosures: J. Robinson: None. **S. Schmid:** None.

Poster

916. Cognitive Learning and Memory Systems IV

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:916.01/DDD16

Topic: F.02. Animal Cognition and Behavior

Support: FCT Grant SFRH/BD/33280/2007

FCT Grant PTDC/PSI-PCO/118776/2010

Title: Neurogenomics of social eavesdropping in zebrafish

Authors: *R. A. DE ABREU[1,2], R. F. OLIVEIRA[1,2]
[1]Champalimaud Neurosci. Programme, Lisbon, Portugal; [2]Unidade de Investigação em Eco-Etologia, ISPA - Inst. Universitário, Lisbon, Portugal

Abstract: In social species individuals need to continuously adjust their behavior as a function of the available social information, which can be collected first-hand by directly interacting with other individuals, or by observing others (social learning). We are using the model system zebrafish (Danio rerio) to study the role of social learning on behavioral plasticity and how changes in neurogenomic states allow for adaptive plastic responses to a fluctuating social environment. This plasticity may be achieved by rewiring or by biochemically switching nodes of the neural network underlying social behavior in response to perceived social information. Therefore, at the molecular level, it depends on the social regulation of gene expression, so that different neurogenomic states correspond to different behavioral responses and the switches between states are orchestrated by signaling pathways that interface the social environment and the genotype. Previous work in our lab has shown that the perceived outcome of agonistic social interactions in zebrafish has a major impact in the brain transcriptome profile that mediate the effects of prior social experience on subsequent behavior (i.e. winner and loser effects). Here we investigate if these effects are also present in bystanders who watch but do not participate in social interactions. For this purpose we established a protocol with three conditions, where isolated male zebrafish either: (1) observe an agonistic interaction between a pair of conspecifics; (2) observe a non-interacting conspecific male dyad; or (3) observe an empty adjacent tank. Brain gene expression levels are analyzed using the Affymetrix GeneChip Zebrafish Genome, which covers over 14.900 zebrafish transcripts. Behavior measures are obtained using an automated video tracking system. This study will help establish the impact of social eavesdropping at the transcriptome level, opening the way to future studies on the mechanisms of social learning in zebrafish by contrasting it with equivalent asocial learning mechanisms (e.g. stimulus enhancement). The comparison of the different profiles of gene expression across these studies will allow to test if social learning shares its neurogenomic mechanism with that of its corresponding asocial learning form.

Disclosures: R.A. De Abreu: None. **R.F. Oliveira:** None.

Poster

916. Cognitive Learning and Memory Systems IV

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:916.02/DDD17

Topic: F.02. Animal Cognition and Behavior

Support: RO1MH086629

F31DA029499

Vanderbilt Kennedy Center

Title: Biochemical and behavioral alterations in the dopamine D2 receptor knockout mouse

Authors: *D. L. GRAHAM[1], E. L. COHEN[1], A. L. FREDERICK[2], F. D. ECHEVARRIA[2], G. D. STANWOOD[1,3]
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Abstract: The striatum (STR) and medial frontal cortex (MFC) have been implicated in the control of motivated behavior. The functional output of STR and MFC circuitry is regulated in very complex ways, in part by the expression and activity of GABAergic interneurons. Both interneurons and projection neurons within these regions receive prominent dopamine (DA) input and express DA receptors as early as mid-gestation. Also, disruption of DA receptors and/or signaling early in development modulates GABAergic neuronal migration and differentiation. Our group and others have previously implicated D1 receptors in these responses; we now investigate potential roles for the D2 receptor (D2R) in interneuron development and function. First, we used immunohistochemistry to identify alterations in neuron-specific markers within the STR and MFC in D2R knockout (KO) mice. Significant increases in GAD67+ (GABAergic) neurons were found in D2R KO mice in both the STR and MFC. Parvalbumin, a marker of a subpopulation of fast-spiking GABAergic interneurons, was also significantly increased in both brain regions. No changes in parvalbumin or GAD67 were apparent in the somatosensory cortex, demonstrating neuroanatomical specificity. We also conducted several behavioral studies in wildtype and D2R KO mice. As previously described, D2R KO mice were significantly hypoactive in an open field. D2R KO mice also expressed reduced immobility in a tail suspension test, suggesting that genetic loss of D2 receptors may produce an antidepressant-like response. Ongoing studies attempt to relate our observed changes in GABAergic neurons with our behavioral findings. Taken together, these data demonstrate that the D2 receptor plays an important role in GABAergic interneuron expression in the MFC and STR and that perturbations of this receptor produce functional outcomes.

Disclosures: D.L. Graham: None. **E.L. Cohen:** None. **A.L. Frederick:** None. **F.D. Echevarria:** None. **G.D. Stanwood:** None.

Poster

916. Cognitive Learning and Memory Systems IV

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:916.03/DDD18

Topic: F.02. Animal Cognition and Behavior

Support: JSPS Research Fellow 4134

Title: Impulsive action is regulated by the infralimbic cortex; $D_{1/5}$ dopamine receptors in the infralimbic cortex are involved in milnacipran-enhanced inhibitory control

Authors: I. TSUTSUI-KIMURA[1], *Y. OHMURA[1], T. IZUMI[1], H. KUMAMOTO[1], T. YAMAGUCHI[2], T. YOSHIDA[1], M. YOSHIOKA[1]
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Abstract: Disruption of inhibitory control of impulsive behavior is an intolerable symptom in various psychiatric disorders and also could be a risk factor for suicide, pressing for understanding the neural basis of inhibitory control. Previous lesion studies have demonstrated that the rat infralimbic cortex (IL), the ventral portion of the medial prefrontal cortex (mPFC), is implicated in inhibitory control. In addition, we previously revealed that nicotine, a major addictive component of tobacco, disrupted inhibitory control via stimulating α4β2 nicotinic acetylcholine receptor in the IL, but not the prelimbic cortex, the dorsal portion of the mPFC. We recently found that acute milnacipran, an antidepressant and a serotonin/noradrenalin reuptake inhibitor, could enhance inhibitory control in rats. However, the neural mechanisms underlying the effects of milnacipran on impulsive action remain unclear. Milnacipran increases not only extracellular serotonin and noradrenalin but also dopamine specifically in the mPFC.Our goals were to confirm the role of the IL in inhibitory control and to identify whether $D_{1/5}$ and/or $D_{2/3}$ receptors in the IL mediate the milnacipran-enhanced inhibitory control in the 3-choice serial reaction time task (3-CSRTT).We recorded single-unit activity in the IL of rats performing the 3-CSRTT. Fifty-six units were isolated from 8 rats and among which 38 (68%) of units were related to inhibitory control. Inactivation of the IL of other 9 rats by bilateral injection of muscimol (0, 0.1, 0.5 μg/side), a $GABA_A$ agonist, significantly disrupted inhibitory control in the 3-CSRTT. Other 20 rats were bilaterally injected with SCH23390, a selective $D_{1/5}$ receptor antagonist, (0.3 or 3 ng/side) or eticlopride, a selective $D_{2/3}$ receptor antagonist, (0.3, 1, 3 μg/side) into the IL after acute administration of milnacipran (10 mg/kg, i.p.). Intra-IL SCH23390 injections reversed the milnacipran-enhanced inhibitory control, while injections of eticlopride into the IL failed to block the effects of milnacipran on inhibitory control in the 3-CSRTT.These results suggest that the rat IL has a critical role in inhibitory control and $D_{1/5}$ receptors in the area are responsible for acute milnacipran-enhanced inhibitory control in the 3-CSRTT.

Disclosures: I. Tsutsui-Kimura: None. **Y. Ohmura:** None. **T. Izumi:** None. **H. Kumamoto:** None. **T. Yamaguchi:** None. **T. Yoshida:** None. **M. Yoshioka:** None.

Poster

916. Cognitive Learning and Memory Systems IV

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:916.04/DDD19

Topic: F.02. Animal Cognition and Behavior

Support: Grant-in-Aid for Scientific Research (B) (No. 22330205)

Title: Dopamine release in the prefrontal cortex is paradoxically smaller when preferred rather than non-preferred reward is delivered during a working memory task - a monkey microdialysis study

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Abstract: Introduction: It has been well documented that optimal level of dopamine in the mammalian prefrontal cortex (PFC) is critical for higher cognitive control of behavior. We previously reported increase of dopamine release in the primate lateral PFC during the working memory (WM) task. Microinjection of dopamine and stimulation of dopamine D1 receptor in the PFC, dose-dependently modulates (enhances or suppresses) WM-related neuronal activity. When reward is delivered, increase of dopamine release is observed in many areas of the mammalian brain including PFC. It has also been indicated that preferred as compared with nonpreferred reward induces enhanced WM-related neuronal activity. We thus thought that there should be more dopamine release in the PFC when more preferred as compared with less preferred reward is delivered during the WM task.
Methods: Three monkeys were trained in a WM (spatial delayed alternation) task. We examined dopamine release while the monkey was (1) performing the WM task with a non-preferred reward (water), (2) performing the WM task with a preferred reward (grape juice) and (3) sitting quietly without task performance and without reward (no-reward). After insertion of the microdialysis probes into the PFC, we obtained dialysate samples for the next two consecutive days during the (1) water reward, (2) juice reward and (3) no-reward periods. Sampling time for each condition was 20 min. The dopamine level in the perfusate was determined by high-performance liquid chromatography and electrochemical detection.
Results and Discussion: Monkeys performed the WM task in more than 98 % correctness with the reaction time significantly shorter in juice than in water reward trials. Contrary to our expectation, dopamine level was higher when water was given than when juice was given as a reward. Considering our previous study indicating no dopamine change during the non-WM control task performance (where cognitive load was small but the reward was delivered),

dopamine release in the PFC appears to be more concerned with cognitive requirement rather than reward per se. It is speculated that when the monkey is less motivated to perform the task during the non-preferred reward condition, more cognitive resources may be required to perform the WM task and thus there would be more dopamine release.

Disclosures: T. Kodama: None. **K. Hikosaka:** None. **Y. Honda:** None. **T. Kojima:** None. **M. Watanabe:** None.

Poster

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Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:916.05/DDD20

Topic: F.02. Animal Cognition and Behavior

Title: [^{18}F]FDG PET as a potential biomarker for mapping dopamine D1 receptor-induced changes in brain regional glucose metabolism associated with improvement of working memory.

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Abstract: Schizophrenia is a disabling neuropsychological illness causing substantial cognitive deficits including working memory (WM) deficits. Dopamine D1-like receptors (D1r) are the most expressed dopamine receptors in the neocortex, including the medial prefrontal (mPFC)/cingulate cortex. Studies in nonhuman primates have shown that WM, studied during delayed response paradigms, is critically dependent on appropriate stimulation of D1 receptors in the mPFC/cingulate cortex (Goldman-Rakic 2000, 2004). This finding suggests that targeting the activation of D1r is a valuable therapeutic intervention for treating cognitive deficits in schizophrenia. [^{18}F]FDG PET provides a non-invasive, *in vivo* measure of relative regional glucose metabolism that can be used to assess regional brain function in a variety of states, including the acute responses to psychoactive drugs. In the current study, we aimed to determine if [^{18}F]FDG PET could detect D1r-modulated changes in glucose metabolism in brain regions associated with WM. Forty-eight male rats were divided into four groups tested with either vehicle, A77363 (1mg/kg, sc) or SKF-38393, (0.32 & 3.2mg/kg, sc), D1-like receptor ligands that have different degrees of intrinsic efficacy (full and partial agonist respectively). Although both drugs showed similar capacity to reverse ketamine-induced impairment in a radial arm maze task, a rodent task of working memory, only A77393 increased locomotor activity in a

dose-responsive manner, while SKF-38393 had no effect. [^{18}F]FDG PET was performed on fasted rats using a Siemens Focus 220 microPET scanner at 60 mins post [^{18}F]FDG injection (~47 MBq, iv). PET data were reported in terms of the standard uptake values (SUV). SUV images for each animal were normalized to each animal's own mean whole brain uptake. PET imaging showed significantly reduced FDG uptake ($p<0.05$) in the mPFC/cingulate cortex of both drug-treated groups as compared to vehicle controls. However, the magnitude of the effect was related to agonist efficacy; A77363 showed significant further reduction in [^{18}F]FDG uptake in mPFC/cingulate cortex compared to SKF 38393. These finding suggest that this method can be used to assess dopamine D1-like agonist activity based on their effect sizes and demonstrates the utility of [^{18}F]FDG PET in mapping D1 agonist-induced changes in brain regional glucose metabolism associated with optimal prefrontal functioning. All animal procedures conducted were approved by an institutional review committee (IACUC).

Disclosures: K. Kuszpit: Employment; Pfizer, Inc. **R. Kozak:** Employment; Pfizer, Inc. **A. Zhu:** Employment; Pfizer, Inc. **G. Currier:** Employment; Pfizer, Inc. **D. McGinnis:** Employment; Pfizer, Inc. **V. Guanowsky:** Employment; Pfizer, Inc. **P.A. Seymour:** Employment; Pfizer, Inc. **K. Zasadny:** Employment; Pfizer, Inc. **L. Chen:** Employment; Pfizer, Inc. **D. Gray:** Employment; Pfizer, Inc..

Poster

916. Cognitive Learning and Memory Systems IV

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:916.06/DDD21

Topic: F.02. Animal Cognition and Behavior

Support: NIH/NIMH MH077298

Title: An astrocyte response to dopamine depletion of the prefrontal cortex

Authors: *P. J. VOLLBRECHT[1], H. WANG[2], A. DEUTCH[2]
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Abstract: Dopamine loss in the prefrontal cortex (PFC), as has been reported in schizophrenia, results in a marked decrease in pyramidal cell (PC) dendritic spine density. The mechanisms through which cortical dopamine loss decreases the number of dendritic spines are not known. Changes in glutamate signaling are a major determinant of dendritic spine morphology and number. Over the past decade it has become clear that astrocytic proteins play a major role in regulating extracellular glutamate levels. In particular, the glial-specific glutamate transporter GLT-1 (EAAT2) and the group II metabotropic glutamate receptors (mGluRs), which are

expressed by both astrocytes and neurons, are key players in modulating extracellular glutamate levels. We determined if PFC dopamine depletion results in altered levels of astrocytic and neuronal proteins involved in extracellular glutamate homeostasis. Adult male rats received 6-hydroxydopamine (6-OHDA) lesions of the A10 dopamine neurons and were sacrificed three weeks later. Prefrontal cortical dopamine depletion significantly increased both GLT-1 and mGluR2/3 levels in membrane fractions from the prelimbic cortex, suggesting an enhanced clearance of extracellular glutamate in the PFC. The magnitude of the changes in GLT-1 and mGluR2/3 levels are inversely correlated with the extent of dopamine depletion in the PFC. Stereological assessment of GFAP-ir astrocytes uncovered no change in the number of astrocytes, nor were GFAP protein levels changed in the PFC. In contrast to the increased levels of the astrocytic protein GLT-1 and mGluR2/3, we did not observe significant changes in the expression of neuronal proteins involved in glutamate signaling, including AMPA and NMDA receptor subunits. These data suggest that dopamine depletion of the PFC leads to alterations in astrocytic regulation of extracellular glutamate, which may in turn determine dendritic spine plasticity.

Disclosures: P.J. Vollbrecht: None. **H. Wang:** None. **A. Deutch:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH

IHMRO

The Staglin Family

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UCSF Neurosciences Graduate Program

Title: Synaptic activity unmasks dopamine d2 receptor modulation of a specific class of layer v pyramidal neurons in prefrontal cortex

Authors: *S. GEE, I. ELLWOOD, V. SOHAL
UCSF, San Francisco, CA

Abstract: Dopamine D2 receptors (D2Rs) play a major role in the function of the prefrontal cortex (PFC), and may contribute to prefrontal dysfunction in conditions such as schizophrenia. Here we report that in mouse PFC, D2Rs are selectively expressed by a subtype of layer V pyramidal neurons that have thick apical tufts, prominent h-current, and subcortical projections. Within this subpopulation, the D2R agonist quinpirole elicits a novel afterdepolarization that generates voltage fluctuations and spiking for hundreds of milliseconds. Surprisingly, this afterdepolarization is masked in quiescent brain slices, but is readily unmasked by physiologic levels of synaptic input which activate NMDA receptors, possibly explaining why this phenomenon has not been reported previously. Notably, we could still elicit this afterdepolarization for some time after the cessation of synaptic stimulation. In addition to NMDA receptors, the quinpirole-induced afterdepolarization also depended on L-type calcium channels and was blocked by the selective L-type antagonist nimodipine. To confirm that D2Rs can elicit this afterdepolarization by enhancing calcium (and calcium-dependent) currents, we measured whole-cell calcium potentials that occur after blocking sodium and potassium channels, and found quinpirole enhanced these potentials, while the selective D2R antagonist sulpiride had the opposite effect. Thus, D2Rs can elicit a calcium-channel-dependent afterdepolarization that powerfully modulates activity in specific prefrontal neurons. Through this mechanism, D2Rs might enhance outputs to subcortical structures, contribute to reward-related persistent firing, or increase the level of noise in prefrontal circuits.

Disclosures: S. Gee: None. **I. Ellwood:** None. **V. Sohal:** None.

Poster

916. Cognitive Learning and Memory Systems IV

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Program#/Poster#:916.08/DDD23

Topic: F.02. Animal Cognition and Behavior

Title: Defining the interspecies exposure-receptor occupancy-cognitive disruption relationship of (±)-ketamine

Authors: *C. L. SHAFFER, S. M. OSGOOD, D. L. SMITH, P. TRAPA, S. GRIMWOOD
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Abstract: Glutamate dysregulation and *N*-methyl-D-aspartate (NMDA) receptor hypofunction are hypothesized key contributors to the psychopathology of schizophrenia and the various cognitive impairments in this disease. The NMDA receptor antagonist (±)-ketamine has been shown to produce schizophrenia-like symptoms and cognitive impairment, including working

memory deficits, in healthy humans. Thus, (±)-ketamine has been used in rats and nonhuman primates (nhp) to replicate specific cognitive deficits in schizophrenia, and such models have been used to study potential nootropic agents. Accordingly, this work evaluates the interspecies (i.e. rat, nhp and human) translatability of (±)-ketamine-mediated cognitive disruption via normalization to NMDA receptor occupancy (RO); both (±)-ketamine and its 7-fold less potent active metabolite (±)-norketamine were evaluated simultaneously following (±)-ketamine administration. To achieve this, unbound compound concentrations (both plasma and brain) and observed NMDA RO from rat in vivo RO studies using [^{3}H]MK-801 were analyzed by both direct response and receptor-binding kinetic models to afford matrix-specific IC_{50} and K_D, respectively. Assuming similar neuropharmacokinetics and respective ligand-receptor binding kinetics across species, these values allowed estimates of NMDA RO over time using (±)-ketamine and (±)-norketamine plasma exposures following the administration of cognitively impairing acute doses of (±)-ketamine in rats (10 mg/kg, SC) or nhp (1 mg/kg, IM). This same methodology transformed published human plasma data at a (±)-ketamine dose (0.23 mg/kg IV bolus + 0.5 mg/kg infusion) impairing working memory clinically. Collectively, the presented data provide insights into the translational pharmacologic relationship of NMDA RO to cognitive disruption for (±)-ketamine in rats, nhp and humans.

Disclosures: C.L. Shaffer: Employment; Pfizer Inc.. Ownership Interest; Pfizer Inc. **S.M. Osgood:** Employment; Pfizer Inc.. Ownership Interest; Pfizer Inc. **D.L. Smith:** Employment; Pfizer Inc.. Ownership Interest; Pfizer Inc. **P. Trapa:** Employment; Pfizer Inc.. Ownership Interest; Pfizer Inc. **S. Grimwood:** Employment; Pfizer Inc.. Ownership Interest; Pfizer Inc..

Poster

916. Cognitive Learning and Memory Systems IV

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:916.09/DDD24

Topic: F.02. Animal Cognition and Behavior

Title: Environmental enrichment: differential effects on drugs on object recognition memory in rats

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Abstract: Drug effects are usually evaluated in animals that are housed under standard conditions. However, it is assumed that rats raised in an enriched environment (EE) may be more

comparable to the human condition as compared to rats raised in a standard laboratory condition. Many studies have shown different expressions of biochemical markers in EE rats as compared to standard housed (SH) rats. Also, drug effects in various animal models differ between rats with different rearing histories Therefore the validity of the use of standard housed animals in drug research could be questioned. Moreover, there are no studies that have studied the effects of cognition enhancers in EE rats. In the present study we examined the effects of scopolamine and a PDE5 inhibitor on object recognition performance in standard housed and EE rats. SH and EE rats performed equally well when using a 1- delay but the EE rats outperformed the SH rats when using a 24-h delay. Scopolamine had similar effects in both standard housed and EE rats at the doses tested (0.03-0.3 mg/kg). As expected, a PDE5 inhibitor improved memory performance in SH rats. However, PDE5 inhibition did not improve memory performance in EE rats when using a 24-h delay (ceiling effect). Even when applying a 48-h interval PDE5 inhibition did not improve object recognition in the EE rats. These data suggest that scopolamine was effective to impair memory performance in EE and SH rats. However, memory enhancement was not found in EE rats. These data question the use of standard housed animals for the development of cognition enhancers in animal research.

Disclosures: A. Blokland: None. **S. Akkerman:** None. **J. Prickaerts:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: FAPESP (2011/03791-3)

AFIP

Title: Analysis of the effects of chronic treatment of ayahuasca on memory and anxiety in rats.

Authors: *V. M. FAVARO[1], M. YONAMINE[2], M. G. M. OLIVEIRA[1]
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Abstract: Ayahuasca is a hallucinogenic beverage that combines the action of the agonist serotonin N,N-dimethyltryptamine (DMT) from Psychotria viridis with inhibition of monoamine oxidase (MAO) induced by beta-carbonyls from Banisteriopsis caapi. In recent years, the legalization of consumption of the drink in a religious context provided an increase in the

consumption of ayahuasca chronically worldwide, arousing interest of the scientific community about the effects of drink on the central nervous system. Thus, this study investigated the effects of chronic treatment of ayahuasca on memory and anxiety in rats using different behavioral models. We used male Wistar rats (3 mo) weighing 250-350g. Ayahuasca was freeze-dried and the doses used were 120, 240 and 480 mg/kg (p.o) and tap water as control (N=12/group). Chronic treatment based on the administration of a daily dose of tea for 30 days. The animals were placed on the elevated plus maze (EPM) for 5 minutes, 48 hours after the last administration of the tea. After an interval of 30 minutes, the animals were trained on tone fear conditioning task (TFC), where they received 5 pairings of sound (5s) + shock (1s - 0.6 mA) for 6 minutes and 24 hours after training, animals were exposed to the same context of training for 5 minutes, and 48 hours after training, the same animals were placed in a different context where the sound (5s) was presented 5 times for 6 minutes. The paradigm of the Morris water maze (MWM) - spatial version was initiated 48 hours after last administration of ayahuasca with a new group of animals and was based on five days of training (4 trials - 1min/animal), followed by a test day (one trial - 1min/animal) in which the platform was removed. We found no significant differences between groups in the number of entries and time spent in open arms of the EPM. In contextual fear conditioning (CFC), the animals that received a dose of 120 mg/kg had a greater total time of freezing when compared to other groups. In TFC there were no significant differences in freezing time between groups. In MWM we found no significant differences in performance between groups. The behavioral data showed that chronic administration of ayahuasca improved the performance of animals that received a dose of 120 mg/kg in the CFC test. Furthermore, there was no change in anxiety levels, or performance of animals in MWM and TFC tests.

Disclosures: V.M. Favaro: None. **M. Yonamine:** None. **M.G.M. Oliveira:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH085607

State of Florida

Title: Central administration of angiotensin IV rapidly enhances novel object recognition among mice

Authors: *J. P. MCLAUGHLIN, S. O. EANS, E. MIZRACHI, K. J. REILLEY, M. L. GANNO, J. J. PARIS
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Abstract: Angiotensin IV (Val^1-Tyr^2-Ile^3-His^4-Pro^5-Phe^6), a metabolite of Angiotensins II and III, has demonstrated potential cognitive-enhancing effects. The present investigation aimed to assess and characterize: (1) angiotensin IV's dose-dependent effects for cognitive-enhancement in a C57BL/6J mouse model of a one-day novel object recognition task, (2) the time-course for these effects, and (3) the identity of residues in the hexapeptide important to these effects. Assessment of C57BL/6J mice in a novel object recognition task demonstrated that prior administration of angiotensin IV (0.1, 1.0, or 10.0 nmol, i.c.v.) significantly enhanced novel object recognition in a dose-dependent manner, with 0.1 nmol angiotensin IV proving the lowest, most efficacious dose as compared to vehicle administration. These effects were time dependent, as improved novel object recognition was observed when angiotensin IV (0.1 nmol, i.c.v.) was administered 10 min prior to the onset of the novel object recognition task, but not when administered 20 or 30 min prior to testing. Rotorod testing demonstrated no alterations of locomotion induced by any dose of angiotensin IV tested. A targeted alanine scan revealed that Ala-Val^1 replacement attenuated effects on novel object recognition, whereas Ala-Pro^5 replacement did not. Pretreatment with the angiotensin IV antagonist, divalinal-angiotensin IV, demonstrated the involvement of the angiotensin IV system in these behavioral effects. Overall, these data demonstrate that angiotensin IV produces a rapid enhancement of associative learning and memory performance in a mouse model, dependent on the valine residue in the first position of the peptide. Given reports of the conserved distribution of angiotensin IV binding in brain regions associated with mnemonic performance, the present results further support the potential of this ligand as a therapeutic to enhance cognition.

Disclosures: J.P. McLaughlin: None. **S.O. Eans:** None. **E. Mizrachi:** None. **K.J. Reilley:** None. **M.L. Ganno:** None. **J.J. Paris:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: CAPES

FAPERJ

CNPq

Title: Early life handling effects in an anxiety disorder model.

Authors: *M. L. RIBEIRO
Rio De Janeiro, Brazil

Abstract: The anxiety behavior is influenced by several factors, including environment, genetic and the interaction between them, which is the aim of this work. The neonatal handling procedure was performed with the Carioca rats, an anxiety disorder Wistar rat model, developed through selective lines matching based in a task of contextual fear conditioning, which gives rise to two different lines: CHF (Carioca High Freezing) and CLF (Carioca Low Freezing). In the neonatal handling study, CHF and Control rat pups were manipulated from PND1-21, daily, for 3min/animal (CHFH and CTRH groups), and evaluated in the Elevated Plus Maze (EPM) at PND28; other two groups did not received handling (CHF and CTR groups). A two-way ANOVA followed by LSD post-hoc test showed that he CHFH rats had the lowest rate entries in BA: CTR (Mean 4, SEM 0 ± 50, 73), CTRH (5.27 ± 0.52), CHF (3.44 ± 0.77) and CHFH (1.71 ± 0.75); $F(3,36) = 4.54$; $p < 0.05$. The CHF group had fewer entries in the BF than the CTR groups, independent of manipulation: CTR (8.70 ± 3.76), CTRH (11.00 ± 1.07), CHF (6.11 ± 0.81), CHFH (4.14 ± 0.86); $F(3,36) = 9.92$; $p < 0.05$. The procedure of handling reduced the relative frequency of entries in the BA in the CHFH group, without effect on group CTRH: CTR (31.87 ± 3.76), CTRH (32.51 ± 1.86), CHF (35.09 ± 5.89), CHFH (19.18 ± 6.98); $F(3,36) = 2.04$; $p < 0.05$. CTRH rats explored longer the center of the EPM: CTR (40.50 ± 3.99), CTRH (62.00 ± 6.79), CHF (27.56 ± 5.58) and CHFH (32.71 ± 11.18); $F(3,36) = 5.42$, $p < 0.05$. The CHF and CHFH groups spent less lime exploring the BA: CTR (14.80 ± 2.89), CTRH (15.30 ± 2.13), CHF (11.19 ± 2.93) and CHFH (4.33 ± 2.22); $F(3,36) = 3.27$; CHFH < CTR = CTRH, no significant difference between CHF and CHFH; $p < 0.05$. Conclusions: Animals of the CHF Group - manipulated or not - have lower rates of exploration of anxiogenic areas of the EPM compared with control groups. Manipulation, although not presenting significant variations in behavior related to anxiety, was able to change the ambulatory behavior (BF) and risk assessment (Time Center), but only in control rats. The handling has significantly changed the measure of entries in the BA group CHFH, reducing it, which is different than expected, indicating that these animals show a different behavior. The handling model was unable to reverse the behavioral pattern of anxiety in the experimental group CHF. The authors thank CAPES, FAPERJ and CNPq for the financial support.

Disclosures: M.L. Ribeiro: None.

Poster

916. Cognitive Learning and Memory Systems IV

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Program#/Poster#:916.13/DDD28

Topic: F.02. Animal Cognition and Behavior

Support: CIHR MOP-89890

Title: Genetic variant BDNF (Val66Met) polymorphism is associated with volumetric brain changes in the mouse

Authors: *J. SCHOLZ[1], A. VOINESKOS[2], F. S. LEE[3], S. SPRING[1], J. P. LERCH[1]
[1]Mouse Imaging Ctr. (mice), Hosp. For Sick Children, Toronto, ON, Canada; [2]Ctr. for Addiction and Mental Hlth., Dept of Psychiatry, Univ. of Toronto, Toronto, ON, Canada; [3]Dept. of Psychiatry, Weill Cornell Med. College, Cornell Univ., New York, NY

Abstract: Introduction
Brain-derived neurotrophic factor (BDNF) is a neurotrophine involved in growth and differentiation of new neurons and synapses. A single-nucleotide polymorphism in the BDNF gene (Val66Met) allows to study how BDNF contributes to affective disorders. In humans a methionine (Met) substitution for valine (Val) has been associated with changes in brain anatomy (Bueller et al, 2006) and memory (Hariri et al., 2003). In the mouse model decreased dendritic complexity in the dentate gyrus and increased anxiety-related behaviour has been observed (Chen et al., 2006). Here we test for volumetric brain changes across the whole brain between wild type (ET), heterozygous, and homozygous mice with post mortem magnetic resonance imaging (MRI). We hypothesize that the previously observed decreased neuronal complexity is associated with decreased volume.
Methods
69 (36 female) fixed mouse brains were examined, 43 WT, 13 (+/Met), 13 (Met/Met). MRI Acquisition: T2-weighted, 3-D fast spin-echo sequence (TR/TE = 2000/14 ms, 2 averages, field-of-view 14 x 14 x 25 mm3, matrix size = 250 x 250 x 450, imaging time = 11 h) (Lerch et al. 2011). Analysis: Images were aligned non-linearly (Lerch et al. 2011). The smoothed (FWHM 0.5 mm) Jacobians of the warp fields were used in an Anova design to test voxel-wise for differences between the three groups. Threshold-free cluster enhancement was used to correct for multiple comparisons (Smith et al., 2009). Voxels that passed the $p<0.05$ threshold were deemed significant.
Results
The voxel-wise test revealed that the three groups differed significantly in the area of the nucleus accumbens bilaterally ($p<0.01$, corrected). Averaging over the two significant clusters showed that the volume of the nucleus accumbens was significantly decreased in homozygous (10%, $p<0.01$), but not in heterozygous (4%, $p=0.21$). The was no significant effect of sex ($p=0.15$), but a significant interaction between sex and gene ($p=0.005$). WT and heterozygous males had smaller nuclei then their female counterparts. This relationship seemed to be reversed in

homozygous.
Conclusion
The nucleus accumbens plays an important role in processing reward and fear stimuli and has been implicated in vulnaribility to addictions. In humans deep brain stimulation of the right nucleus accumbens has shown to decrease the severity of anxiety disorders (Sturma 2003). It is possible that the polymorphism in the BDNF gene affects the structural organization of the nucleus accumbens resulting in increased anxiety. Future studies will investigate this link between behaviour and brain structure and might help to improve treatments of anxiety and addiction in the future.

Disclosures: J. Scholz: None. **A. Voineskos:** None. **F.S. Lee:** None. **S. Spring:** None. **J.P. Lerch:** None.

Poster

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Program#/Poster#:916.14/DDD29

Topic: F.02. Animal Cognition and Behavior

Title: The object retrieval test in the non-human primate: effects of rolipram and scopolamine on cognitive performance

Authors: T. WOLINSKY[1,2], C. CRUZ[3], I. HUBERT[3], M. LEMAIRE[1,2], *D. J. VIRLEY[1,2]
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Abstract: The Object Retrieval Test (ORT) or 'detour reaching test' in non-human primates (NHPs) is an established cognitive task thought to engage attention, response inhibition and planning (i.e. executive function). Importantly this test, involving prefrontal mechanisms, has been shown sensitive to both cognition-enhancing and cognition-impairing agents from distinct pharmacological classes.These findings suggest that the ORT in NHPs is relevant for assessing the pharmacological efficacy and safety of novel compounds on cognitive function thereby providing a useful translational bridge from rodent to human clinical studies.
The aim of the present study was to investigate the effects of rolipram and scopolamine using a simplified version of the ORT. 4 cynomolgus monkeys (*Macaca fascicularis*) were trained from their home cages to retrieve food rewards from a small clear box over the course of 24 trials per test session. Sessions containing 12 easy and 12 difficult trials were presented to each monkey in a balanced order. The level of task difficulty was determined by the positioning of the food

reward and the orientation of the box. The measure taken was the percent success at first-reach at each difficulty level. Rolipram (0.003 - 0.03 mg/kg i.m.) and scopolamine (0.010 - 0.040 mg/kg i.m.) were compared to saline (i.m.) on performance in the ORT for each monkey using a randomized within-subjects design. Using a 'best dose' analysis, rolipram was shown to significantly improve the percentage of accuracy of first-reaches during difficult trials in each of the 4 monkeys, whereas a clear impairment in performance was observed with scopolamine. These data suggest that our version of the test in a small number of monkeys together with a 'best dose' analysis to control for individual differences in response to specific doses provides a sensitive evaluation of the potential efficacy and safety of new chemical entities (NCEs) on cognition. This simple test of executive function should therefore have utility in guiding dose-prediction of NCEs for clinical assessment, with respect to neuropsychiatric disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease and Schizophrenia.

Disclosures: T. Wolinsky: None. **C. Cruz:** None. **I. Hubert:** None. **M. Lemaire:** None. **D.J. Virley:** None.

Poster

916. Cognitive Learning and Memory Systems IV

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:916.15/DDD30

Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS-08-43175

NSF IOS 10-52464

Title: Extracellular levels of BDNF in the hippocampus measured with microdialysis, change differentially during and after place and response learning.

Authors: *D. L. KOROL[1], P. E. GOLD[1], C. J. SCAVUZZO[2]
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Abstract: The role of brain-derived neurotrophic factor (BDNF) in neural plasticity, learning, and memory has been well established by many approaches including the delivery of antagonists and agonists, genetic manipulations, and analysis of tissue levels of the protein, its mRNA, and activation of downstream targets. To study BDNF functions across multiple memory systems, we previously examined tissue levels of mature BDNF (mBDNF) after rats were tested on a hippocampus-sensitive working memory task or on a striatum-sensitive response learning task.

The results showed that mBDNF levels increased in the hippocampus or striatum in a task-dependent manner. To test more directly the role for endogenous BDNF release in modulating learning and memory processing in these brain regions, we used in vivo microdialysis methods in the current study to measure changes in the concentration of mBDNF in the extracellular fluid (ECF) of the hippocampus, while rats were trained on a hippocampus-sensitive place task or a striatum-sensitive response task. Both tasks were conducted on the same 4-arm radial maze using food reward. The microdialysate was collected into micropipette tips (0.5 µl / min, 90 min samples) and diluted with sample buffer prior to analysis with ELISAs to determine BDNF concentration. The results indicate that ECF concentrations of hippocampal mBDNF increase from baseline during place training and continue to increase during the 90 min following training. In contrast, ECF mBDNF in the hippocampus falls below baseline and continues to decrease during the 90 min after response training. Because we interpret changes in ECF BDNF to reflect release of the protein, these data show that extracellular levels of mBDNF can be measured in awake, behaving animals and that mBDNF release is sensitive to cognitive demands. Moreover, the striking dissociation in the dynamics of extracellular hippocampal BDNF after place and response training suggests that brain site-specific patterns in BDNF release may reflect the cognitive strategies engaged by training in different tasks.

Disclosures: D.L. Korol: None. **P.E. Gold:** None. **C.J. Scavuzzo:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS-08-43175

NSF IOS 10-52464

Title: Engagement in a spatial working memory task enhances subsequent place and response learning through BDNF signaling

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Abstract: This study examines a possible mechanism through which learning can be enhanced by prior experience. Rats were tested on a spontaneous alternation (SA) task using spatial

working memory immediately, 1 hr, or 24 hrs prior to learning a hippocampus-sensitive place task or a striatum-sensitive response task. Place and response learning were both assessed in a 4-arm maze for food reward. SA testing immediately and 1 hr, but not 24 hr, prior to training enhanced learning in both place and response versions of the maze. Previously we showed that improvements in place and response learning following physical activity relied on BDNF signaling during learning; blockade of TrkB receptors just prior to place and response testing attenuated improvements seen after three weeks of voluntary exercise. Because mature BDNF (mBDNF) release and signaling have been implicated in learning and memory, we tested whether, like physical activity, the cognitive activity induced by SA testing primes later place and response learning through BDNF-dependent events. First, rats were sacrificed immediately, 1 hr, and 24 hrs following SA testing to measure whole tissue concentrations of mBDNF in the hippocampus and striatum using ELISAs. Increased concentrations were detected in the hippocampus within 1 hr after SA, returning to baseline within 24 hrs. Striatal mBDNF concentrations increased at 1 hr after SA testing, increasing further at 24 hrs. Second, the importance of the changes in BDNF signaling to learning was examined using an inhibitor of the TrkB receptor, K252a. Rats trained on the place and response tasks 1 hr after SA testing received injections of K252a or vehicle into hippocampus and striatum, respectively, 30 min after SA testing, i.e. 30 min prior to place or response training. K252a infusions attenuated enhancements in place and response learning produced by prior SA testing. Blockade of TrkB signaling in rats without prior SA training did not impair performance. These results showing increased BDNF expression and attenuation of learning enhancements by K252a after cognitive activity are analogous to those seen when place and response learning are enhanced by prior physical exercise, suggesting common mechanisms to the effects on learning from both cognitive and physical activity.

Disclosures: C.J. Scavuzzo: None. **D.L. Korol:** None. **P.E. Gold:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS-08-43175

NSF IOS-05-20876

Title: The effects of exercise on learning and hippocampal succinate dehydrogenase histochemistry: Sex differences and the interaction of estradiol

Authors: *K. L. MITTERLING[1], K. ANDERSON[2], D. L. KOROL[1]
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Abstract: Epidemiological studies show that exercise and estrogen treatment enhance learning during healthy aging. Both treatments may also slow age-related memory decline or the progression of neurodegenerative diseases. Interventions started in young adulthood may confer even greater protection in old age and estrogen therapy may be more effective in women that are fit. Physical activity and exposure to estrogens can modulate brain metabolism, a possible shared mechanism for the neuroprotective effects of these treatments. Male and female young adult Long-Evans rats were trained on a hippocampus-sensitive spatial learning task following three weeks of voluntary running wheel access or sedentary housing in standard cages. Males were left gonadally intact while females were ovariectomized one week prior to separation into exercise (EX) or sedentary (SED) groups. Females received subcutaneous injections of 17β-estradiol benzoate (45 μg/kg) or sesame oil 48 and 24 hours prior to testing. Immediately after testing, rats were euthanized and brains harvested for hippocampal succinate dehydrogenase (SDH) histochemistry. Exercise significantly enhanced place learning in males compared to male sedentary controls. In females, the group that received EX+estradiol had enhanced learning relative to EX+oil, SED+oil, and SED+estradiol groups; learning in the latter three groups did not differ from each other. The finding that enhanced learning was evident only in the combined EX+estradiol groups suggests a synergistic effect between the treatments. In the female exercise conditions, estradiol enhanced total distance run in the 48 hours prior to testing compared to the EX+oil group. It is possible that the increase in physical activity contributed to the enhanced learning in this group. Unlike previous studies using Sprague-Dawley rats, estradiol alone failed to enhance place learning in Long-Evans females. The lack of enhancement by estradiol in sedentary females may be due to strain differences in estradiol sensitivity or may have been obscured because many sedentary rats failed to reach criterion within the 75 trial maximum; this ceiling effect may have precluded the ability to detect differences in criterion performance. After maze training, prior exercise significantly increased SDH staining density in the hippocampus in males vs their sedentary controls but did not significantly change SDH staining density in any female groups. Thus, while increased SDH expression may contribute to the ability of exercise to enhance place learning in males, a different cellular or metabolic pathway in females appears to contribute to the enhanced learning after EX+estradiol.

Disclosures: K.L. Mitterling: None. **K. Anderson:** None. **D.L. Korol:** None.

Poster

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NSF IOS 10-52464

Alzheimer's Association Award

NIH Training Grant HD007333

Title: A role for astrocytes in metamodulation of memory: Working memory and hippocampal extracellular lactate levels vary based on prior training

Authors: *P. E. GOLD[1], L. A. NEWMAN[1,2], C. J. SCAVUZZO[3], D. L. KOROL[1]
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Abstract: Astrocytic glycogen provides an energy reserve that can supplement total energy available to neurons. Particularly during times of high demand such as during memory processing, astrocytes provide energy by breaking down glycogen to lactate and then supplying the lactate to neurons. Recent findings suggest that lactate is an important modulator of memory processing and show that extracellular (ECF) lactate levels increase selectively in brain regions engaged during learning and memory. Moreover, experience-dependent changes in glycogen stores are task specific: training on a hippocampus-sensitive spatial task can lead to long-lasting increases (>30 days) in glycogen content in the hippocampus while training on a striatum-sensitive response task leads instead to similar increases in glycogen content in the striatum. The present experiment was designed to determine whether these changes in glycogen resources are functionally significant. Rats were trained for food reward on either a place or response version of a 4-arm radial maze; controls underwent the same food restriction schedule but were not trained. After training, ad libitum access to food was returned for 28 days. At that time, rats were tested for spatial working memory on an unrewarded spontaneous alternation task. During alternation testing, ECF levels of lactate in the dorsal hippocampus were measured in 1-sec intervals using biosensor probes. Rats that had received prior place training had higher alternation scores on the spatial working memory task and had higher lactate release during alternation testing than did rats that had received prior response training. Second-by-second analyses of hippocampal lactate during alternation testing revealed significantly greater increases in lactate after completion of an alternation in previously place-trained rats as compared to response-trained rats. These findings reveal a form of metamodulation in which prior experience

primes subsequent memory in a task-specific manner with concomitant potentiation of energy reserves in specific brain regions. Thus, long-lasting changes in astrocytic glycogen reserves and lactate provision to neurons may play a substantial role in modulation and metamodulation of memory. Importantly, the response of ECF lactate levels to a working memory task is differentially affected by an experience at least 28 days before, in a manner consistent with the long-lasting changes in glycogen levels we have seen previously. Moreover, the present findings indicate that both long- and short-term changes in ECF levels of lactate are significantly related to past and current experience, respectively.

Disclosures: P.E. Gold: None. **L.A. Newman:** None. **C.J. Scavuzzo:** None. **D.L. Korol:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOB 0520876

NIA PO1 AG024387-04

NCAAM ODS NCI P50 AT006268

Title: Site- and task-specific ERK activation following genistein treatment corresponds to temporal aspects of learning

Authors: *S. L. PISANI[1], V. E. JUNG[2], D. L. KOROL[3]
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Abstract: The hippocampus and striatum undergo specific changes in cell signaling, gene transcription, and partitioning of metabolic substrates following place and response learning respectively. We previously found that oral treatments of genistein, a botanical estrogen found in soy, to young ovariectomized rats mimics estradiol-induced shifts in learning. Estrogens enhanced hippocampus-sensitive place learning but impaired striatum-sensitive response learning. Because local signaling via ERK plays an important role in estrogenic enhancement of hippocampal-sensitive memory tasks, we examined whether changes in ERK activation in hippocampus and striatum align with the magnitude and direction of shifts in place and response learning. Using quantitative Western blot analysis, ERK activation was measured in the

hippocampi and striata of rats trained on place or response learning tasks with or without genistein treatment. Simple increases and decreases in ERK activation in hippocampus and striatum following genistein were not observed. However, hippocampus to striatum (H:S) ratios of ERK activation corresponded to several features of learning. Placed-trained rats had approximately 2-fold higher H:S ratios of ERK activation than did response-trained rats, representing greater contribution of the neural structure critical for each respective task. Interestingly, in rats from the highest-performing treatment groups in each task, namely genistein-treated rats on the place task and vehicle-treated rats on the response task, faster task acquisition was associated with lower H:S ERK activation. We hypothesize that this greater striatal, or lower hippocampal, contribution represents a shift to habit/procedural memory through the over-training of rats that acquired the tasks quickly. In rats that acquired the tasks more slowly, i.e. the vehicle-treated rats on the place task and the genistein-treated rats on the response task, H:S ERK activation was correlated with the total duration of training. Specifically, rats with the slowest learning had the highest H:S ratios. Thus, the hippocampus appears to be engaged during early phases of acquisition of both place and response tasks, while the striatum dominates later in training after substantial learning. These results suggest a dynamic temporal relationship, rather than strictly competition or independence, between these neural systems in the acquisition and performance of different types of learning.

Disclosures: S.L. Pisani: None. **V.E. Jung:** None. **D.L. Korol:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH T32HD007183

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NIH NS20498

NIH P30 HD02274

Title: Targeted deletion of ERK5 MAP Kinase in adult neurogenic regions attenuates hippocampal adult neurogenesis and hippocampus-dependent memory formation

Authors: *Y.-W. PAN[1], H. SAKAGAMI[2], M. G. GARELICK[1], G. C. K. CHAN[1], C. T. KUO[3], X. WANG[4], C. TOURNIER[4], D. R. STORM[1], Z. XIA[1]
[1]Univ. of Washington, Seattle, WA; [2]Kitasato Univ. Sch. of Med., Sagamihara, Japan; [3]Duke Univ. Med. Ctr., Durham, NC; [4]Univ. of Manchester, Manchester, United Kingdom

Abstract: Recent studies have led to the exciting idea that newly generated neurons in the adult dentate gyrus of the hippocampus may play a role in hippocampus-dependent memory formation. However, signaling mechanisms that regulate adult hippocampal neurogenesis are not well defined. Here we report that ERK5, a MAP kinase, is selectively expressed in the two neurogenic regions of the adult mouse brain. This unique pattern of expression distinguishes ERK5 from other signaling molecules implicated in adult neurogenesis, such as Wnt, PI3K-Akt and BDNF, which are more widely expressed in the brain. It also suggests that ERK5 may be critical in regulating adult neurogenesis. Indeed, shRNA knockdown of ERK5 in cultured aNPCs or conditional deletion of the ERK5 gene specifically in the neurogenic regions of the adult mouse brain reduces neurogenesis *in vitro* and *in vivo*, respectively. By contrast, ectopic activation of endogenous ERK5 signaling via expression of caMEK5 promotes neurogenesis in cultured aNPCs as well as in the dentate gyrus of mouse brains. These data suggest that ERK5 signaling is an important regulator of adult neurogenesis in SGZ cells *in vitro* and *in vivo.* Importantly, targeted deletion of ERK5 specifically in neural progenitors of the adult mouse brain caused deficits in the more challenging forms of hippocampus-dependent learning and memory including contextual fear when a weaker foot shock was administered. Conditional ERK5 KO mice are deficient in fear extinction and reversal water maze training and spatial memory, suggesting that ERK5 and ERK5-mediated adult neurogenesis is important for those forms of learning that require active forgetting of a prior memory. Furthermore, our data suggest a critical role for ERK5 and ERK5-mediated adult neurogenesis in pattern separation, a form of dentate gyrus-dependent learning and memory. Finally, conditional ERK5 KO mice have no remote memory in the passive avoidance test, suggesting for the first time a pivotal role for adult hippocampal neurogenesis in the maintenance of long term memory. Together, our results implicate ERK5 as a novel signaling molecule regulating adult neurogenesis and provide strong evidence that adult neurogenesis is critical for hippocampus-dependent memory formation and for the maintenance of long-term memory.

Disclosures: Y. Pan: None. **H. Sakagami:** None. **M.G. Garelick:** None. **G.C.K. Chan:** None. **C.T. Kuo:** None. **X. Wang:** None. **C. Tournier:** None. **D.R. Storm:** None. **Z. Xia:** None.

Poster

916. Cognitive Learning and Memory Systems IV

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH57683

NSF Predoctoral Fellowship

Title: Basolateral amygdala stimulation produces heterosynaptic suppression of inputs from other temporal cortical structures in the prefrontal cortex

Authors: *H. A. TEJEDA[1], T. SHIPPENBERG[2], P. O'DONNELL[3]
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Abstract: The prefrontal cortex (PFC) is critical for executive control of behavior, behavioral flexibility, and working memory. These functions are dependent on highly interconnected circuits that include limbic substrates, such as the amygdala and hippocampus, as well as primary and associative cortices. Afferents arriving from the basolateral amygdala (BLA) likely provide information related to emotional states and may adjust PFC function. It is conceivable that strong amygdala activation reduces PFC synaptic responses to other afferents. Here, we examined the interactions between BLA inputs and those from other temporal cortical nuclei including the ventral hippocampus and the amygdalopiriform transition area (APiri), an associative cortical structure, using in-vivo intracellular recordings in anesthetized rats. Using sharp microelectrodes, medial PFC (mPFC) neurons were recorded intracellularly, and synaptic responses were generated by electrical stimulation of the BLA and fimbria (the fiber bundle carrying hippocampal projections to the PFC) or APiri. To determine whether BLA train stimulation attenuated hippocampal or APiri-evoked responses in the mPFC, a baseline test pulse (S1) was applied to the fimbria or APiri followed by conditioning burst stimulation of the BLA (10 pulses; 10, 20, or 50 Hz) 500 ms later. A test synaptic response (S2) was evoked at varying delays (50, 100, 150, 300, or 500 ms) after the last BLA pulse in the train. BLA burst stimulation attenuated fimbria- and APiri-S2-evoked EPSP amplitudes in a time- and frequency-dependent manner, with S2 responses at short delays being inhibited more robustly than those at longer delays. These effects were also observed using optical stimulation of the fimbria in rats expressing a light-sensitive cation channel in ventral hippocampal neurons. This effect is activity-dependent as single pulse BLA stimulation is without effect on fimbria- or APiri-evoked S2 responses at all delays. Fimbria and APiri train stimulation did not alter BLA-evoked responses, suggesting that heterosynaptic suppression of temporal cortical inputs is unidirectional and only produced by activation of the BLA-mPFC pathway. A BLA-evoked heterosynaptic suppression of other temporal cortical inputs could be critical for the selection of the appropriate behavioral response to fear-inducing or conditioned stimuli that would activate the amygdala. The mechanisms involved in this interaction remain to be discovered.

Disclosures: H.A. Tejeda: None. **T. Shippenberg:** None. **P. O'Donnell:** None.

Poster

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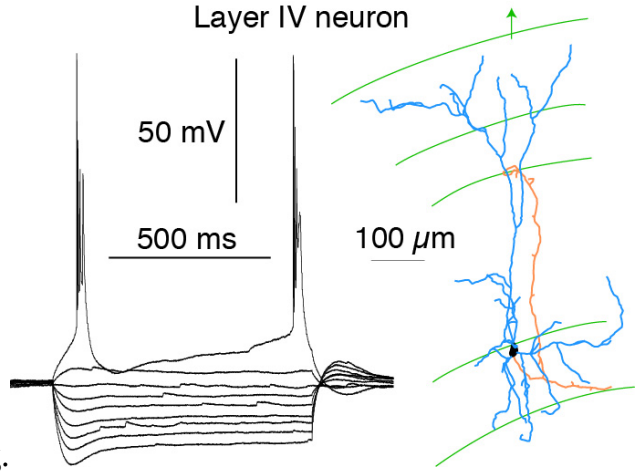
Support: ANRJCJC 10206DD

Title: Functional neuroanatomy of rat presubiculum

Authors: J. SIMONNET, E. EUGÈNE, I. COHEN, R. MILES, *D. FRICKER
CHU Pitié-Salpêtrière, CRICM, Paris, France

Abstract: The presubiculum, at the transition from hippocampus to cortex, is a key area for spatial information coding. We used patch clamp recordings from horizontal slices of rat (p21-35) hippocampus to establish the first detailed neuroanatomical description of this 6-layered cortex. Neurons were filled with biocytin for post hoc analysis. Principal neurons were classified on the basis of 15 anatomical and 14 electrophysiological parameters. Neuronal subpopulations were defined using Ward's unsupervised clustering from 58 completely characterized presubicular cells.
We found three main cell types that followed the laminar distribution of somata. Cluster 1 contained principal neurons in layers II and III (n = 24), small pyramidal shaped regular spiking cells. This group was homogeneous, with the most negative resting membrane potential (RMP, -77.5 ± 0.8 mV), compared to all other groups. Dendrites were confined to superficial layers only. Cluster 2 contained intrinsic bursting pyramidal cells (n = 6) in layer IV, previously not described. These low resistance neurons had a RMP of -62.1 ± 1.4 mV, close to firing threshold. Bursting neurons had a high sag index, 1.23 ± 0.03, suppressed by ZD7288 (20 µM). Cluster 3 contained regular spiking neurons of layers V and VI cells with large action potential widths. Two subclusters rapidly emerged: 3.1 with diverse shapes, including pyramidal, multiform and inverted pyramidal cells (n = 17); 3.2 with high resistance pyramids, more excitable than the other clusters (n = 11).
Some presubicular cells provide maintained head direction signals in vivo. Similarly, firing frequency adapted very little during maintained depolarization in vitro, for most regular spiking presubicular cells. A late adaptation index was calculated for 20 Hz maintained firing over 800 ms, showing a mean diminution of firing frequency of less than 10 %.
Thus, the presubiculum contains the cellular elements of cortical circuits. Regular spiking

neurons in superficial and deep layers possess an unusually weak firing adaptation, while layer IV neurons are intrinsically bursting.



Disclosures: J. Simonnet: None. **E. Eugène:** None. **I. Cohen:** None. **R. Miles:** None. **D. Fricker:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Title: New features in the 2012 release of the open source (para)hippocampal connectome of the rat.

Authors: *N. L. CAPPAERT[1], M. P. WITTER[2], N. M. VAN STRIEN[3]
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Abstract: Interactions between the hippocampal formation (HF) and the parahippocampal region (PHR) contribute to encoding, consolidation and retrieval of declarative memories. Knowledge of the neuroanatomical connections of the HF and PHR is a prerequisite to understand how these neural interactions affect for example memory processing. To provide this knowledge in a comprehensive and transparent way, we constructed a connectome in which the three-dimensional organization of the projection patterns between and within the 14 sub-regions of the HF and PHR are displayed in an interactive visualization. This connectome has several unique

features. First, the three-dimensional topographical detail is as high as current knowledge permits. Second, the connectome is interactive, i.e. connections can be switched on and off. Third, through decades of neuroanatomical research, the nomenclature has changed significantly. This connectome provides a translation of one nomenclatures into another via a 'Rosetta Stone'. The 2012 update of this project introduces a new project website with an online interactive literature search tool, advanced PubMed queries aimed at finding the right connectivity literature and a more detailed description of the 14 sub-regions incorporated in the interactive connectome. The search tool allows one to interactively search for literature describing connections between a set of brain regions that are part of the connectome. In addition, access to advanced PubMed search queries is provided to help researchers create more effective searches. The expanded section on hippocampal anatomy allows both students and experienced researchers to experience new things about hippocampal anatomy. The poster will be accompanied with a live demonstration of the new online resources.

Disclosures: N.L. Cappaert: None. **M.P. Witter:** None. **N.M. van Strien:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: BFU 2006-12964

BFU 2009-14705

Title: Brainstem afferents to the hippocampal formation: Comparative immunohistochemical study in the Macaca fascicularis monkey

Authors: *D. H. MOMBIELA[1], M. MUÑOZ[1], M. ARROYO-JIMÉNEZ[1], A. MOHEDANO-MORIANO[1], E. ARTACHO-PÉRULA[1], E. LEGIDOS-GARCIA[1], M. UBERO[1], J. GONZÁLEZ-FUENTES[1], M. LAGARTOS-DONATE[1], S. CEBADA-SÁNCHEZ[1], N. LOGOTHETIS[2], R. INSAUSTI[1]
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Abstract: The synaptic plasticity of the Hippocampal Formation (HF, which includes the dentate gyrus -DG-, CA3, CA2, CA1, subiculum, pre-parasubiculum and the entorhinal cortex -EC) is strongly influenced by neurotransmiters (presumably Dopaminergic -DA, Ventral Tegmental

Area-VTA; Noradrenergic -NA, Locus Coeruleus-LC and Serotoninergic -5-HT, Raphe Nuclei-RN, respectively), (Otmakhova and Lisman, 1996; Katsuki et al., 1997), although the anatomical basis of the chemical modulation of memory in the HF is far from being understood.
The neuroanatomical connections between the brainstem and in the HF in the nonhuman primate are still unclear. Previous tracer studies showed retrogradely labeled neurons in the brainstem areas including the VTA, LC and RN, after deposits in the hippocampus (Amaral and Cowan, 1980), as well as in the EC (Insausti et al., 1987). In order to characterize the neurochemical nature of those projections, as well as their topographic and laminar differences, we studied comparatively the distribution on those substances in the HF using immunohistochemical techniques.
Immunohistochemistry for each DA (Tyrosine Hydroxylase, TH), NA (Dopamine Beta Hydroxylase -DBH-, and 5-HT) as well as double-immunohistochemical techniques using Alexa 488 (5-HT detection) and Alexa 568 (TH or DBH labeling) disclosed that:
•The polymorphic layer of the DG had fibers with the three neurotransmitters, whereas the molecular layer showed only TH and 5-HT immunolabeling, without double-stained processes.
•The pyramidal layer of CA3 showed denser 5-HT fiber labeling than TH; CA1 showed only scattered TH and 5-HT fibers, without double labeling profiles.
•The subiculum and presubiculum showed fibers immunoreactive for TH, SER and BHD in the molecular layer. No double-labeled TH-5HT or DBH-5HT fibers were seen.
•The superficial layers of the rostral EC (I and II) displayed TH- or 5-HT-labelled processes, while the most lateral subdivisions of EC (ELR/ELc) had TH- or DBH-positive fibers; they did not show co-localization.
The preferential location of these positive fibers in ELR/ELc is significant, as this portion of the EC receives abundant unimodal and polymodal sensory input and innervates the body and tail of the hippocampus, and therefore it might be an important step for the monoaminergic modulation memory consolidation. Our preliminary anatomical results suggest that the HF function may be modulated independently by monoaminergic neurotransmitters.

Disclosures: D.H. Mombiela: None. **M. Muñoz:** None. **M. Arroyo-Jiménez:** None. **A. Mohedano-Moriano:** None. **E. Artacho-Pérula:** None. **E. Legidos-Garcia:** None. **M. Ubero:** None. **J. González-Fuentes:** None. **M. Lagartos-Donate:** None. **S. Cebada-Sánchez:** None. **N. Logothetis:** None. **R. Insausti:** None.

Poster

916. Cognitive Learning and Memory Systems IV

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:916.25/DDD40

Topic: F.02. Animal Cognition and Behavior

Support: Supported by grants BFU 2006-12964 and 2009-14705 from Ministry of Education, Spain.

Title: A tract-tracing study of entorhinal cortex afferents to the dorsolateral frontal cortex in the nonhuman primate (Macaca fascicularis).

Authors: *M. LEGIDOS GARCIA[1], M. M. ARROYO-JIMÉNEZ[1], E. ARTACHO-PERULA[1], S. CEBADA-SÁNCHEZ[1], J. GONZALEZ-FUENTES[1], D. HERNÁNDEZ-MOMBIELA[1], M. IÑIGUEZ DE ONZOÑO[1], M. J. LAGARTOS-DONATE[1], P. MARCOS[1], A. MOHEDANO-MORIANO[1], M. MUÑOZ[1], M. RAMOS[1], C. RUIZ-LOPEZ[1], M. M. UBERO[1], D. AMARAL[2], R. INSAUSTI[1]
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Abstract: Retrograde tracing studies in the nonhuman primate have shown that the dorsolateral prefrontal cortex (DLPFC) presents a paucity of afferents to the entorhinal cortex (EC, Insausti et al., 1987). Further experiments with anterograde tracers (Insausti et al., 2005) confirmed this fact. In contrast, the rodent EC provides a broad innervation of all frontal cortex (Swanson and Köhler, 1986; Herrero et al., 1987). Given the importance of the DLPFC in different cognitive and behavioural functions such as encoding and retrieval of declarative memory, it is striking that the nonhuman primate DLPFC is scarcely connected by direct projections to the EC, the main gateway to the hippocampus. In order to clarify whether the EC projects to the DLPFC in a reciprocal fashion to the same areas it gets afferents from, or whether it is able to influence to a higher degree the DLPFC, we studied the prefrontal afferents to the EC by neuroanatomical anterograde tracing techniques.
We analyzed nine cases of Macaca fascicularis with byotinilated Dextran Amine (BDA, mw 10,000) deposits in various portions along the medio-lateral and rostro-caudal axes of the EC. A magnetic resonance (MR) examination to localize the stereotaxic coordinates of the injection site was performed in all the animals. After 2 weeks survival, animals were perfused through the heart with 4% paraformaldehyde. Other cases were injected with 3H-aminoacids and PHA-L resulting in deposits in various portions along the EC.
The main finding is that, relative to other prefrontal regions as the orbitofrontal (OFC) and medial frontal (MPFC) cortices, the EC afferents were overall, negligible. BDA deposits in the intermediate portion of the EC resulted in the densest projection, which was located in Areas 9 and 8, in reciprocity with retrograde studies. Other cases, with deposits placed at other locations of the EC, showed only scattered fibers in the white matter, some of the which, seemed to reach layer VI. The distribution of labeled fibers after mid entorhinal cortex portions showed labeled fibers in layers I and VI of Areas 9 and 8. The weakest labeling resulted after deposits in caudal portions of the EC, suggesting that little or no direct interplay between this portion (which projects to the body and tail of the hippocampus), and the dorsolateral prefrontal cortex is present in the nonhuman primate.

Our results suggest that the hippocampal output to the DLPFC is not direct, but mediated by the relay of other prefrontal regions as MPFC and OFC.
The functional significance of this particular pattern of connectivity between the hippocampal formation and the dorsolateral prefrontal cortex warrants further investigation.

Disclosures: M. Legidos Garcia: None. **M.M. Arroyo-Jiménez:** None. **E. Artacho-Perula:** None. **S. Cebada-Sánchez:** None. **J. Gonzalez-Fuentes:** None. **D. Hernández-Mombiela:** None. **M. Iñiguez de Onzoño:** None. **M.J. Lagartos-Donate:** None. **P. Marcos:** None. **A. Mohedano-Moriano:** None. **M. Muñoz:** None. **M. Ramos:** None. **C. Ruiz-Lopez:** None. **M.M. Ubero:** None. **D. Amaral:** None. **R. Insausti:** None.

Poster

916. Cognitive Learning and Memory Systems IV

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Program#/Poster#:916.26/DDD41

Topic: F.02. Animal Cognition and Behavior

Support: MH084053

Title: Connections between the medial temporal lobe and the lateral mediodorsal thalamic nucleus in macaque monkey

Authors: *V. L. MCCOY[1], D. S. MELCHITZKY[1,2], S. L. ERICKSON[2], D. A. LEWIS[2,3]
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Abstract: The mediodorsal thalamic nucleus (MD) is the principal source of subcortical input to the prefrontal cortex (PFC) with different domains within MD targeting particular prefrontal cortical areas. The medial temporal lobe (MTL) is comprised of the hippocampal formation and the perirhinal and parahippocampal cortices and plays a fundamental role in memory functioning. Understanding the topography of the projections from the MTL to different domains within MD will help determine the specific types of information available to each MD domain and subsequently to the PFC. In this study, injections of the retrograde tracer cholera toxin b subunit (CtB) were made into discrete locations of the lateral portion of the MD in four macaque monkeys. Two of the animals had CtB injections in both hemispheres, resulting in six injections sites for analysis. Bilateral coronal sections through the MTL in each animal were processed immunocytochemically to visualize the CtB. Drawings of CtB- immunoreactive cells in the MTL were made from sections spaced 50 microns apart using a camera lucida drawing tube attached to a microscope, and Nissl- and Geimsa- stained sections were used to delineate the borders between the various areas of the MTL. Each injection site had a unique distribution of

retrogradely-labeled cells throughout the MTL and the proportion of labeled neurons in each component of the MTL differed greatly across injection sites. For example, an injection in the ventrolateral MD resulted in a large proportion of labeled neurons in the presubiculum (77%) with very few cells in the CA fields (1.3%). In contrast, the largest proportion of labeled cells following an injection in the caudoventrolateral MD was found in the CA fields (61%). Initial inspection of an anterodorsal injection site suggested a more evenly distributed pattern of labeling between the CA fields and subicular areas. Differences in the proportion of labeled neurons in both the perirhinal and parahippocampal cortices were also seen across the injection sites, although none of these cortical areas contained the majority of labeled neurons from any injection site. These findings suggest that individual locales within the lateral MD receive topographically distinct projections from the MTL, implying that a different complement of information from the MTL is relayed to specific domains of MD.

Disclosures: V.L. McCoy: None. **D.S. Melchitzky:** None. **S.L. Erickson:** None. **D.A. Lewis:** None.

Poster

916. Cognitive Learning and Memory Systems IV

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Program#/Poster#:916.27/DDD42

Topic: F.02. Animal Cognition and Behavior

Support: NSF Research Grant BCS-0843027 (APB)

RO1 MH62122 (MSF)

Title: Neural mechanisms mediating the use of imagery and inferential reasoning by rats in ambiguous situations.

Authors: *C. D. FAST, M. M. FLESHER, S. R. STERLACE, N. A. NOCERA, M. S. FANSELOW, A. P. BLAISDELL
Univ. of California - Los Angeles, LOS ANGELES, CA

Abstract: Fast & Blaisdell (2011) demonstrated that prior learning influenced how rats behave in ambiguous situations. Rats that had learned a challenging, non-linear negative patterning task (where two cues are reinforced when presented individually but not when they are presented in compound) responded significantly differently when one cue was occluded from view (ambiguously absent) compared to when it was explicitly absent. Specifically, rats behaved as if they were unable to determine if an ambiguous trial constituted an elemental or compound trial

from training by emitting significantly fewer instrumental lever presses when the cue was ambiguous compared to when it was explicitly absent - suggesting an image of the ambiguous cue was active. Rats that had learned a simple linear positive patterning discrimination (individual presentations of each cue are not reinforced while the compound presentation is), on the other hand, did not respond differently in the two test situations. Interestingly, rats trained on a positive patterning task do show sensitivity to the ambiguous test situation if they had also been trained on a negative patterning task. After learning both discriminations, rats responded significantly more during ambiguous test trials with cues that had previously served in the positive patterning discrimination compared to test trials when the cue was explicitly absent. These results suggest that the more complex task of negative patterning engaged representational processing capacities necessary for sensitivity to ambiguity that were otherwise not engaged by positive patterning. Acetylcholine is known to regulate cognitive tasks through its impact on both the processes of attention and plasticity (Reber, Luechinger, Boesinger, & Henke, 2012; Zeithamova, Schlichting, & Preston, 2012). Therefore, as an initial step toward identifying the brain mechanisms responsible for this behavioral sensitivity to ambiguity, we tested subjects after administering the muscurinic cholinergic antagonist scopolamine. In a follow-up experiment, we specifically investigated the role of the hippocampus through temporary inactivation. The results have interesting implications for representational processes and the underlying neural mechanisms engaged in problem solving.

Disclosures: C.D. Fast: None. **M.M. Flesher:** None. **S.R. Sterlace:** None. **N.A. Nocera:** None. **M.S. Fanselow:** None. **A.P. Blaisdell:** None.

Poster

916. Cognitive Learning and Memory Systems IV

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R15N2072870

KBRIN Grant P20RR016481

Title: Enzymatic analysis of glutathione reductase in wistar rats with moderate traumatic brain injury: A dosage dependent approach

Authors: *J. T. JOHNSON[1,1], Z. P. SELLERS[1], T. T. REED[1], P. G. SULLIVAN[2], A. SEBASTIAN[2]
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Abstract: Imbalances in the production of reactive oxygen species (ROS) and native antioxidant mechanisms result in oxidative stress(1). Glutathione is a key antioxidant in the neutralization of the ROS, and it is hypothesized that when γ-glutamylcysteine ethyl ester (GCEE), a glutathione mimetic, is administered a decrease in oxidative stress will be shown in the moderate traumatic brain injury rat model. Glutathione reductase is responsible for reducing oxidized glutathione to allow further protection against oxidative stress via the oxidation of β-nicotinamide adenine dinucleotide phosphate. Reduced glutathione can initiate ROS reduction to protect against oxidative stress. GCEE was administered using a dosage dependent approach, and the significance of each dose was determined by examining the activity of glutathione reductase in brain samples treated with GCEE and brain samples treated with saline at time intervals of either 30 or 60 minutes post injury. Each sample was analyzed using a glutathione reductase assay modified from Sigma Aldrich (St. Louis, Missouri, USA). Enzyme kinetics analysis for the assay was examined using spectrometric analysis. Brain samples treated with a dosage of 150 mg/kg of body weight of GCEE yielded an increase in the activity of glutathione reductase compared to those treated with saline, indicating the presence of increased levels of reduced glutathione. The increased activity correlates with elevated antioxidant capacity. This lowers overall oxidative stress making this a potential post injury therapeutic strategy for moderate traumatic brain injury. Ongoing future research is being conducted to determine how GCEE affects other key enzymes such as glutathione peroxidase, glutathione S-transferase, and glucose-6-phosphate dehydrogenase.
1.News Medical.Net. What is Oxidative Stress? http://www.news-medical.net/health/What-is-Oxidative-Stress.aspx (accessed March 15, 2012)

Disclosures: J.T. Johnson: None. **Z.P. Sellers:** None. **T.T. Reed:** None. **P.G. Sullivan:** None. **A. Sebastian:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: R15NS072870

P20RR016481

Title: 3-nitrotyrosine as an oxidative stress indicator in wistar rats involving moderate traumatic brain injuries for proteomic analysis

Authors: *M. T. HENDERSON, J. JOHNSON, J. VANZANT, Z. SELLERS, T. REED
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Abstract: 3-Nitrotyrosine(3-NT) is one of the most frequently observed byproducts from reactive nitrogen species (RNS) reacting with proteins. As a biomarker of nitrosative stress, elevated levels of 3-NT signify the presence of oxidative stress and decreased levels of antioxidant enzymes. Traumatic brain injury (TBI) is profoundly related to oxidative stress, which has been indicated as a mechanism of secondary neuronal injury in TBI that can ultimately result in numerous related neurological maladies. Therefore, 3-NT can be utilized as an *in vivo* marker of oxidative nitric oxide damage following TBI. By using a moderate traumatic brain injury model with Wistar rats, it is hypothesized that the role of 3-NT formation as an intermediate will predict the involvement of protein nitration and oxidative stress in the brain. In this experiment, the levels of 3-nitrotyrosine were significantly elevated in TBI injured, saline treated rats compared to those who sustained an injury and were treated with the glutathione mimetic, GCEE. Determining the existence of elevated 3-NT levels provides insight into the relationship between protein nitration and oxidative stress which can determine the pathogenesis and progression of specific neurological diseases.

Disclosures: M.T. Henderson: None. **J. Johnson:** None. **J. VanZant:** None. **Z. Sellers:** None. **T. Reed:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R15NS072870

KBRIN Grant P20RR016481

Title: Gamma-glutamylcysteine ethyl ester as a post therapeutic treatment for moderate traumatic brain injury in rat: Insights into glutathione elevation

Authors: *Z. SELLERS, T. T. REED
Chem., Eastern Kentucky Univ., Richmond, KY

Abstract: Glutathione is a multifunctional tripeptide found in millimolar concentrations in the human brain. As a detoxifying agent, glutathione plays an integral role in maintaining cellular

integrity, serving as both an antioxidant and a conjugant for exporting toxic species (*1*). Traumatic brain injury (TBI) causes initial tissue damage in the brain, but also leads to secondary injuries mediated in large part by free radicals (*2*). Thus, glutathione requirements in the brain are greatly increased following TBI. Gamma-glutamylcysteine ethyl ester (GCEE) is an analog to a natural glutathione precursor that has been shown to increase intracellular glutathione levels in the human brain (*3*). Using a cortical contusion model for moderate traumatic brain injury in male Wistar rats, we are measuring the effect of post-TBI GCEE treatment on oxidative stress parameters in rat brain. We hypothesize that GCEE treatment will significantly increase overall glutathione concentration and the ratio of reduced glutathione to oxidized glutathione in the brains of male Wistar rats following moderate TBI. To test this hypothesis, the brain samples are first standardized by measuring their protein concentrations photometrically via the bicinchoninic acid assay method. Kinetic assays are then performed on each sample to test for reduced glutathione, oxidized glutathione, and total glutathione concentrations. These assays employ the reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) via the oxidation of reduced glutathione, which is measured photometrically at 412 nm. For measuring total glutathione levels, glutathione is recycled by glutathione reductase and reduced nicotinamide adenine dinucleotide phosphate during the assay. Oxidized glutathione is measured by first neutralizing reduced glutathione using 2-vinylpyridine, then assaying the sample in combination with the glutathione recycling method. By testing our rat models for glutathione elevation, we hope to offer insight into the development of post therapeutic treatment options for traumatic brain injury patients.

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Disclosures: Z. Sellers: None. **T.T. Reed:** None.

Poster

917. Social Effects on Fear Processing

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH072897

NIH Grant MH090541

Title: N-methyl-d-aspartic acid receptor distribution in fear-conditioned, socially partnered and socially isolated Wistar rats

Authors: E. HUSAIN[1], J. DASILVA[1], Y. LEI[1], A. MORRISON[2], *S. M. TEJANI-BUTT[1]
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Abstract: Resilience or vulnerability to stress involves both genetic and environmental factors. Compared to other out-bred rat strains, the Wistar (WIS) rat exhibited reduced freezing behavior to predator odor, suggesting that the WIS rat may be a more resilient strain. We had previously reported that cued fear-conditioning (CFC) increased freezing behavior in socially isolated WIS rats with little effect on sleep microarchitecture. While the freezing response to CFC was attenuated on Day 1 in socially partnered WIS rats, social partnering induced a fragmented rapid eye movement sleep pattern that was independent of CFC. Given that extensive connections between the limbic and forebrain regions are thought to regulate fear responses, the goals of the present study were to investigate the effects of CFC on N-methyl-D-aspartate (NMDA) receptors in socially isolated (CFC-SI) and socially partnered (CFC-SP) environments in WIS rats. The binding of [^{3}H] MK-801 to NMDA receptors was measured in the prefrontal cortex (PFC), basolateral amygdala (BLA), central amygdala (CeA), CA1/CA2, CA3, dentate gyrus (DG), ventral tegmental area (VTA), nucleus accumbens core (NAcc), nucleus accumbens shell (NAcsh), caudate putamen (CPU) and substantia nigra (SN). The results indicated that while CFC-SI rats exhibited decreased binding of [^{3}H] MK-801 to NMDA receptors in the PFC, BLA, CA1/CA2, DG, NAcc, VTA, CPU and SN as compared to control WIS rats, CFC-SP rats exhibited increased binding in the PFC, BLA, CeA, CA1/CA2, CA3, DG, NAcc, NAcsh, CPU and SN as compared to the CFC-SI group. It has been hypothesized that glutamatergic inputs to the amygdala from the PFC function to modulate fear output pathways via activation of inhibitory interneurons within the amygdalar microcircuitry. Decreased NMDA receptor densities in the PFC, BLA and hippocampus of CFC-SI rats may reflect a mechanism of receptor down-regulation in response to increased glutamatergic activation and inhibition of the amygdala, supporting our previous observations that CFC did not alter sleep in socially isolated WIS rats. In contrast, social partnering increased NMDA receptor density in the PFC, amygdala and hippocampus of CFC-SP rats. These results suggest that complex interactions between the PFC, amygdala and the hippocampus as well as several limbic regions are differentially involved in the modulation of freezing behavior and sleep disruptions in WIS rats.

Disclosures: E. Husain: None. **J. DaSilva:** None. **Y. Lei:** None. **A. Morrison:** None. **S.M. Tejani-Butt:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: USPHS grant MH072897

USPHS grant MH090541

Title: N-methyl-d-aspartic acid receptor distribition in fear-conditioned, socially partnered and socially isolated wistar-kyoto rats

Authors: *J. K. DASILVA[1], E. HUSAIN[2,1], Y. LEI[1], G. L. MANN[3], A. R. MORRISON[3], S. TEJANI-BUTT[1]
[1]Pharmaceut. Sci., Univ. of the Sci., Philadelphia, PA; [2]Applied Med. Sci., Univ. of Tabuk, Tabuk, Saudi Arabia; [3]Animal Biol., Univ. of Pennsylvania Sch. of Vet. Med., Philadelphia, PA

Abstract: Social environment has a profound effect on the susceptibility to developing stress-induced disorders. The Wistar-Kyoto (WKY) rat strain is known for its stress-sensitive phenotype. We have previously reported that socially partnered WKY rats exhibited a reduced effect on sleep-wake behavior in response to cued fear conditioning (CFC) compared to their socially isolated counterparts. Extensive connections between limbic regions and the forebrain are thought to regulate fear responses, and altered glutamatergic neurotransmission in these regions has been implicated in stress-induced psychiatric disorders. The goals of the present study were to investigate the effects of CFC on N-methyl-D-aspartate (NMDA) receptor binding in socially partnered (CFC-SP) and socially isolated (CFC-SI) WKY rats. The binding of [3H] MK-801 to NMDA receptors was measured in the prefrontal cortex (PFC), basolateral amygdala (BLA), central amygdala (CeA), CA1/CA2, CA3, dentate gyrus, ventral tegmental area, nucleus accumbens (NAc), caudate-putamen (CPU) and substantia nigra (SN). The results indicated that CFC in a socially isolated environment increased [3H] MK-801 binding in the PFC and SN, but CFC in a socially partnered environment increased binding in the BLA, CeA, and NAc. It has been hypothesized that glutamatergic projections from the PFC modulate fear expression by inhibiting amygdalar activity. Increased NMDA receptor density in the PFC of CFC-SI rats may reflect a mechanism of receptor up-regulation in response to decreased glutamatergic activation and disinhibition of the amygdala, supporting our previous observations that CFC-SI WKY rats exhibited increased freezing behavior and disrupted sleep. Furthermore, social partnering normalized PFC receptor density in CFC WKY rats and increased NMDA receptor density in the BLA and CeA, consistent with our previous observations of reduced freezing behavior and an absence of sleep disruption in these animals. No effect was observed in the hippocampal NMDA

receptor density of CFC-SP rats suggesting that the decreased behavioral response to conditioned fear may not involve NMDA receptor mediated mechanisms in the hippocampus. Thus, the attenuated conditioned fear response in socially partnered WKY rats may be occurring via the engagement of the PFC, amygdala and NAc but not the hippocampus or CPU.

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Poster

917. Social Effects on Fear Processing

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Program#/Poster#:917.03/DDD48

Topic: F.02. Animal Cognition and Behavior

Title: Juvenile social defeat alters fear extinction learning during adolescence and impairs social behavior into adulthood.

Authors: *L. A. FARNBAUCH, A. M. JASNOW
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Abstract: Anxiety is important for health and survival, but can become excessive, as exemplified by Post Traumatic Stress Disorder (PTSD); arising after trauma and eventually leading to a host of maladaptive behaviors, causing memory disturbances and impairing decision making. Anxiety disorders can present early in life, during brain development, and can influence judgment and interfere with social relationships. Moreover, there is a very strong relationship between the level of childhood trauma and adult anxiety disorder symptoms in humans. In the current study, we used acute social defeat as an animal model of trauma and stress to investigate the effects of social stress on subsequent social behavior, anxiety-like behavior, as well as fear learning and extinction in adolescent and adult mice. Juvenile (PND 28±4) male C57Bl6/J mice were subjected to social defeat and tested 24 hours and thirty days later for social interaction. 24 hours after social interaction testing the mice were tested for anxiety-like behavior using an elevated-plus maze. One week or five weeks post-defeat we used a standard cued fear conditioning procedure to examine the effects of prior social defeat on the acquisition, expression and extinction of fear. Juvenile mice exhibited normal social interaction compared with non-defeated controls when tested with an age-matched CD1 stimulus mouse, but interacted less when tested with an adult CD1 mouse 24 hours after defeat. When these mice were tested as adults (30 days post defeat), defeated mice still exhibited significantly reduced social interaction compared with control mice. Mice defeated as juveniles displayed normal fear acquisition during

adolescence as well as in adulthood, but extinction learning was impaired during adolescence only. These effects were observed without apparent alterations in anxiety-like behavior. We demonstrate that acute social defeat during the juvenile period produces long-term, yet differential effects on social behavior and cued fear during adolescence versus adulthood.

Disclosures: L.A. Farnbauch: None. **A.M. Jasnow:** None.

Poster

917. Social Effects on Fear Processing

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Program#/Poster#:917.04/DDD49

Topic: F.02. Animal Cognition and Behavior

Title: Sex differences in the generalization of contextual fear

Authors: *J. F. LYNCH, III, M. LATSKO, P. K. CULLEN, A. M. JASNOW, D. C. RICCIO
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Abstract: There is a high prevalence of anxiety disorders among adults,and females are 60% more likely to suffer from an anxiety disorder than males. One hypothesis for these findings may be that females exhibit higher rates of fear generalization than males. It has been demonstrated that the generalization gradient for contextual cues flattens over time whereas retention of the learned response remains intact in male subjects.The current experiment was designed to investigate the possibility of sex differences in the generalization of contextual cues. 184 male and female Long-Evans rats were trained and tested in a passive avoidance procedure to examine sex differences in the forgetting of stimulus attributes, as inferred from flattening of the generalization gradients. Rats were tested at different retention intervals (1, 3, 5, or 7 days) in either the same context as training or in a significantly altered context. At 1 day, male and female rats displayed equivalent levels of reduced fear when tested in a novel context, as expected (i.e., the context shift effect). Males continued to express reduced fear in a novel context across all retention intervals, whereas females exhibited comparable levels of fear in either context after 3 days. Overall, females showed no significant differences in the expression of fear between contexts at the 3, 5, and 7 day retention intervals. These results demonstrate that the generalization gradient for contextual cues flattens at a faster rate in female rats. Current work in the lab is attempting to elucidate the role of estrogens in the forgetting of contextual cues.

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Poster

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Howard Hughes Medical Institute

Title: Cell-type specific optogenetic activation mediates fear inhibition in the basolateral amygdala

Authors: *A. M. JASNOW[1], D. EHRLICH[2], D. C. CHOI[2], M. E. BOWERS[2], D. G. RAINNIE[2], K. J. RESSLER[3,2]
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Abstract: Previous research identified distinct neuronal circuits within the basolateral amygdala (BLA) that differentially correlate with fear expression and inhibition. Here we examine if optogenetic activation of a cellular subpopulation within the BLA would differentially support fear conditioning vs. fear inhibition / extinction. Using transgenic mice, we demonstrated that a Thy1-channelrhodopsin-2 (Thy1-ChR2-EYFP) expressing subpopulation of BLA pyramidal neurons was activated by fear expression and extinction. Optogenetic stimulation of these neurons inhibited a subpopulation of medial central amygdala (CeM) neurons and shunted excitation from the lateral amygdala (LA). Brief activation of these neurons during training disrupted fear memory when tested 24 hr later. Furthermore, optogenetic activation during unreinforced stimulus exposure enhanced extinction retention, but not within-session extinction, and had no effect on fear expression, locomotion, or open-field behavior. Furthermore, optogenetic-induced enhanced extinction consolidation also inhibits renewal of fear. These data suggest that Thy1-ChR2 expressing BLA neurons provide an important molecular and pharmacological target for inhibiting fear and enhancing extinction in fear-related disorders.

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Poster

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Program#/Poster#:917.06/DDD51

Topic: F.02. Animal Cognition and Behavior

Title: Impaired social interaction and fear extinction in a mouse social defeat model of PTSD.

Authors: *J. D. MEDURI, B. N. DULKA, A. M. JASNOW
Psychology, Kent State Universty, Kent, OH

Abstract: Posttraumatic stress disorder (PTSD) is an anxiety disorder that results after single or repeated exposure to a traumatic event. In humans, this disorder is associated with significant deficits in the ability to extinguish fear responses. Social stress is one of the most prevalent stressors experienced by many animal species. For instance, interpersonal violence is a significant contributor to the development of PTSD in humans. Several studies demonstrated that the experience of social defeat produces drastic and prolonged alterations in subsequent agonistic behavior. However, it is unclear how social stress modifies subsequent responses to other emotionally relevant stimuli. In the current study, we used acute social defeat as an animal model of trauma and stress to investigate the effects of social stress on subsequent social behavior, anxiety-like behavior, as well as fear learning and extinction. Mice were subjected to social defeat and tested 24 hours later for social interaction. 24 hours after the social interaction test, mice were tested for anxiety-like behavior using an elevated-plus maze. One week post-defeat we used a standard cued fear conditioning procedure to examine the effects of prior social defeat on the acquisition, expression and extinction of fear. Acute social defeat produced long-term effects on social behavior both 24 hours and 30 days post-defeat, with no apparent alterations in anxiety-like behavior. In addition, acute social defeat had no significant effect on the acquisition of fear conditioning but disrupted the within-session extinction and extinction retention. We demonstrate here that acute social defeat produces long-term changes in both social behavior and subsequent cued fear extinction. These data suggest that exposure to social stress may act to reorganize fear circuits resulting in prolonged alterations in subsequent emotional learning.

Disclosures: J.D. Meduri: None. **B.N. Dulka:** None. **A.M. Jasnow:** None.

Poster

917. Social Effects on Fear Processing

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:917.07/DDD52

Topic: F.02. Animal Cognition and Behavior

Title: The role of presynaptic inhibition in the generalization of context memory

Authors: *P. K. CULLEN, S. TRASK, B. DULKA, D. C. RICCIO, A. M. JASNOW
Psychology, Kent State Univ., Kent, OH

Abstract: Contextual fear conditioning involves pairing a novel context (conditioned stimulus) with several footshocks (unconditioned stimulus) that serve to condition fear to that context. Many studies have demonstrated that shifting contextual cues following context fear conditioning results in reduced freezing, (i.e., the context shift effect). At early time points rodents can discriminate between a training and novel context. However, these shifts in contextual cues become less disruptive as the retention interval between training and testing increases (context specificity is lost by 14 days post-training). In other words, the fear memory is no longer precise or context-specific, but has generalized to novel contexts. In an attempt to investigate potential mechanisms underlying the generalization of fear responses over time, we have identified presynaptic inhibition as playing a role in the precision of context memory. Specifically, we discovered that mice lacking a GABAB1 receptor subtype, GABAB1a, exhibit a loss of context discrimination compared to wild-type animals. Animals lacking GABAB1a receptors showed a significant, but not complete loss of context specificity 24 hours post-training. However, GABAB1a knock out mice exhibited a complete loss of context discrimination (i.e. generalization) by 5 days post-training. Our results indicate that presynaptic inhibition is required for the discrimination of context and identify GABAB1a receptors as a potential mechanism underlying context fear generalization. We are currently investigating the specific neural location that these receptors are required for context discrimination.

Disclosures: P.K. Cullen: None. **S. Trask:** None. **B. Dulka:** None. **D.C. Riccio:** None. **A.M. Jasnow:** None.

Poster

917. Social Effects on Fear Processing

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:917.08/DDD53

Topic: F.02. Animal Cognition and Behavior

Title: Dopamine controls the precision of long-term fear memory

Authors: *C. A. LEPPLA, S. TRASK, A. M. JASNOW
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Abstract: It is well established that the hippocampus plays an important role in the formation and consolidation of contextual memory, but the underlying biological mechanisms of retention and precision in this system are still not fully understood. Many studies have demonstrated that shifting contextual cues following context fear conditioning results in reduced freezing, (i.e., the context shift effect). At early time points rodents can discriminate between a training and novel context. However, these shifts in contextual cues become less disruptive as the retention interval between training and testing increases (context specificity is lost by 14 days post-training). In other words, the fear memory is no longer precise or context-specific, but has generalized to novel contexts. Rossato et al. (2009) demonstrated that a weak context memory persisted up to 14 days in rats after a 12-hour post-training hippocampal infusion of a D1 agonist was administered, whereas control animals lost the memory after 2 days. This study provided evidence that dopamine is required for the persistence of a context memory, but it is unclear if dopamine is involved in the maintenance of the precision of the memory. The current study explores the role of dopamine in regulating contextual memory precision in long-term memory. C57BL/6 mice were given bi-lateral infusions of a D1 agonist (SKF38393, 0.50μL 25μM) into the dorsal CA1 region of the hippocampus 12 hours after context fear training. Mice infused with SKF38393 exhibited greatly reduced context generalization when tested 14 days later in a novel context compared with vehicle controls. This suggests that dopamine not only plays an important role in regulating the persistence of contextual fear memory, but also the precision of those memories.

Disclosures: C.A. Leppla: None. **S. Trask:** None. **A.M. Jasnow:** None.

Poster

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Support: NIH Grant DC009910 to RMS

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Title: Adult fear expression as a function of early life attachment and motherhood

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Abstract: In a fearful situation, individuals can respond passively (i.e. freezing, risk assessment) or actively (i.e. fleeing, exploring, fighting), with fear expression being dependent on context and prior experience. Early life abuse and motherhood have been shown to alter later life fear expression, although the mechanisms by which early life experience and motherhood shape fear expression are not fully understood. Here we examine how infant abuse and motherhood program the adult to be primed for active fear expression.
Methods: Rat pups were reared with either a normal or abusive mother (PN8-12), and were permitted to reach adulthood and give birth. Fear expression in virgin controls and mothers (with and without pups), was assessed with two tests: 1) Naturally fearful odor presentation (predator odor) and 2) Learned fearful odor presentation (after odor-0.7mA shock conditioning). For both tests, we explored active and passive fear responses and maternal behavior.
Results: Early life abused and mother rats differed significantly from control rats in both tests. In the predator odor test, mothers and early life abused rats spent more time near the predator odor and less time hiding compared to virgin controls. Mothers and early life abused rats exhibited more active fear responses in both tests. In the learned fear cue test, mothers abused in infancy displayed decreased active fear responses compared to normally reared mothers.
Conclusion: Early life abuse and motherhood change fear expression from passive (freezing, risk assessment) to active (fleeing, and approaching danger) fear. These data suggest that both early life abuse from the caregiver and becoming a caregiver may alter the response selection process for fear expression.

Disclosures: R.E. Perry: None. **R.M. Sullivan:** None.

Poster

917. Social Effects on Fear Processing

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Program#/Poster#:917.10/DDD55

Topic: F.02. Animal Cognition and Behavior

Support: FCT Grant- SFRH / BD / 51261 / 2010

Fundação Champalimaud

Fundação Bial

Title: A blast from the past: How prior experience modulates transmission of fear

Authors: *A. CRUZ, M. A. MOITA
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Abstract: In natural populations social cues are often used to avoid danger. This transmission of information can account, in part, for the success of social species, as an animal does not need to experience the threat in order to avoid it.
Previous studies showed that prior experience with shock is necessary for robust transmission of fear during a social interaction between rats.
What happens during prior experience with shock that makes an animal respond to the distress of others? Is it just the experience of an aversive event? Is learning involved? To test these possibilities, we performed experiments where receiving footshock could be dissociated from fear learning. We found that rats that receive shocks but were not allowed to express fear responses prior to the social interaction did not display vicarious fear, even when they learned to fear the environment where footshocks were delivered. Thus, experiencing footshocks or contextual fear learning per se was not sufficient to facilitate vicarious fear.
One possibility is that stress during prior experience sensitizes the amygdala, thus determining the strength of the transmission of fear during the social interaction. To test this we measured corticosterone levels from animals that experienced immediate shocks (without fear learning), and compared them to animals that received spaced shocks in a familiar environment (yields fear learning and facilitates vicarious fear). We found that shock alone is a weak stressor, which might account for the absence of response of these animals to the display of fear by their con-specifics. Future experiments will be conducted to further elucidate the role of stress in this process.

Disclosures: A. Cruz: None. **M.A. Moita:** None.

Poster

917. Social Effects on Fear Processing

Location: Hall F-J

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Program#/Poster#:917.11/DDD56

Topic: F.02. Animal Cognition and Behavior

Support: Military Operational Medicine Research Program

Title: Adolescent traumatic stress experience shapes adult fear conditioning responses in rats

Authors: *N. L. MOORE, S. GAUCHAN, R. F. GENOVESE
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Abstract: Recent studies have brought attention to the possibility that early-life traumatic stress exposure may influence the response to stress in adulthood. In order to better characterize these potential developmental alterations, a traumatic stress paradigm (underwater trauma, UWT) was used to model an intense developmental stress event during adolescence and evaluate behavioral effects lasting into adulthood. Adolescent rats (postnatal day 37) were exposed to UWT or swim without submersion. Behavioral evaluations using an elevated plus maze (EPM) and acoustic startle tests were conducted at baseline, immediately after UWT, and seven days later. Rats were then allowed to reach adulthood and fear conditioned using light and sound (conditioned stimulus) paired with a footshock (unconditioned stimulus). Testing was conducted the day before footshock, and immediately afterward. UWT during adolescence decreased open-arm time on the EPM through the seven-day testing timepoint. Testing before fear conditioning revealed similar pattern of behavior on the EPM in early adulthood regardless of adolescent treatment condition. However, following footshock, preliminary data suggest a differential change in both serum corticosterone and EPM activity across adolescent treatment conditions. These data suggest that alterations in fear conditioning response may be found in adult rats exposed to a developmental traumatic stress, relative to rats that were not stressed during the adolescent developmental period.
Supported by the Military Operational Medicine Research Program, US Army Medical Research and Materiel Command. NLTM is a National Research Council Fellow.
Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 and/or 2011 editions. All procedures were reviewed and approved by the WRAIR Institutional Animal Care and Use Committee, and performed in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Disclosures: N.L. Moore: None. **S. Gauchan:** None. **R.F. Genovese:** None.

Poster

917. Social Effects on Fear Processing

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Topic: F.02. Animal Cognition and Behavior

Support: FCT Grant: SFRH/BD/51257/2010

Fundação Champalimaud

Title: Oxytocin in the central nucleus of the amygdala mediates social buffering of fear in rats

Authors: *E. M. RICKENBACHER[1], M. MOITA[2]
[1]Radiology, [2]Behavioral Neurosci., Champalimaud Fndn., Lisboa, Portugal

Abstract: Social interactions can decrease anxiety and fear in a variety of circumstance, a phenomenon known as social buffering. Even tough oxytocin has been implicated in this process, its underlying neural mechnisms remain poorly understood. We use auditory fear conditioning, during which an animal can learn to fear a neutral tone when it is paired with aversive footshocks, to test the effect of social buffering on fear conditioned rats. We conditioned rats to fear a tone and the next day, we exposed them to the tone in the presence or absence of their cagemate. We found that rats tested in the presence of their cage-mate showed less freezing than if tested alone. In addition, when tested again, now alone, rats that were previously exposed in the presence of their cagemate still froze less than the ones exposed alone showing that social buffering has long lasting effects on fear. Finally, we are currently testing the role of oxytocin in the central nucleus of the amygdala (CeA), a major output station that controls several defense responses. Preliminary data suggests that blocking oxytocin in CeA blocks the immediate and long lasting effect of social buffering on freezing.

Disclosures: E.M. Rickenbacher: None. **M. Moita:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

Location: Hall F-J

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOB-0517575

NSF IOS-1121969

Title: Neural activity of Purkinje cells and IPN neurons covaries with theta oscillations in the cerebellum during trace eyeblink conditioning

Authors: *C. HOU, L. HOFFMAN, S. BERRY
Psychology Dept., Miami Univ., Oxford, OH

Abstract: The cerebellum is an essential brain structure for acquisition of trace eyeblink conditioning. Purkinje cells in the cerebellar cortex receive input from climbing fibers and parallel fibers, projecting to the interpositus nucleus (IPN), which facilitates the acquisition of conditioned responses (CRs). The firing patterns of Purkinje cells and neurons in the IPN reflect cerebellar information processing during training, which predicts how well-learned responses are executed. In the current study, theta oscillations in the IPN were used as the criteria for triggering training trials. For cerebellar theta-triggered rabbits (T+), a trial was initiated when the proportion of theta was high for ~1 second immediately before the trial. For cerebellar nontheta-triggered rabbits (T-), a trial was initiated when there was virtually no theta oscillation for a ~1 second pretrial period. Upon reaching behavioral asymptote, Purkinje cells from both T+ and T- groups were observed to increase their firing rates after the onset of the tone, compared to their responses at the beginning of training. Purkinje cell activity during the trace period, however, varied according to T+ or T- training. More Purkinje cells displayed excitatory activity during the trace in the T+ group, compared to the T- group. Also, cerebellar T+ or T- training was found to affect when the inhibition of Purkinje cells would occur, with T+ inhibition of cortex occurring more often in the second half of the trace period when IPN cells must fire if CRs are to occur. Many theories suggest that the cortical control of IPN is involved in precise, adaptive timing of the behavioral responses, with IPN disinhibition allowing CRs during the second half of the trace interval. Thus, increased IPN firing rates permitted by T+ training can activate significantly greater responses in the IPN late in the trace period, supporting the execution of adaptive, well-timed CRs. These results demonstrate that significant theta-related modes of cerebellar information processing accompany substantial differences in behavioral eyeblink conditioning.

Disclosures: C. Hou: None. **L. Hoffman:** None. **S. Berry:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOB-0517575

NSF IOS-1121969

Title: Theta-dependent hippocampal field potentials during delay eyeblink conditioning

Authors: M. A. SPIES, L. C. HOFFMANN, *S. D. BERRY
Psychology Dept., Miami Univ., Oxford, OH

Abstract: Previous research has demonstrated significant learning rate improvements during hippocampal theta during delay and trace eyeblink classical conditioning (EBCC). Neural correlates of these behavioral effects during trace EBCC have been reported, however these analyses have yet to be extended to delay EBCC. In the present study, two groups of rabbits received daily trials of delay EBCC in the explicit presence or absence of naturally occurring theta, while two additional groups were used as yoked behavioral controls. For rabbits trained in the presence of theta, local field potential analysis showed rhythmic time-locked post-trial theta across animals, corresponding to important behavioral learning milestones. Rhythmic oscillations were absent in the post-trial period in those animals trained without theta. Analysis of the yoked controls revealed the presence of time locked theta in response to conditioning stimuli, although the phase relationship varied across animals. These findings provide new insights into hippocampal functional and non-functional states during learning that is modulated by, rather than essentially dependent upon, hippocampal processes.

Disclosures: M.A. Spies: None. **L.C. Hoffmann:** None. **S.D. Berry:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOB-0517575

NSF IOS-1121969

Title: Comparison of identified neural response profiles in CA1 and CA3 during theta-contingent eyeblink conditioning.

Authors: *J. J. CICCHESE, S. D. BERRY
Psychology, Miami Univ., Oxford, OH

Abstract: The hippocampus has proven to be a critical structure in the acquisition of trace eyeblink classical conditioning (EBCC). Many studies on the role of the hippocampus in associative learning have focused on neurobiological oscillations, particularly the hippocampal theta rhythm. Our lab has developed a brain-computer interface (BCI) capable of initiating conditioning trials in the presence (T+) or absence (T-) of hippocampal theta. Utilizing this BCI in conjunction with single-unit recording techniques, we have previously shown that interneuron firing behavior is highly correlated to hippocampal theta state in CA1. While these patterns have been identified in CA1, it is still unclear if theta-contingent training affects single-unit response profiles in other hippocampal subfields. In the present study, custom-made drives were used to lower multiple tetrodes into CA1 and CA3 of four groups of rabbits (Oryctolagus cuniculus) to allow for simultaneous recording from both subfields. Units were sorted and each was categorized based on firing properties as pyramidal cell or one of the known interneuron types. Response profiles of identified cell types were characterized across paired and unpaired presentation of the conditioning stimuli in both T+ and T- theta conditions. The results of the present analyses provide additional insight into our current understanding of theta modulated response properties of hippocampal CA1 and CA3 cell populations engaged during hippocampal-dependent forms of EBCC.

Disclosures: J.J. Cicchese: None. **S.D. Berry:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOB-0517575

NSF IOS-1121969

Title: Oscillatory potentials reveal differential information processing in hippocampus-dependent versus independent tasks based on theta state

Authors: *L. C. HOFFMANN, M. A. SPIES, S. D. BERRY
Dept Psychology, Miami Univ., OXFORD, OH

Abstract: The hippocampus plays a modulatory role in acquisition of delay eyeblink classical conditioning (EBCC), but as task demands increase in complexity, such as in trace EBCC, it becomes essential. Our lab has developed a brain-computer interface (BCI), which allows for the comparison of two naturally-occurring extremes of hippocampal functioning (theta and non-theta). Using this technology, we have demonstrated that the absence of pretrial theta impedes learning rate during delay EBCC, while the presence of theta significantly enhances learning rate during trace EBCC. The behavioral effect of hippocampal theta triggering is twice as strong for trace as it is for delay EBCC. Here, we present an analysis of differences in the hippocampal local field potentials that accompany the two-fold learning rate differences in these two behavioral paradigms. The neural data provide evidence that theta activity represents a specific, active state of the hippocampus that becomes more beneficial when the hippocampus is essential to the task (as in trace EBCC). In contrast, the non-theta condition is more disruptive to delay EBCC than it is to trace which may suggest a different role for hippocampal theta states depending upon whether the hippocampus is essential or part of a parallel, modulatory circuit. In full, these data support the growing evidence for the influential role of theta frequency oscillations in hippocampus-involved associative learning processes.

Disclosures: L.C. Hoffmann: None. **M.A. Spies:** None. **S.D. Berry:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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NARSAD young investigator award

CIG marie Curie Fellowship

Fondation Fyssen

CNRS

Université de Strasbourg

Title: Dialogue between the lateral habenula and the hippocampo-cortical system during the sleep-wake cycle and a spatial recognition test

Authors: *R. GOUTAGNY[1], J. JACKSON[2], M. LOUREIRO[1], J. CHAUMONT[3], S. WILLIAMS[2], P. ISOPE[3], J.-C. CASSEL[1], L. LECOURTIER[1]
[1]Univ. de Strasbourg, CNRS UMR7237, Strasbourg, France; [2]Dept. of Psychiatry, Douglas institute research center-McGill Univ., Montréal, QC, Canada; [3]INCI - UPR3212-CNRS, Strasbourg, France

Abstract: Recently, the lateral habenula (LHb), wich is directly or indirectly connected with the hippocampo-cortical system, has been postulated to play a role in hippocampo-cortical dependent processing of contextual informations and elaboration of spatialmemories. To test this hypothesis, we recorded local field potentials (LFP) simultaneously within the LHb, the CA1 area of the dorsal hippocampus (dHPC) and the medial prefrontal cortex (mPFC) in freely moving male Long-Evans rats (n=5), during a continuous 24-hour recording session as well as during a spatial recognition test in an open-field. During the 24-hour recording session, we first showed that local activity within the LHb was modulated according to brain states, with prominent theta rhythm during wake (W) and REM sleep. During these states, LHb theta oscillations were significantly coherent with both dHPC (0.72±0.022 during REM vs 0.64±0.03 during W, p=0.009) and mPFC (0.57±0.01 during REM vs 0.66±0.025 during W, p=0.0871). To test if the LHb can genuinely generate theta oscillations, we performed single-cell recordings in urethane anaesthetized rats (n=4) using glass microelectrodes and found LHb cells (n=5) which fire selectively during episods of cortical theta oscillations induced by tail pinch (theta-on cells) suggesting that local LHb circuits operate at theta rhythm. To further test whether these coherent theta oscillations might have a functional role in a dHPC-mPFC dependant task, we performed LFP recordings within the three structures during a spatial recognition test designed to evaluate reactions to changes in the spatial layout of three objects within an open-field (n=5 rats). To that matter, following three consecutive sessions during which the objects remained in the same location (habituation sessions), in a subsequent session (test session), whereas one of the objects was left in place (“unmoved” object), one object was placed at the former location of the remaining one (“replace” object), the latter being moved to a location previously free of any object (“moved to a new location” object). During the test session, rats preferentially explored the “moved to a new location” object ($p<0.01$), showing that they remembered the previous layout of the context. Interestingly, this preference for the “moved to a new location” object was correlated to a specific and significant increase in theta power within the LHb ($p<0.05$). In conclusion, we have shown for the first time that the LHb is functionally connected with the hippocampo-cortical system and that it plays a role in the processing of contextual informations and the elaboration of spatial memory.

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Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

Support: EFA 188726

NIH NIDCD T32DC008553

Title: Evidence that focal hippocampal interneuron loss disrupts theta- and gamma- band activity

Authors: *C. A. ROSSI, M. J. LEHMKUHLE, F. E. DUDEK
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Abstract: Hippocampal theta (6-12 Hz) and gamma (40-100 Hz) activity are oscillatory local field potentials (LFPs) that are thought to play a critical role in the encoding and storage of new information. GABA-ergic interneurons are hypothetically involved in the generation and pacing of these oscillatory patterns of activity. The current study aimed to directly test the hypothesis that interneurons are responsible for local gamma and theta generation in the dorsal CA1 region of the hippocampus in mice.
Selective focal interneuron lesions were made by intrahippocampal injection of the targeted neurotoxin SSP-Saporin into dorsal CA1 in the hippocampus of GAD67-GFP transgenic mice. Chronic recording electrodes were also implanted in the lesion area. LFPs were monitored continuously, along with video recordings of the subjects, for a period of several weeks. LFP recordings were analyzed over 24-hour periods for the occurrence of theta- and gamma-band activity.
Analysis of LFP data revealed attenuation of both local theta and gamma activity in SSP-saporin-injected animals compared to controls.
These results suggest a direct role of GABA-ergic interneurons in the generation of local rhythmic activity in these two frequency bands, and, by extension, an important role in learning and memory processes.

Disclosures: C.A. Rossi: None. **M.J. Lehmkuhle:** None. **F.E. Dudek:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

Support: Alzheimer Association Grant NIRG-10-173332

Title: Oscillation-modifying drugs modify cognition in J20 Alzheimer mouse models and WT mice.

Authors: *A. E. BIBBIG[1], W. ZHAO[1], H. LEE[1], E. CEPEDA[1], H. MORENO[1], A. A. FENTON[1,2]
[1]Physiol. & Pharmacol., SUNY Downstate Med. Ctr., Brooklyn, NY; [2]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Gamma (30 - 80 Hz) and beta (13 - 30 Hz) frequency oscillations are found in neocortex and hippocampus and are thought to bind together different aspects of the stimuli and thus thought to be the substrate of cognition. With an interdisciplinary approach of a behavioral place avoidance task to test cognitive ability, injection of several drugs which influence gamma and beta oscillations, EEG and hippocampal local field potential (LFP) recordings, and a detailed hippocampal computer model, we showed that oscillation-modifying drugs change cognitive behavior in *in vivo* Alzheimer mouse models and WT mice just as predicted by computer simulations modeling their effects on gamma and beta oscillations: (1) J20 Alzheimer mouse models show slower learning curves than WT mice in the *in vivo* place avoidance task. (2) This is true as early as at 4 months of age, while robust Alzheimer pathologies have so far only been shown in J20 mice at 6 months of age. This seems to point away from static pathologies (like plaques) and point towards more fluid, physiological deficits (like oscillation deficits) as the origin of cognitive deficits in J20 Alzheimer mouse models. (3) *In vivo* EEG and hippocampal LFP recordings show reduced gamma and beta oscillations in J20 compared with WT mice (4) As predicted by the computer model the 5-HT_4 agonist RS67333, which depolarizes pyramidal cells and increases gamma and beta oscillation power, significantly improves learning in J20 mice. (5) 5-HT_4 agonists also influence learning in WT mice: they significantly improve cognition in bad learners, but reduce the cognitive ability of the best learners. This could be explained with the effect of 5-HT_4 agonists on gamma and beta oscillations and the assumption that cognition functions best with an optimal level of gamma and beta oscillations: According to this hypothesis the best learners should have an optimal level of gamma and beta oscillations. Further depolarizing pyramidal cells and increasing the oscillation power above this 'optimal' level will generate too synchronous oscillations which should impair information processing. (6) Contrary to its normal use to impair cognitive ability, but as predicted by its action on oscillations, the cholinergic antagonist scopolamine can *improve* learning under very special circumstances: Performance of the very best mice in the place avoidance set-up was impaired by the 5-HT_4 agonist RS67333 but is increased again by addition of scopolamine. This is consistent with the hypothesis that the 5-HT_4 agonist RS67333 and the cholinergic antagonist scopolamine influence cognition via gamma and beta oscillations.

Disclosures: A.E. Bibbig: None. **W. Zhao:** None. **H. Lee:** None. **E. Cepeda:** None. **H. Moreno:** None. **A.A. Fenton:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: TSRI Molecular & Integrative Neurosciences Departmental funds

Title: Hippocampal EEG power changes associated with novel object recognition

Authors: *S. HUITRON-RESENDIZ, A. J. ROBERTS
Dept Molec and Integ Neurosci MB-18 & Mouse Behavioral Assessment Core, The Scripps Res. Inst., LA JOLLA, CA

Abstract: Theta and gamma oscillations in the hippocampus have been related with attention and learning processes. While many studies have largely focused on the role of these EEG rhythms on working memory, only a few studies have attempted to evaluate these neuronal rhythms during a simple recognition task. Therefore, the goal of this experiment was to examine theta and gamma power in mice during the novel object recognition test. Under isoflurane anesthesia, a standard set of stainless steel screw electrodes was implanted in male C57BL/6J for chronic EEG recordings. The EEG was recorded from electrodes placed in the frontal and parietal bone over the hippocampus. One week after surgery, mice were habituated individually to their own recording chambers during 48 hours with food and water available ad libitum. After the habituation period was over, the EEG was recorded throughout novel object testing. One object (small plastic toy) was placed in one corner of the chamber, approximately 2cm from the walls. Mice were allowed to explore the object for five minutes, and then the object was removed from the chamber for one minute. The same object was moved back into the chamber in the same location, and then the mice were allowed to explore it for another 5 minutes. Then object was moved out for one minute. Four trials were done using the same object, whereas for the fifth trial a novel object was introduced into the chamber in the same position as the previous object. Mice were allowed to explore the object for 5 minutes. All behavior was video recorded and then the number of contacts each mouse made with the object was scored along trials. A contact was defined as a nose/object touching interaction or nose pointing at the object with the vibrissae moving, and within 0.5 cm of the object. The EEG was displayed on a computer monitor and stored with a resolution of 128 Hz in the hard drive of a computer for the off-line analysis of the

vigilance states and spectral analysis. In the object habituation trials, mice showed a reduction in the number of contacts along the trials, whereas during the novel object trial, the number of contacts was increased. The EEG power analysis showed a significant increase in the fast gamma power associated with detection of novelty (45.1-60 Hz), while both the slow gamma (30-45 Hz), and theta power (6-12 Hz) did not show significant differences, compared to baseline conditions. These data suggest that the fast gamma oscillations are a component of the hippocampus processes of novel object recognition.

Disclosures: S. Huitron-Resendiz: None. **A.J. Roberts:** None.

Poster

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UK Medical Research Council

Wellcome Trust

Title: Theta phase precession of grid and place cell firing in 2d.

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Abstract: Theta-phase precession (O'Keefe and Recce, 1993; Skaggs et al., 1996), a remarkably robust example of temporal coding in the brain, is found in two types of spatially tuned neurons in the hippocampal formation. It has been explored in some detail in place cells, and to a lesser degree in grid cells, with most analyses concentrating on firing on linear tracks. Here we characterize the theta-phase of firing of grid cells and place cells in rats foraging in open field environments. Firing phase relative to the theta rhythm of the local field potential is described as a function of the animal’s location within the current firing field relative to the running direction

during reasonably straight runs, extending the idea of “directional rate zones” (Burgess et al., 1993; Huxter et al., 2008). Consistent with data from linear tracks (Hafting et al., 2008), phase precession is most reliable in non-directional grid cells from superficial layers, while directional (or conjunctive) cells exhibit no or extremely weak phase precession. Place cells and grid cells with phase precession show firing phases that transition from “late” phase (and low phase variance) in the first half of the field, to an earlier phase (and high phase variance) after the animal moves through the field by a distance equivalent to the field radius. This relatively sharp transition is obscured when combining runs into “linearized” plots that ignore the lateral displacement of the run from the field centre.

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O'Keefe J, Recce ML (1993) Phase relationship between hippocampal place units and the EEG theta rhythm. Hippocampus 3:317-330.

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Disclosures: A. Jeewajee: None. **C. Barry:** None. **C. Lever:** None. **V. Douchamps:** None. **D. Manson:** None. **N. Burgess:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

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NARSAD 2009 Independent Investigator Award to DGR

Yerkes P51RR165/P51OD011132

Title: Delta phase modulation of gamma oscillations in the BLA and mPFC during fear learning and expression in freely moving rats

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Abstract: In mice, fear memory recall has recently been shown to correlate with the appearance of a low theta (4-6 Hz) oscillation in local field potentials (LFPs) that is expressed coherently between the basolateral complex of the amygdala (BLA) and the medial prefrontal cortex (mPFC, reviewed in Pape & Pare 2010). However, in rats, we have previously demonstrated that a similar increase in power and coherence between LFPs in the BLA and mPFC occurs instead in a tightly tuned delta (1-4 Hz) frequency band during fear memory acquisition and recall. Moreover, spectral analysis further revealed a strong increase in gamma (45-65 Hz) power in LFPs in both the BLA and mPFC, especially immediately after the shock during fear acquisition and to a lesser extent at the end of the cue presentation early in extinction training. In the hippocampus, the amplitude of gamma can be modulated by the phase of theta oscillations. Furthermore, a 4 Hz phase modulation of gamma amplitude has recently been reported in the prefrontal cortex of rats performing a working memory task. Hence, we hypothesized that gamma may be similarly modulated by delta in the rat BLA and mPFC during fear learning and expression.

Here, we report that the amplitude of the gamma oscillations is indeed dramatically modulated by the phase of the delta oscillations, specifically during and after the presentation of conditioned tones. The raw LFPs were first corrected for frequency-varying timing delays that occur as a result of analog filters in the headstage and pre-amplifier using Plexon's FPAlign. Custom Matlab scripts, adapted from those provided by the Kopell lab, were then used to filter the aligned LFPs into delta and gamma bands and then perform a Hilbert Transform to extract the phase and amplitude envelope, respectively. The Modulation Index (MI) and its significance were then calculated by the method described in Tort *et al* 2010. In four of the five rats tested, the amplitude envelope of the gamma (45-65 Hz) filtered LFPs was significantly modulated by the phase of delta (1-4 Hz) filtered LFPs, especially in the mPFC during fear recall. Specifically, the gamma amplitude was highest approximately 20° past the trough of the delta filtered LFP. The fifth rat showed no peak in the gamma frequency range of its power spectra.

To our knowledge, this is the first demonstration of cross-frequency coupling within the amygdala. With an eye to demonstrating a functional relevance for this phase-amplitude modulation in affective processing, ongoing analysis is focused on examining the change in MI over time, both within and across trials and phases of fear and extinction learning.

Disclosures: T.E. Madsen: None. **D.G. Rainnie:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

Support: MRC Industrial Collaborative PhD Studentship

Title: Hippocampal-prefrontal cortical neuronal assemblies in a delayed non-match to position operant working memory task

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Abstract: Rhythmic network activity supports cognitive processing in hippocampus and prefrontal cortex and is presumed to underpin behavior-dependent coordination of neuronal assemblies. However, the nature of assembly formation and coordination across multiple brain regions, learning stages and cognitive task phases remains largely undocumented.
Simultaneous tetrode recordings from dorsal CA1 and the medial prefrontal cortex (mPFC) of six adult rats learning and performing a working memory-dependent, delayed-non-match-to-position (DNMTP) operant task were used to detect groups of co-activated neurons using methods described by Lopes-dos-Santos et al. (2011). Sample, delay and response phase-selective activity included hippocampal units showing discriminative encoding of position and prefrontal units that maintained differential firing on sample specific trials throughout entire delay periods (up to 16s). Of 350 CA1 and 372 mPFC units isolated, approximately 30% and 20% respectively participated in coactive assemblies: distinct assemblies included 37 intra-CA1, 26 intra-mPFC and 26 incorporating units from both CA1 and mPFC. Individual assemblies were consistently and preferentially active during either sample, delay or choice stages of the task. Accordingly, assembly-active units showed significantly more task phase-specific firing than other task-active neurons. The number of delay-selective mPFC assemblies detected increased as delay was extended from 0-16s.
The spike-timing of units contributing to hippocampal-prefrontal cortical assemblies was highly correlated, and unit pair cross-correlations strongly modulated in the theta frequency range (approximately 5Hz). This rhythmic modulation of assembly activity was disrupted by CP55940, an agonist of cannabinoid receptors that severely reduced the number of cross-structure assemblies and impaired DNMTP performance (cf Kucewicz et al. 2011).
Our results suggest that loading, maintenance and retrieval of working memory is reflected by

coordinated assemblies of highly specialized neurons within and across cortical-limbic networks.
Kucewicz et al. 2011 'Dysfunctional Prefrontal Cortical Network Activity and Interactions following Cannabinoid Receptor Activation', J Neurosci. 31(43):15560-8
Lopes-dos-Santos et al. 2011 'Neuronal assembly detection and cell membership specification by principal component analysis', PLoS One 6(6):e20996

Disclosures: M.T. Kucewicz: None. **M.D. Tricklebank:** None. **E.S.J. Robinson:** None. **M.W. Jones:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

Support: Canadian Institutes for Health Research (CIHR)

Natural Sciences and Engineering Research Council (NSERC)

Title: An improved permutation test to detect significant spike-LFP phase-locking reveals frequency-specific synchronization patterns of single ACC/PFC neurons

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Abstract: We develop a novel permutation test to detect at which frequency-bands single neurons reliably synchronize to Local Field Potential (LFP) oscillations. In many applications, the preferred frequencies of spike-LFP locking are not a priori known, e.g. because multiple rhythms co-occur in the same area.
The structure of the statistical algorithm is as follows. A permutation distribution is constructed under the null hypothesis of no significant spike-LFP phase-locking such that statistical dependencies between spike phases (Vinck et al 2011, J Comp Neurosci) and across frequencies are preserved. We then identify clusters of significant frequencies (Rayleigh $p<0.05$), and compute for each cluster the sum of PPCs (Pairwise Phase Consistency) (Vinck et al 2010, NeuroImage), a statistic that is unbiased by spike number and controls for dependencies between spike phases within trials. Significance is then determined based on PPC cluster-mass or on the maximum PPC value. A peak-detection algorithm based on local minima and maxima of the PPC spectrum is employed to detect significant peaks within significant frequency bands. Our

procedure has two main advantages relative to standard available techniques to detect significant phase-locking. By incorporating that many dependent (across frequencies) statistical (Rayleigh) tests are performed the algorithm shows enhanced sensitivity compared to other Bonferroni or FDR multiple comparison correction techniques. In addition, it avoids the problem that the Rayleigh test is sensitive to non-Poissonian history effects (e.g., bursting) within the same trial, meaning that the underlying assumptions of the Rayleigh test are typically violated.
We use the technique to analyze population-wide synchronization patterns in an ACC/PFC dataset (Kaping et al 2011, PLoS Biol) containing >800 neurons. In the majority of cells (>70%), significant clusters of frequencies are detected. For >20% of cells, multiple clusters of significant frequencies are detected. The distribution of preferred frequencies in the ACC/PFC reveals frequency-specific synchronization patterns relative to the same and different electrodes within the ACC/PFC.

Disclosures: T. Womelsdorf: None. **S. Ardid:** None. **S. Everling:** None. **M. Vinck:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Program#/Poster#:918.13/DDD70

Topic: F.02. Animal Cognition and Behavior

Support: NIH-NS20686

Title: GABA blockade in the medial septum disrupts hippocampal theta and leaves place cell coding intact

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Abstract: As an animal explores its environment, individual 'place cells' in the hippocampus demonstrate location-specific firing, representing a neural substrate for the computations involved in spatial navigation. Also during active exploration, the hippocampal local field potential is dominated by a 5-12 Hz oscillation known as theta rhythm. Place cell firing patterns are exquisitely organized in relation to the field theta oscillation, raising the possibility that this temporally-structured activity contributes to spatial processing. Hippocampal theta can be disrupted by lesioning or inactivating the medial septal area in the basal forebrain, and these manipulations affect place cell firing characteristics. However, the medial septum serves a dual role in modulating hippocampal activity as the rhythmic pacemaker for theta oscillations and the

primary source of cholinergic input to the hippocampus. To assess the relative importance of these functions for location-specific coding, hippocampal local field potentials and place cell activity were monitored after intraseptal injection of tetracaine (local anesthetic), muscimol (GABA-A agonist), or gabazine (GABA-A antagonist). Septal inactivation by injection of either tetracaine or muscimol reduced theta power in the local field potential and place cell activity in the CA1 and CA3 regions of the hippocampus. Injection of gabazine robustly disrupted hippocampal theta oscillations while increasing power in the delta and fast gamma (50-90 Hz) ranges. These results indicate that GABAergic transmission in the medial septum is necessary for theta rhythmic activity and that septal output influences a broad range of oscillatory activity in the hippocampus. During extended periods of minimal theta power in the hippocampus, place cell activity was not substantially altered. This observation limits the role for theta oscillations in the expression of location-specific coding and emphasizes the influence of cholinergic modulation on spatial processing in the hippocampus.

Disclosures: K.A. Bolding: None. **S.E. Fox:** None. **R.U. Muller:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

Support: FP7-ICT/2007-2011 Grant 217148

Title: Hippocampal theta reflects on anticipation of food localization and consumption

Authors: *E. WASSERMAN, T. PELC, M. MINTZ
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Abstract: The dorsal hippocampus has a key role in construction of cognitive spatial maps, as evidenced by spatially-related activity of its place-cells. Analysis of the related place-fields demonstrates skewed distribution with high resolution around zones with emotive significance. This 'remapping' may be induced by emotive appraisal toward objects in the space, for example by the amygdala, or by emotive appraisal of the spatial context, for example by the ventral hippocampus. However, the mechanism of spatial remapping by emotion is presently not known. We argue that the mediating mechanism is the hippocampal theta rhythm that paces the place cell firing, and which disruption compromises place cell functionality and disorganizes behavioral navigation. Locomotion and theta rhythm activity in the dorsal hippocampus was monitored in rats foraging for food in a maze consisting of home-base and target chambers

connected by an alley. Rats experienced four consecutive conditions: no food in the maze; small food pellet found and consumed in the target chamber; large food pellet found in the target chamber and carried to the home-base chamber where it was consumed, i.e., hoarding; and finally, no food in the maze. HHT analysis demonstrated an increase in theta frequency along the trajectory toward the small and large food pellet in the target chamber and along the trajectory back toward the home-base chamber where the large food pellet was consumed. In contrast, theta frequency decreased along the trajectory to the target chamber when food was omitted. These findings suggest that theta rhythm projects on the dorsal hippocampus the emotive appraisal of the spatial trajectories. Currently we aim to test the impact of the observed changes in theta frequency on the representation of the trajectories by the hippocampal place-cells.

Disclosures: E. Wasserman: Research Grant; FP7-ICT/2007-2011, grant agreement no. 217148. **T. Pelc:** None. **M. Mintz:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

Support: Engineering and Physical Sciences Research Council

Biotechnology and Biological Sciences Research Council

Title: Multiplexing of theta-nested gamma oscillations and grid firing fields in an attractor network model of layer II of the medial entorhinal cortex

Authors: *L. SOLANKA, H. PASTOLL, M. C. W. VAN ROSSUM, M. F. NOLAN
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Abstract: Grid cells in the medial entorhinal cortex (MEC) encode location through firing fields that form grid-like maps of the environment. At the same time network activity in the MEC is dominated by oscillations in the theta (4-12 Hz) and gamma (30-100 Hz) bands. The relationship between oscillatory activity and grid firing is not known. Our recent experimental data established that feedback inhibition between excitatory stellate cells and inhibitory fast spiking interneurons dominates the synaptic connectivity in layer II of the MEC. To determine if this circuit is sufficient to explain both the network oscillations and grid firing fields, we constructed a network of model stellate cells and interneurons. In this model, stellate cells connect exclusively to interneurons, while interneurons contact only stellate cells. We show that external

excitatory conductances can drive the network into an attractor state. Feedback inhibition onto the model stellate cells has a synchronizing effect. Synaptic input to both populations of neurons is synchronized in the gamma frequency range (30 - 100 Hz). When coupled with a theta modulated external drive (8 Hz), we observed network synchronization during the trough of the theta signal only. When the borders of the network are connected with a twisted torus topology (Guanella et al. 2007) and velocity modulated inputs are applied to the circuit, excitatory neurons in the circuit generate grid-like firing fields. Due to limited stability of the attractor, the grid fields were blurred. However, it was possible to obtain stable grid fields by including a place cell resetting mechanism as an additional input to the network (Guanella et al. 2007). These results demonstrated that the same local circuit architecture - feedback inhibition - supports emergence of attractor states and nested gamma oscillations. This has implications for mechanisms of both formation of grid-like receptive fields as well as population coding and information transmission between brain areas.

Guanella, A., Kiper, D., & Verschure, P. (2007). A model of grid cells based on a twisted torus topology. International journal of neural systems, 17(4), 231-40.

Disclosures: L. Solanka: None. **H. Pastoll:** None. **M.C.W. van Rossum:** None. **M.F. Nolan:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

Support: NSF 0919783

Title: Theta dynamics: The relationship between theta frequency and locomotor speed in familiar and novel environments

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Abstract: Changes in theta amplitude and frequency have been linked to the execution of ongoing motor programs, as well as cognitive processes such as attention, encoding and retrieval. Theta in the rat hippocampus exhibits significant variation across the laminarly arranged somatodendritic field of hippocampal neurons, as well as across the septotemporal (long) axis. This variation can be correlated to ongoing changes in a rat’s behavior, environment and

cognitive performance. We have previously demonstrated that the relationship between locomotor speed and theta amplitude systematically decreases across the long axis. In contrast, the relationship between speed and theta frequency is invariant across the long the axis. Previous demonstrations of the relationship between speed and theta frequency have been limited to a single recording condition, yet there are numerous demonstrations of theta frequency changing across conditions (e.g. novelty).
We asked whether the relationship between speed and theta frequency changes across multiple sessions in a familiar and a novel environment. Electrodes were implanted at multiple septotemporal levels of CA1 and local field potential theta signals were recorded as rats (N = 10) ran laps around a rectangular linear track. Three recording sessions (10 minutes in length) were conducted each day for multiple days in a familiar environment and then multiple days in a novel environment. Despite performing a well-learned task in a familiar room, theta frequency systematically increased within sessions, as well as across sessions within a single day at sites across the long axis. Upon exposure to the novel environment theta frequency decreased compared to the familiar environment, although this decrease was relatively short lived. By the second session in the novel room theta frequency had returned to levels similar to the familiar room. A multiple regression analysis was conducted that included time within a session and speed as predictors of theta frequency and it was observed that the relationship between speed and theta frequency positively covaried in both the novel and familiar environments. Yet in both environments higher intercepts were associated with lower slopes, thus indicating that the relationship between locomotor speed and theta frequency is not constant. This may be a fundamental feature of the relationship between speed and theta frequency.

Disclosures: J.R. Hinman: None. **L.L. Long:** None. **M.A. Escabi:** None. **J.J. Chrobak:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

Support: NSF 0919783

Title: Theta dynamics: Speed, velocity, acceleration and contribution to cognition

Authors: *L. L. LONG[1], J. R. HINMAN[1], C.-M. CHEN[1], M. A. ESCABI[2,3], J. J. CHROBAK[1]
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Abstract: The neural substrate of memory formation resides within the function connectivity of ensembles of neurons and their dynamical interactions. The laminar and afferent organization of the hippocampus allows for the observance of synchronizing rhythms such as theta (6-12 Hz). Theta is observed in the extracellular local field potential (LFP) where these potentials reflect the summation of synaptic potentials that determine neuronal discharge and orchestrate ensemble interactions. Endless evidence now demonstrates that variation in the theta signal is predictive of memory encoding and subsequent cognitive performance during retrieval or recognition tasks. Previously, we have reported a dominant relationship between speed and theta power at septal hippocampal sites and that this relationship diminishes systematically with distance from the septal pole of the hippocampus in both CA1 and DG. The current data focus on the relationship between acceleration and velocity (directionality) and how these indices contribute to variability in the theta signal. Recordings were obtained while rats shuttled between both ends of a linear track for a palatable food reward. As previously shown, theta amplitude was positively modulated by speed at septal CA1 sites with speed accounting for 40-80% of the variability. In addition to speed, positive acceleration significantly accounted for 5-15% of theta variability, although no significant relationship was observed for deceleration (negative acceleration). Velocity (directionality) did not account for any significant modulation of theta amplitude at CA1 sites. Currently we are assessing the relationship between acceleration and velocity at DG sites as well as variability at distinct laminar positions within CA1 (eg, stratum oriens, radiatum and stratum lacunosum moleculare). These findings demonstrate that locomotor speed and positive acceleration are main predictors of theta amplitude in septal hippocampus. An open question remains on whether speed and acceleration are constant contributors to variation in the theta signal or whether behavioral/cognitive context alter the relationship between speed/acceleration and theta amplitude. Importantly, a better understanding of the relative contribution of these highly quantifiable variables and their potential dynamic variation should facilitate analyses of how variation in theta relates to cognitive/memory processes.

Disclosures: L.L. Long: None. **J.R. Hinman:** None. **C. Chen:** None. **M.A. Escabi:** None. **J.J. Chrobak:** None.

Poster

918. Learning and Memory: Gamma and Theta Activity

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Topic: F.02. Animal Cognition and Behavior

Support: NSF 0919783

Title: Theta dynamics: Septotemporal differences in response to habituation, spatial novelty and the absence of expected reward

Authors: *J. CHROBAK[1], L. L. LONG[1], M. A. ESCABI[2,3], J. R. HINMAN[1]
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Abstract: Novel and unexpected experiences engage neural circuits including the hippocampus. The once-thought regular drumbeat of the hippocampal theta signal has experienced a resurgence of research interest with evidence demonstrating its dynamic qualities and its predictive relation to memory processing in humans. The theta signal exhibits variation within the laminarly arranged somatodendritic field of hippocampal neurons, as well as across the septotemporal axis. Local theta signals correlate with changes in a rat's behavior, environment, experience and memory performance. The present study examined theta local field potential signals at sites across the septotemporal axis of the CA1 region while rats (N = 10) ran unidirectional laps around a rectangular linear track over repeated exposures to both a familiar room and a novel room, as well as to the novel experience of the absence of expected reward. To ensure that any changes in theta power did not result from changes in running speed (Hinman et al 2011), we conducted a multiple regression analysis to take the speed of the animal into consideration.
In agreement with previous findings (Hinman et al., 2011), theta power habituated at temporal CA1 sites, but not at septal sites, across multiple sessions within a single day in a familiar room. Exposure to the novel room resulted in a significant increases in theta power at both septal and temporal CA1 sites compared to the familiar room. Theta power habituated in both septal and temporal CA1 across sessions on the first day in the novel room, although habituation was greater in temporal CA1 as compared to septal CA1. Theta power in the novel room stabilized to levels observed in the familiar room within three days. Exposure to the novel experience of the absence of an expected reward resulted in a substantially greater increase in theta power at temporal as compared to septal CA1 sites. The findings demonstrate dynamic septotemporal variation in the theta signal: they highlight conditions that may engage the temporal hippocampus and provide clues into functional differentiation across the septotemporal axis of the hippocampus.

Disclosures: J. Chrobak: None. **L.L. Long:** None. **M.A. Escabi:** None. **J.R. Hinman:** None.

Poster

919. Learning and Memory: Systems and Aging

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Topic: F.02. Animal Cognition and Behavior

Support: NIA Grant RO1AG28488

NIA Grant T32AG000114

Title: The role of L-type voltage-gated calcium channels in age-related cognitive decline

Authors: *S. J. MOORE[1], J. N. SLATER[1], G. G. MURPHY[1,2]
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Abstract: Although cognitive impairments in the aged population are generally associated with neurodegenerative disorders like Alzheimer's disease, they more often occur in the absence of any overt neurological pathology. However, the neurobiological mechanisms underlying this age-related cognitive decline remain relatively poorly understood. In rodent models, aging is associated with an up-regulation of L-type voltage-gated calcium channels (L-VGCCs) and this increase has been correlated with poorer performance in a hippocampus-dependent learning and memory task. We have used a novel transgenic mouse line in which the αCamKII promotor drives over-expression of the CaV1.3 subtype of L-VGCCs in young animals (CaV1.3 OE) to directly examine the relative contribution of this increased expression to cognitive deficits. While CaV1.3 OE mice exhibit normal sensory, motor, and anxiety-like behaviors, they show impairments in performance on several learning and memory tasks, including the Morris water maze and discrimination in contextual fear conditioning. Taken together, our results suggest that the age-dependent up-regulation of CaV1.3 contributes to cognitive deficits in learning and memory. Because this increase precedes many other age-related changes, CaV1.3 may represent a novel therapeutic target for the amelioration of age-related cognitive decline.

Disclosures: S.J. Moore: None. **J.N. Slater:** None. **G.G. Murphy:** None.

Poster

919. Learning and Memory: Systems and Aging

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Topic: F.02. Animal Cognition and Behavior

Support: NIH-NINDS R01 #NS059873

Title: The PKCβ selective inhibitor, Enzastaurin, impairs learning and memory without changes to hippocampal transcription

Authors: *M. TURK[1,2,3], S. E. MENNENGA[1], A. SINIARD[2,3], J. J. CORNEVEAUX[2,3], L. HEWITT[1], C. TSANG[1], J. CASELLI[1], B. B. BRADEN[1], H. A. BIMONTE-NELSON[1,3], M. J. HUENTELMAN[2,3]
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Abstract: Enzastaurin (ENZ) is a Protein Kinase C-β (PKCβ) selective inhibitor currently in Phase I/II trials as a treatment for various types of difficult to treat chemo-resistant tumors. PKCβ is an important enzyme in modulating a variety of neuronal functions. In particular, previous rodent studies have reported a deficit in spatial and fear-conditioned learning and memory with decreased endogenous concentration of PKCβ. Due to ENZ's mechanism of action, the present study investigated the consequences of ENZ on learning and memory in 12-month-old Fischer-344 male rats. Rats were treated daily with either vehicle (polyethylene glycol, n=10) or subcutaneous injections of ENZ (25mg/kg; 0.4mL, n=8), and tested using a Morris Water Maze (MWM) 30 minutes post-injection. The MWM is a spatial memory task that is sensitive to age-related memory changes, and depends on hippocampal integrity. Swim distance to the platform was calculated using tracking software (Ethovision XT 5.1, Noldus). Each rat underwent six trials a day for three days. A probe trial was performed on the final day as a seventh trial. The group treated with ENZ had higher swim distance on days two and three ($p<0.05$), indicating impaired spatial reference memory, and also showed decreased overnight retention ($p<0.05$).. However, there were no differences during the probe trial, in which the platform was removed from the maze, indicating localization to the target maze quadrant. Immediately after the final day of testing, rats were anesthetized with inhalent isofluorane and brains were rapidly dissected and frozen. RNA from the CA1/CA2 region of the left hippocampus was extracted, isolated, and purified for mRNA next generation sequencing (Illumina) to examine the potential transcriptional differences between the two groups. Data were analyzed using Cufflinks to quantitate gene expression in Fragments Per Kilobase of transcript per Million mapped reads (FPKM). No significant transcriptional changes were found at the gene expression level (using a Kruskal Wallis test) indicating that the consequences of ENZ are exerted primarily through a direct effect on the target kinase, and not at the transcriptional level. These results suggest that PKCβ inhibition impairs spatial memory performance in middle-aged animals, and support the incorporation of cognitive testing in future human clinical trials that utilize this drug to determine if impairments are noted concordant with ENZ administration in humans as well.

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Poster

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TGen Foundation

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Tulane COBRE in Hypertension and Renal Biology (NCRR 2P20RR017659)

State of Arizona and Arizona DHS

Title: Regional brain network of MRI gray matter with gradual induction of hypertension in the Cyp1a1-Ren2 transgenic rat

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Abstract: It is well established that hypertension (HTN) in humans can lead to regional brain atrophy and cognitive decline. With a high prevalence in the community-dwelling elderly population, HTN may be an important factor influencing the development and progression of cognitive aging. We sought to investigate the effects of HTN on gray matter volume from magnetic resonance imaging (MRI) scans of the brain obtained in a rodent model of HTN. Since the onset and progression of HTN in humans often occurs gradually after middle-age, we used transgenic rats that allow for the gradual induction of HTN in middle-aged animals (Mitchell et al., 2006). The inbred male rats, with a Fischer 344 background, have the cytochrome P450 promoter (Cyp1a1) inserted to up-regulate the expression of the mouse renin (Ren2) gene. Administration of 0.15% indole-3-carbinol (I3C) activates the Cyp1a1 promoter to induce a gradual onset of HTN. We administered an I3C augmented diet at 16 months of age over a 6-week interval to produce a HTN group of Cyp1a1-Ren2 rats (N = 5), whereas the control group

of transgenic rats (N = 5) received normal food pellets. Volumetric T2-weighted MRI scans were acquired at 7.0 T with 150-micron isotropic voxel resolution for both the HTN and control groups. Multivariate network analysis with voxel-based morphometry (VBM) and the Scaled Subprofile Model (SSM; Alexander and Moeller, 1994) was used to identify a regional network pattern of MRI gray matter that differed between the groups. The HTN group had higher mean systolic (p < 0.009) and diastolic (p < 0.016) blood pressures during the 6-week interval than controls. Additionally, when analyzed as a proportion of body weight, heart and kidney weights were increased for the HTN group compared to controls (p < 0.009). SSM analysis of MRI VBM with bootstrap re-sampling of the HTN and control groups combined identified two regional network patterns of gray matter that distinguished the hypertensive rats from controls (p < 0.009). This linearly combined pattern was characterized by gray matter reductions in the vicinity of the thalamus, basal ganglia, cerebellum, and a region of the hippocampus with relative increases observed in selective frontal and temporal areas. Together, these findings provide preliminary support for the use of the Cyp1a1-Ren2 rat to help advance translational research on the cerebrovascular effects of HTN during aging. The use of such transgenic rodent models combined with high resolution MRI and network analyses may aid efforts in the evaluation of new treatments and prevention therapies for the brain changes associated with healthy and pathological aging.

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Poster

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Support: University of Arizona Start-up Funds

McKnight Brain Research Foundation

Title: Changing characteristics of neural stem cells across the lifespan during aging

Authors: *K. SMITH, C. A. BARNES, M. J. CORENBLUM, L. MADHAVAN
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Abstract: In the context of understanding plasticity in the brain during aging, we have begun to characterize the properties of neural stem cells (NSCs) obtained from the subventricular zones of Fisher 344 rats in vitro over an aging continuum of 2 (adolescent), 9 (mature), 15 (middle-aged) and 24 (old) months. Characterization in this manner provides a comprehensive view of the population as it changes throughout a lifetime rather than comparing only the young and old endpoints. To begin with, significantly greater numbers of NSCs were isolated from the more younger (2, 9 months) than the more older (15, 24 months) animals. The isolated adolescent, mature, middle-aged and old NSCs differed in size and morphology although being immunophenotypically similar with no difference in the expression of NSC markers, Nestin or Sox2. The proliferative capacity of the NSCs was next determined by quantifying sphere formation using a serial dilution assay as well as incorporation of the thymidine analog bromodeoxyuridine (BrdU). The dilution assay indicated that adolescent and mature younger cells formed significantly greater number of neurospheres than the middle aged and old cells at every dilution level. Concurrent with this, a significant decline in the fraction of BrdU labeled cells was observed with increasing age. Interestingly, in both the dilution and BrdU assays, NSCs isolated from the middle-aged (15 months) animals showed greater impairment in proliferative ability compared to the cells from old (24 months) animals. In support of the proliferation data, survival of NSCs measured using a live/dead cell assay did not show any significant differences between adolescent, mature and old age groups, but the middle-aged cells displayed a considerably greater fraction of dead cells. Further, adolescent, mature, and old cells were found to be capable of being propagated in culture for a greater number (>15) of passages than middle-aged cells (<10). NSC differentiation was also examined. While the number of RIP+ oligodendrocytes remained fairly constant, TUJ1+ neurons declined, and S100B+ astrocytes increased with age. Overall, the results indicate (1) increased senescence, (2) reduced survival, proliferation, and neuronal differentiation of NSCs as aging progresses, and (3) suggests a particular vulnerability of cells during middle-age which needs to be analyzed further. These ongoing studies will help establish baseline changes in characteristics of NSCs throughout the natural lifespan, and provide a framework for further investigations of underlying factors, as well NSC potential to modify the aged brain.

Disclosures: K. Smith: None. **C.A. Barnes:** None. **M.J. Corenblum:** None. **L. Madhavan:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

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Title: Aging does not affect the proportion of dorsal medial entorhinal cortex cells active during track running behavior.

Authors: *J. LIANG[1,2], J. P. LISTER[1,2], C. A. BARNES[1,2]
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Abstract: Previous work has shown that hippocampal place cells in aged rats show changes in their population dynamics: in CA1 there are errors in retrieving the correct map for a known location (e.g., Barnes et al., 1997), and in CA3 they exhibit difficulty in establishing a new map for a novel location (Wilson et al., 2005). The entorhinal cortex provides input to the hippocampus and could contribute to these observed age-related changes in the population code for space. The medial entorhinal cortex (MEC) contains "grid cells" that fire in a highly spatially regular fashion (e.g., Hafting et al., 2005). These cells fire in repeating patterns across an entire environment and are believed to contribute significantly to the spatial signal within downstream hippocampal regions. The current experiment investigated whether aging affects population activity in MEC during track running, by quantifying expression of the immediate early gene *Arc* using the "catFISH" method (Guzowski et al., 1999). The rigid kinetics of *Arc* localization to distinct cellular compartments allows this gene to mark neuronal activity during discrete behavioral epochs. Young (9 months) and old (24 months) Fischer 344 male rats (N = 24 at each age) were divided into three groups: caged control (CC), maximal electroconvulsive shock control (MECS), and track running behavior. Rats learned to run alternating clockwise and counterclockwise laps on a circular track (48 inch diameter) for food reward. For the experiment, CC rats were sacrificed immediately from home cages, and the MECS group was sacrificed following a shock that activates Arc expression in all neurons capable of expressing the gene. Rats in the running group performed two 5 min sessions of track running, separated by a 20 min rest period in their home cage. Immediately after the second session, animals were sacrificed. After sacrifice, brains were flash-frozen to preserve mRNA, sectioned on a cryostat, stained for *Arc* expression with fluorescence *in situ* hybridization, and the dorsal MEC was examined in each rat (3 sections per brain). Neurons were quantified as nuclear positive (Nuc+), cytoplasmic positive (Cyto+), or double-labeled (Dbl+). Repeated measures ANOVA revealed a significant ($p<0.01$) effect of the *Arc*-compartment factor - there was a significantly higher proportion of Dbl+ neurons than Nuc+ or Cyto+ in both age groups. The age factor was not significant,

indicating that the ability of MEC to reactivate the same populations during the two sessions of track running in the same environment is not affected by age. This suggests that at least the cellular composition of the path integration input to the hippocampus is preserved during aging.

Disclosures: J. Liang: None. **J.P. Lister:** None. **C.A. Barnes:** None.

Poster

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Support: McKnight Brain Research Foundation

NIH Grant AG033460

Title: Effect of aging on the activity pattern of Arc expression in the deep layers of lateral entorhinal cortex.

Authors: *J. P. LISTER[1,2], S. J. CLASEN[1,2], A. L. HARTZELL[1,2], S. N. BURKE[1,2], C. A. BARNES[1]
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Abstract: Age-related changes in hippocampal function have been studied extensively, but less is known about the effect of aging on input from entorhinal cortex. Neurons of lateral entorhinal cortex (LEC) lack spatial specificity in their firing properties (e.g., Hargreaves at al., 2005) and are thought to provide the hippocampus with information about the contents of the environment. Data from earlier experiments indicate that neither age nor changing environmental features altered the proportion of cells with *Arc* activation in superficial layers of LEC (Lister et al., SfN 2011). These data were not consistent with the suggestion that object identity information was carried by LEC cells in this layer, leaving open the possibility that perhaps cells in the deep layers may do so. Here we report results from deep layers of LEC in 24 young and 24 old F344 rats. There were four groups: 1) caged control, 2) positive control with electroconvulsive shock, inducing maximal expression of the immediate early gene *Arc*, 3) an A/A behavioral group that explored for two 5 min sessions in a box populated with the same set of 5 objects during both sessions, and 4) an A/B group, identical to A/A with entirely dissimilar objects during the second session compared to the first. Behavior rats had a 20 minute rest period between sessions and were sacrificed immediately after the second session. This design tests the hypothesis that the

identity of objects will be critical to individual neuron activity and that this function will be affected by aging. The kinetics of *Arc* mRNA within discrete intracellular compartments (nucleus or cytoplasm) allows each *Arc*+ neuron to be identified as active within a specific temporal window, including the ability to identify cells active in both sessions. 3 sections per brain from posterior LEC were stained for *Arc* expression via fluorescence *in situ* hybridization and imaged using confocal microscopy. *Arc*+ neurons were classified as foci-positive (Foci+), cytoplasmic-positive (Cyto+), and double-labeled (Dbl+) using the ImageJ software package. Repeated measures ANOVA showed that there were no significant differences in age or object change treatment (AB) factors. About a third of the cells counted in this layer were active in response to the behavioral treatment overall, and half of these were active in both epochs of behavior, regardless of object identity. The proportion of neurons active in both sessions (Dbl+) was significantly greater than either Foci+ or Cyto+ positive neurons alone. Thus, while half of the responsive cells do show stable activity across behavioral treatment sessions, these cells do not appear to carry information about distinct object identities.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH Grant AG003376

Title: The effects of age and environmental change on Arc transcription in perirhinal cortical ensembles following object exploration

Authors: *S. N. BURKE[1,2], A. L. HARTZELL[1,2], J. P. LISTER[1,2], L. T. HOANG[1,2], C. A. BARNES[1,2]
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Abstract: Object recognition memory requires the perirhinal cortex (PRC) and this cognitive function declines during normal aging. Specifically, old animals are able to retain the memory of a familiar object but they are more likely to falsely recognize a novel stimulus (Burke et al.,

2010). This 'false memory' for novel stimuli observed in aged rats also occurs when the environmental context is changed between the familiarization and test phase of the spontaneous object recognition task. Recent electrophysiological recordings from awake-behaving rats have shown that neurons in the PRC of young rats are activated by 3-dimensional objects (Burke et al., 2012). Thus, it is possible that age-related object recognition deficits could be due to alterations in how PRC neuron activity is modulated by objects in older animals. The present study used cellular compartment analysis of temporal activity by fluorescence *in situ* hybridization (catFISH) with confocal microscopy to monitor subcellular distributions of activity-induced *Arc* RNA in the PRC. Activity was monitored during two distinct epochs of object exploration. In one group of rats (6 young and 6 aged) an animal was placed in a familiar testing arena and was allowed to explore five different 3-dimensional objects for two 5-min sessions separated by a 20-min rest (AA condition). The second group of animals (6 young and 6 aged) explored the same five 3-dimensional objects for two 5-min sessions but the environment was changed between the first and the second epoch of exploration (AB). Behavioral data showed that both young and aged rats spent less time exploring objects during the second epoch of behavior in both conditions, indicating successful object recognition. Interestingly, the reduction in object exploration during the second epoch relative to the first was not associated with reduction of cells that expressed *Arc* between epochs. This indicates that similar populations of neurons are activated by exploring the same objects during two distinct epochs, even when the objects are familiar and the environment changes. When numbers of *Arc* positive neurons were compared between age groups, the old rats had significantly lower proportions of *Arc*-positive PRC neurons for both the AA and AB behavioral conditions. The age-related reduction in the population of PRC neurons that express *Arc* during object exploration could reflect reduced glutamate levels in aged compared to young rats that have been reported for this brain region (Rushaidhi et al., 2012). Together these data support the hypothesis that age-associated functional alterations in the PRC contribute to declines in stimulus recognition over the lifespan.

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Poster

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BIO5

HHMI 52006942

Title: The influence of advanced age on noradrenergic-dependent novelty detection

Authors: *J. FRIEL[1,2], J. J. FERNG[1,2], A. L. HARTZELL[1,2], S. N. BURKE[1,2], C. A. BARNES[1,2]
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Abstract: Previous research has shown that age-associated object recognition deficits are not due to aged rats 'forgetting' a previously experienced stimulus, but because old animals are more likely to identify novel objects as familiar (Burke et al., 2010). The extent that this impairment can be explained by object discrimination problems, or a problem in novelty detection has not been examined. Previous studies have suggested that a functionally intact noradrenergic system is necessary to facilitate novelty detection (Sara et al., 1995). The main source of noradrenaline in the brain, the locus coeruleus (LC), shows several age-associated changes including, altered activity levels (Olphe & Steinmann, 1982; Shirokawa et al., 2000), differences in gene expression (Zhu et al., 2005), and changes in its metabolic pathways (Shirokawa et al., 2003; Matsunaga et la., 2004). The current experiment tested the hypothesis that these alterations in the LC may partially account for the inability of aged rats to correctly respond to novel objects using a task that is known to require noradrenergic activity (Sara et al., 1995). Young (9 months) and old (24 months) rats were placed in a hole board apparatus for a habituation and novel-object detection phase separated by a 3-hour period. The 9 holes of the apparatus were left empty for the habituation phase. In contrast, during the novelty-detection phase the holes were filled with 4 identical objects. Novelty detection was quantified by comparing the number and duration of nose pokes in holes with objects relative to the holes without objects. Because rats were not required to discriminate between novel and familiar objects, this task allows one to measure the ability of animals to detect novelty in the absence of stimulus discrimination requirements. Contrary to the idea that age-related changes in the LC contribute to impairments in object recognition, there was no significant difference in the performance of young and old animals on this task. These data suggest that the decline in the ability of aged rats to appropriately identify novel objects is due to a reduced capacity to discriminate between novel and familiar stimuli, which critically relies on the perirhinal cortex, rather than to an overall decline in LC-dependent novelty detection.

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Poster

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Support: McKnight Brain Research Foundation

NIA Grant AG036053

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Title: The effect of peripheral E. coli infection on behaviorally-induced Arc expression patterns in hippocampal granule cells.

Authors: *A. L. HARTZELL[1,2], R. M. BARRIENTOS[3], L. T. HOANG[1,2], J. P. LISTER[1,2], S. F. MAIER[3], C. A. BARNES[1,2]
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Abstract: Aged animals exhibit a sensitized neuroinflammatory response to a peripheral increase in pro-inflammatory agents relative to adult animals (Abraham et al., 2008; Barrientos et al., 2006, 2009; Chen et al., 2008; Godbout et al., 2005). This increased neuroinflammatory response to peripheral infection occurs in conjunction with hippocampus-dependent learning and memory impairments (Barrientos et al., 2006, 2009). However, the mechanism by which this neuroinflammatory response undermines memory processes in older animals is not understood. The immediate-early gene *Arc* is known to play a critical role in the regulation of synaptic plasticity and long-term memory in the hippocampus (i.e., Guzowski et al., 2000; Plath et al., 2006; Miyashita et al., 2008). Previously, it has been shown that a peripheral *Escherichia coli* infection suppresses basal *Arc* gene expression in the whole hippocampus as measured by RT-PCR, as well as *Arc* gene expression induced by contextual fear conditioning, in the hippocampus of aged rats (Frank et al., 2010). We investigated the effect of peripheral *E. coli* infection on *Arc* expression patterns in individual cell regions within the dorsal hippocampus after contextual fear conditioning in adult and aged rats, using compartmental analysis of temporal activity by fluorescence *in situ* hybridization (catFISH) (Guzowski et al., 1999). This technique permits inferences about the subcellular distribution of *Arc* mRNA based on the activity history of cells. Populations of adult and aged rats were injected with *E. coli* four days before contextual fear conditioning. *Arc* expression patterns revealed that a smaller proportion of granule cells within the dentate gyrus of aged rats express *Arc*, possibly due to changes in methylation status of the Arc gene during aging as suggested by previous studies (Small et al.,

2004; Penner et al., 2011). Additionally, peripheral *E. coli* infection had no effect on the proportion of cells expressing *Arc* in the dentate gyrus in response to contextual fear conditioning in aged or adult animals. This result in conjunction with the finding that peripheral *E. coli* infection suppresses *Arc* gene expression (Frank et al., 2010) suggests that it may reduce the amount of *Arc* mRNA transcribed on an individual cell basis but does not effect the total number of cells transcribing *Arc* in response to contextual fear conditioning in the dentate gyrus. The CA regions are currently under investigation.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH Grant AG012609

Title: Diminished place field density and direction-dependent learning in the aged rat

Authors: *L. A. SCHIMANSKI[1,2], P. LIPA[1,2], C. A. BARNES[1,2]
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Abstract: Remembering the locations of specific stimuli within an environment becomes more difficult during senescence. The changes in neural information processing underlying this age deficit are not yet fully understood. In this study, the simultaneous activity of ensembles of hippocampal CA1 principal neurons (‘place cells’) was recorded from 6 young adult (9-12 month) and 6 aged (26-28 month) male F344 rats, while they learned the locations of eye-blink stimuli on a circular track. Rats were trained to run alternating clockwise and counterclockwise laps on this circular track with a barrier at 12 o’clock, where they received food reward. Using electrical stimuli applied to the eyelid, the animals were conditioned to blink at two distinct locations on the track: one eye-blink stimulus location was conditioned in only the clockwise running direction while the other one was only administered in the counterclockwise direction. As reported by Schimanski et al. (2011), young and old rats exhibited equivalent accuracy of the blink response at both conditioned locations, but old rats were less successful in restricting the

blink response to the conditioned running *direction*. Here we examine place field firing properties of young and old pyramidal cells under these conditions in more detail. When the number of place fields identified per CA1 pyramidal cell was measured in both running directions, the aged rats exhibited fewer place fields per cell than did young rats. These data suggest a subtle difference between age groups in how the CA1 'map,' or population code represents the same environmental experience. Moreover, when we applied a random sampling algorithm to the place field data (Monte Carlo method), the outcome showed that the number of bidirectional fields observed in young and old rats was close to what one would expect from 'accidental' overlap of firing in opposite direction trajectories, given the situation that each unidirectional field was randomly positioned to occur in either the clockwise or counterclockwise direction. Taken together, these findings imply that because the number of CA1 place fields per cell is reduced during aging, the number of bidirectionally-active place fields also must decline accordingly. These age-dependent alterations of the population code may contribute to the reduced ability of aged rats to discriminate one running direction from another in learning this location- and direction-dependent version of eye-blink conditioning.

Disclosures: L.A. Schimanski: None. **P. Lipa:** None. **C.A. Barnes:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH Grant AG012609

Title: Spiking of theta-firing neurons in medial prefrontal cortex precedes population activity during behavior and rest

Authors: *N. INSEL[1,2], C. A. BARNES[1,2]
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Abstract: Neural activity in the medial frontal cortex can become synchronized within a 6-10 Hz "theta" oscillation, which is thought to underlie communication with more posterior brain regions during selection of actions, emotions, or memories. Cortical circuits are made-up of multiple cell types, and the dynamics among these with respect to the theta oscillation are not

well established. We identify a small (1-3%) proportion of neurons, recorded primarily from dorsal anterior cingulate and dorsal prelimbic cortices, which exhibit regular bursts of action potentials at theta frequency. Action potentials in these neurons had shorter peak-to-trough latencies compared with averages across the populations of regular- and burst-firing neurons, but they were wider than fast-spiking neurons. When rats performed a decision task, theta-firing was coherent with local field potentials recorded from the dorsal hippocampus, with burst intervals averaging 8.4 Hz. During rest epochs, theta-firing was observed in a largely-overlapping set of neurons, and average burst intervals were reduced to approximately 7.5 Hz. Cross correlations revealed that activity among theta-firing neurons preceded activity in fast-spiking and regular-firing neurons by tens of milliseconds. Theta-firing neuron activity also appeared to precede increases in theta-frequency bursting among other burst-firing neurons. Individual theta-firing neurons were selective for trial phases and session epochs; on average, however, these neurons were most active during the choice phase of a decision task. Neither trial phase nor session epoch influenced the temporal relationships between theta-firing neurons and other neurons in the population. One interpretation of these data is that theta-firing neurons represent a specific component of the cortical circuit that are the first to respond to presynaptic regions oscillating at the theta frequency, and may contribute themselves to local theta synchronization. If they do play a role in synchronization, then the observation that each neuron exhibits specific selectivity patterns may indicate that increased network synchronization reflects increased numbers of theta-firing neurons becoming active, as opposed to graded changes across all theta-firing neurons.

Disclosures: N. Insel: None. **C.A. Barnes:** None.

Poster

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Title: Grey matter volume in the orbital prefrontal cortex correlates with reinforcer devaluation but not reversal learning performance in bonnet macaques

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Abstract: Behaviors that rely on the prefrontal cortex are particularly vulnerable to the process of normal aging, but the neurobiological changes that occur over the lifespan in the different subregions of this brain structure are not completely understood. In the current experiment, young (n = 7) and aged (n = 9) bonnet macaques were trained on reversal learning and reinforcer devaluation tasks using a Wisconsin General Testing Apparatus. The reinforcer devaluation task tests the degree to which response selection can be guided by reward value, by pairing two different food rewards (food 1 and food 2) with distinct sets of objects. Monkeys learn this association over weeks and are then satiated on food 1. Successful performance occurs when the animal selects the objects associated with the non-satiated food 2. In contrast, reversal learning tests an animal's ability to learn that a previously rewarded stimulus is no longer associated with a reward on an object discrimination task. Data from lesion studies suggest that the orbital prefrontal cortex (OFC) is necessary for successful performance on both of these tasks (e.g., Gallagher et al., 1999; Bohn et al., 2003). To assess the extent that OFC structural integrity predicts performance on these two tasks, anatomical MRIs were obtained from 7 young and 6 aged monkeys that had completed both behavioral conditions. Boundaries of the OFC were manually determined by three independent observers blind to the age and behavioral performance of the animal (high inter-rater reliability was obtained for all comparisons, $r_{[12]} > 0.7$, $p < 0.01$). Although as a group the aged monkeys performed significantly worse than did young animals on both the reinforcer devaluation ($T_{[14]} = 2.07$, $p < 0.05$) and the reversal learning tasks ($T_{[14]} = 3.80$, $p < 0.01$), there was no significant difference in total OFC volume between age groups ($T_{[12]} = 0.88$, $p = 0.15$). While there was no significant correlation between OFC volume and reversal learning performance ($r_{[12]} = -0.22$, $p = 0.46$), a significant correlation was found between OFC volume and performance on the reinforcer devaluation task ($R_{[12]} = 0.61$, $p < 0.05$). Together these data suggest that OFC grey matter volume is associated with an animal's ability to guide its behavior based on predicted reward value, but not to reverse a previously learned association. Because reinforcer devaluation performance requires functional connectivity between the amygdala and the OFC (Baxter et al., 2000), these data suggest that alterations in structural integrity of the OFC may be involved in defective communication between these two brain structures, which disrupts an animal's ability to use reward value to guide behavior.

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Poster

919. Learning and Memory: Systems and Aging

Location: Hall F-J

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

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Title: Senescence modifies the structure of information encoding in the medial temporal lobe

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Abstract: Primates are remarkably adept at categorizing and remembering visual objects. The ability for organisms to discriminate and recall previously encountered information is critically dependent on the integrity of the medial temporal lobe (MTL) and inferior temporal cortex (ITC) (e.g., Mahut et al., 1982; Zola-Morgan et al., 1994; Buffalo et al., 2000). Lesions of these structures produce profound deficits on a variety of recognition memory and perceptual discrimination tasks. Data across a number of species indicate that these processes are similarly disrupted in senescence (e.g., Moss et al., 1988; Burke et al., 2011). However, the neural mechanisms underlying age-related deficits are poorly understood.
Information processing in the ventral visual stream is believed to proceed along a hierarchy of association, in which the responses in one region reflect the conjunctions of stimulus features represented discretely in upstream cortical areas (e.g., Barlow, 1961; Mishkin et al., 1983). Single-unit recording studies in primates have demonstrated that neurons in these regions represent information via population codes (e.g., Tanaka et al., 1991; Gross, 1992; Logothetis et al., 1995). However, the stability of a population code rests on the reliability of the responses of individual neurons. One theory of cognitive aging proposes that deficits in perceptual discrimination may be tied to deficits in sensory processing (e.g., Baltes and Lindenberger, 1997; Li and Lindenberger, 2002). Selective degradation of sensory information in lower level sensory areas may result in instability of information representation in higher level association areas. A number of theoretical and empirical findings now indicate that behavioral accuracy may rely on the stability of these responses.
To test this hypothesis we recorded the activity of 1633 units from 2 old and 3 young chronically implanted rhesus macaques (*macaca mulatta*) during two different visual tasks. A variety of

analytical techniques were used to examine the tuning characteristics on individual neurons, as well as the underlying signal to noise ratio. These data show that in aged animals there exists a significant decrease in quality of information encoding across the hippocampus (CA3/CA1) as well as upstream cortical structures (EC/PRC/TF). These results provide not only validation of a number of theoretical proposals and behavioral results, but further open new avenues for the development of cognitive and therapeutic approaches.

Disclosures: A. Thome: None. **P. Lipa:** None. **C.A. Erickson:** None. **C.A. Barnes:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

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California National Primate Research Center Grant P51 RR00169

McKnight Brain Research Foundation

Title: Mapping the spatial navigation network of young and aged rhesus macaque monkeys: A positron emission tomography study.

Authors: *J. R. ENGLE[1,3], C. J. MACHADO[3], A. P. MAURER[1,2], M. PERMENTER[3], J. VOGT[3], C. A. BARNES[1,2,3]
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Abstract: Activation of the neural substrates devoted to spatial navigation is largely dependent upon behavioral and environmental cues. Much of our current understanding of how such cues engage these neural substrates comes from studies of freely moving rodents, as it is technically more difficult to study this problem in the nonhuman primate brain. To gain a better understanding of nonhuman primate spatial navigation networks and how they change with age, four rhesus monkeys were trained to freely or passively traverse a long enclosure, and to walk on a treadmill. We measured cerebral glucose metabolism with the radiotracer 2-deoxy-2[^{18}F]-fluoro-D-glucose (FDG) and high-resolution positron emission tomography (microPET). Two of the four monkeys have been successfully scanned after the four conditions given in

pseudorandom order: 1) freely traversing the enclosure, 2) passive traversals in the same enclosure not excluding optic flow, 3) walking on a treadmill without optic flow, 4) sitting in a cage (control condition). We used ROI analysis to assess the contributions of behavioral state and age on spatial navigation network activity. Our preliminary results from two monkeys (12 and 27 years old) suggest that ROIs in the right hemisphere overall had a significantly greater uptake of FDG than ROIs in the left hemisphere (t-test, $p < .001$). The magnitude of the percent change across the behavior conditions, however, interacted with age. The difference in FDG uptake compared to the control condition was more pronounced when the young monkey was considered separately, although movement behavior was matched across age. This interaction was found in the right medial temporal lobe, area TE, anterior cingulate, retrosplenial and medial occipitoparietal area ROIs. These preliminary results suggest that the right hemisphere of the rhesus monkey may contribute to spatial navigation, and may undergo similar age-related changes in the spatial navigation network that has been reported in aged rodents.

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Poster

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Program#/Poster#:919.15/EEE6

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant #AG026505 from NIA to Paul E. Gilbert

Title: Age-related changes in spatial pattern separation in rats

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Abstract: Pattern separation is described as a mechanism for separating partially overlapping patterns of activation so that one pattern may be retrieved as separate from other patterns. Recent studies have reported that spatial pattern separation may be impaired in older adults; however, little is known about age-related changes in spatial pattern separation in rats. Therefore, the current study tested young and old Fischer/Brown Norway rats 6 mo ($n = 18$) and 24 mo ($n = 18$) of age on a task shown to be dependent on the DG subregion of the hippocampus (Morris et al., 2012). Each rat was randomly assigned to an adjacent or a separate condition and tested on a

place-learning task using a radial eight-arm maze (McDonald & White, 1995). Rats were trained to discriminate between a reward arm and a non-reward arm that either were adjacent to one another (high interference/ high demand on pattern separation) or separated by a distance of two arm positions (low interference/ low demand on pattern separation). Each rat received 10 trials per day and was tested until the animal reached a criterion of nine correct choices out of 10 consecutive trials across two consecutive days of testing. The results did not reveal significant differences between young and old rats in the number of trials required to reach the learning criterion in the adjacent or separate condition. However, rats required significantly more trials to reach the learning criterion in the adjacent condition compared to the separate condition. An examination of the total errors committed before reaching the learning criterion revealed that old rats make significantly more errors than young rats. In addition, more errors were committed in the adjacent condition than the separate condition. In the adjacent condition, there may be high overlap among the external cues associated with the non-reward and reward arms, potentially requiring more pattern separation due to heightened interference. In contrast, there likely is less interference among the external cues in the separate condition resulting in less need for pattern separation. The results suggest that spatial pattern separation may be less efficient in old rats and are consistent with recent human studies.

Disclosures: E.I. Gracian: None. **L.E. Shelley:** None. **A. Morris:** None. **A. Najjar:** None. **P.E. Gilbert:** None.

Poster

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Program#/Poster#:919.16/EEE7

Topic: F.02. Animal Cognition and Behavior

Support: P01 AG 09973

Title: Somatostatin-positive interneuron loss in the aged memory-impaired rat dentate hilus

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Abstract: Accumulating evidence indicates that a failure to maintain balance in excitatory and inhibitory processes is a central component of memory loss associated with aging and Alzheimer’s disease (AD) in humans. Studies examining individuals with mild cognitive impairment or others at risk for AD show increased activation in the hippocampus that is localized to the denate gyrus/CA3 subregions. Similarly, patterns of aberrant hippocampal

activity occur in a memory-impaired subgroup of a rodent model of neurocognitive aging. In this model aged Long Evans rats exhibit stable individual differences in cognitive outcomes, similar to humans, when trained on a hippocampus dependent spatial memory task. In aged impaired animals, increased activity of the DG/CA3 network correlates with impaired pattern separation function, resulting in an inability to rapidly encode new information. It is postulated that a decline in inhibitory control within the aged hippocampus may contribute to excess activation in the network. In support of that idea, we have shown that memory impairment in the aged Long Evans rat is associated with a specific reduction in hilar interneuron integrity (Spiegel & Gallagher, SFN 2011). The hilus contains a functionally heterogeneous population of interneurons providing one of the strongest inhibitory controls over principal cell activity found in any cortical region. Given this diversity, identifying the integrity of specific subpopulations of hilar interneurons in conditions of cognitive impairment is important for understanding what components of this inhibitory circuitry are most susceptible to the effects of aging. Somatostatin (SOM) and Neuropeptide Y (NPY) expressing hilar interneuron subgroups regulate granule cell excitability at the perforant path termination zone and contribute to pattern separation processes. We studied the condition of NPY- and SOM-immunoreactive interneurons in relation to cognitive status in behaviorally characterized young and aged Long Evans rats. Using immunohistochemistry with stereological quantification, we found that SOM-positive hilar interneurons were reduced in aged rats with memory impairment relative to both young adults and aged cohorts with intact cognitive function, while NPY-positive hilar interneuron number did not differ between young and aged rats. These data coincide with evidence from models of AD and risk for AD, (e.g. carriers of apoE4) which also demonstrate early decline in SOM positive interneurons. Together these data highlight the importance of SOM-positive hilar interneurons in maintaining proper memory function and inhibitory processing in the aged hippocampus.

Disclosures: A.M. Spiegel: None. **M. Gallagher:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH grants R01MH078194

NIH grants R43MH076309

NIH grants R43 NS073311

Title: Declines in social interaction and social recognition memory are associated with deficits in synaptic plasticity in aged mice

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Abstract: Social withdrawal and decline of social recognition memory manifest with aging in humans. Here we tested the hypothesis that aged mice display decreases in social interaction and social recognition memory are associated with deficits in hippocampal synaptic plasticity.
Social cues (strange mice taken from different homecages) were introduced to the test mice (3-4 month old vs 12-14 month old, female, n=8) in a homecage after habituation. Time spent for investigation of each social cue was automatically quantified using the SmartCage™ (AfaSci, Inc). Direct contact social test with a social cue was conducted using manual scoring. Field synaptic potential was recorded in CA1 and dentate gyrus (DG) of hippocampal slices from the young and aged mice.
In the automated social interaction test, aged mice spent significantly less time interacting with stranger 1, indicating a lack of sociability, although their social novelty preferences to the stranger 2 were comparable to young mice. However, if the social novelty test was delayed in 10 min, the social novelty preference disappeared in aged mice. In contrast, young mice recognized the Stranger 1 for at least 1 h and disappeared in 24 h. Moreover, the aged mice could not recognize their littermates after separation for 20 min, while young mice retention was up to 24 h. In the direct contact test, aged mice spent significantly less time interacting with strangers compared to young mice. Their social recognition retention in this direct contact setting disappeared in 3 h, whereas young mice preserved in 3 h and disappeared in 24 h.
The input-out curves and population spike in CA1 area was significantly lower (1.4 ± 0.1mV, mean ± SEM, in old vs 3.7 ± 0.6mV in young,), and paired-pulse facilitation was significantly less in DG (123.3 ± 4.8% in old vs. 259.6 ± 22.1% in young). Long-term potentiation (LTP) could not be induced in DG of old mice (103.5 ± 14.9% of baseline), while the amplitude of population spike in the young DG increased to 217.7 ± 46.8% of baseline at 45 min after two trains of 100 Hz stimulation. These results indicate that aging significantly decreases social interaction and social recognition memory, and is associated with deficits in basal synaptic neurotransmission and plasticity in the hippocampus.
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Disclosures: **X.S. Xie:** Employment; AfaSci Research Laboratories, AfaSci, Inc.. Research Grant; NIH grants R01MH078194, R43MH076309 and R43 NS073311. Ownership Interest; X Xie is the founder and owner of AfaSci, Inc.. **L. Yang:** None. **B. Zou:** None. **C. Pascual:** None. **A. Malik:** None. **J. Xie:** None.

Poster

919. Learning and Memory: Systems and Aging

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Topic: F.02. Animal Cognition and Behavior

Support: 5R01AG026607-06

Title: Hippocampal transcriptomic analysis provides insight into molecular mechanisms of physiological and cognitive brain aging

Authors: *W. M. FREEMAN[1], H. D. VANGUILDER STARKEY[2], G. V. BIXLER[1], J. A. FARLEY[3], H. YAN[3], W. E. SONNTAG[3]
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Abstract: In both humans and animal model organisms, advanced brain aging is accompanied by molecular alterations that represent impairment of processes ranging from metabolism to inflammation to synaptic signaling. Deficits of hippocampal spatial learning and memory are also evident in a subset (~50%) of subjects, although not all age-related changes in cellular and molecular function are associated with cognitive impairment. The goal of this study was to comprehensively compare and contrast age-related and cognitive decline-specific molecular changes that occur in the hippocampus at both the cellular and synaptic levels. Rats were assessed for spatial learning and memory performance by Morris water maze testing before sacrifice. Whole-genome gene expression analysis was performed to define the transcriptome of hippocampal synaptic preparations, subregion dissections (CA1, CA3, DG), and whole, unfractionated hippocampus derived from mature adult (12m) and aged (24-26m) Fischer 344 x Brown Norway hybrid rats. This evaluation enabled classification of aged rats as cognitively intact or cognitively impaired relative to adult group performance. As expected, fewer transcriptomic alterations were observed specifically with cognitive impairment than with aging, and substantial commonality was evident between expression changes in CA1 and CA3. Meta-analysis of the resultant data demonstrates that transcripts indicative of glial activation (*e.g.*, inflammation and immune-response pathways) are induced across hippocampal subregions with advanced aging and are not directly associated with deficits of spatial learning and memory. Markers of complement activation and STAT3 signaling are induced with aging in pyramidal cell-containing regions (CA1 and CA3) to a greater extent than in the granule cell-containing dentate gyrus (DG). Distal (synaptosome) mRNA changes associated with advanced aging include downregulation of neurotransmission-related and upregulation of antigen presentation-

related genes, whereas whole hippocampal changes recapitulated the neuroimmune activation evident in subregion analyses. Smaller numbers of gene expression changes were associated specifically with cognitive deficits, and included transcripts for growth factor signaling, transcription regulation, and protein modification. Interestingly, the direction of expression change often differed between CA1/CA3 and DG. These studies indicate a potentially additive process of age-related neuroimmune activation and impaired neurotransmission that combine with cognitive impairment-specific inhibition of neuronal maintenance and synaptic plasticity.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: DFG grant DFG DE 792/2-4

Title: Blockade of NK3-receptors interacts with Senktide-mediated behavioral effects and ACh release in aged rats

Authors: S. SCHÄBLE, *J. P. HUSTON, A. M. DE SOUZA SILVA
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Abstract: NK3-receptors (NK3-R) have been localized in brain regions which have been implicated in processes related with learning and memory, including the frontal cortex, hippocampus, amygdala and medial septum in rats and mice. Previous studies displayed that administration of the NK3 receptor agonist senktide promoted both age-dependent and pharmacological-induced memory deficits. There is also evidence that senktide increased ACh release in aged rats. Given the profound effects of NK3-receptor activation on behaviour and cholinergic activity of the basal forebrain, we examined whether NK3-receptors mediate learning and memory processes and whether cholinergic mechanism underlie these effects. Our aim in this study was to assess the effects of systemic administration of the NK3-R antagonist SR142801 (3 mg/kg or 6 mg/kg) on NK3-R activation by senktide (0.2 mg/kg) in the object-place recognition and water maze task in aged Wistar rats (17 months old). Thereafter, the aged animals were submitted to the in vivo microdialysis procedure with HPLC to evaluate the effect of these treatments on ACh levels in frontal cortex, amygdala and hippocampus.
Results: Senktide (0.2 mg/kg) was found to reinstate age-dependent deficits in object-place

recognition task and to enhance water-maze learning. Application of 6 mg/kg of the NK3-R antagonist, SR142801, blocked these memory-enhancing effects of senktide in the aged animals. Blockade of NK3-receptors by 6 mg/kg SR142801 diminished the senktide-induced increase in ACh neurotransmission in the frontal cortex, amygdala and hippocampus.
Our results indicate that the behavioral performance within the object-place recognition and water maze task affected by senktide treatment seems to be directly mediated via the NK3-receptors. In summary, these results provide further insight into behavioral and neurochemical functions of the NK3-R in learning and memory processes in aged rats.

Disclosures: S. Schäble: None. **J.P. Huston:** None. **A.M. de Souza Silva:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Intramural Research Program of the NIA

Title: Effects of the novel histone deacetylase inhibitor, EVX001688, on synaptic connectivity in young and aged rats.

Authors: *A. S. SEWAL[1,2], H. PATZKE[3], E. J. PEREZ[1], P. PARK[1], W. DRIWECH[1], P. R. RAPP[1]
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Abstract: Previous reports indicate that treatments targeting epigenetic regulation, such as histone deacetylase (HDAC) inhibitor administration, can enhance hippocampus-dependent memory and synaptic connectivity. HDAC inhibition prolongs histone acetylation, inducing a permissive environment for the transcription of genes critical for memory and synaptic plasticity. Here we evaluated the effect of the histone deacetylase inhibitor (HDACi) EVX001688 on synaptic markers in the hippocampus of young (7-9 months; veh n=4; drug n=6) and aged (26-28 months; veh n=7; drug n=7) male F344 x BN rats. We also tested whether HDACi mediated changes in synaptic connectivity are regionally selective across the principal connectional zones of the hippocampus: CA1 stratum radiatum, CA3 stratum lucidum and stratum radiatum, and laminar subdivisions of the dorsal dentate gyrus molecular layer. EVX001688 is brain-penetrant and inhibits a spectrum of HDACs, selective for classes I, IIb, and IV in the nanomolar range.

Rats received a single injection (i.p.) of vehicle or 30mg/kg of drug 90 minutes before sacrifice - a dose we previously demonstrated yields modest improvement in long-term spatial memory in young and aged rats. Histone H4 acetylation was robustly increased across all hippocampal principal cell layers in both young and aged subjects, confirming the efficacy of HDACi treatment. Selected synaptic markers, including pre-, post-, and intrajunctional proteins (synaptophysin, spinophilin, and N-cadherin) were analyzed by quantifying regional immunofluorescence labeling intensity. Under resting, baseline conditions at 90 minutes post-treatment, no drug effect was detected on synaptic protein levels in the young or aged hippocampus. Experiments currently in progress are designed to test the prediction that the reported effects of HDAC inhibition on connectivity are instead mediated by a permissive mechanism that requires concurrent memory-related neural signaling in the hippocampus.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: AG 09973

Intramural Research Program of the NIA

Title: A fine balance: Regulation of Arc/Arg3.1 transcription and translation in a model of normal cognitive aging.

Authors: *B. R. FLETCHER[1], M. GALLAGHER[2], M. L. SHAPIRO[3], P. R. RAPP[1]
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Abstract: Age-related cognitive decline is thought to arise from a constellation of neuronal alterations including changes in structure, connectivity and cellular plasticity mechanisms. We examined both behaviorally induced transcription and translation of the immediate early gene Arc in relation to chronological and cognitive aging. Spatial learning capacity was used to classify aged rats as either impaired (AI) or unimpaired (AU) relative to young adults. Several weeks later rats were tested in a redundant place-cue (RPC) version of the water maze and

sacrificed either 30 or 120 minutes post-training, i.e., at timepoints appropriate for assessing behaviorally induced Arc transcription and translation, respectively. RPC training in young adult and AU rats led to significantly increased Arc mRNA transcription in the hippocampus relative to baseline control levels. Arc transcription in the hippocampus of trained AI rats, in contrast, failed to exceed baseline control values. Next we examined Arc protein by quantitative western blotting to determine if the blunted behavioral induction of Arc mRNA in AI rats is associated with correspondingly low protein levels. Surprisingly, behaviorally induced increases in Arc protein were preserved in both CA1 and CA3 in AI rats. We then examined known regulators of Arc translation as a potential basis for this unexpected sparing. Whereas phosphorylation of elongation factor 2 (EF2) was increased by behavioral training in CA1 and CA3 in all groups, the increase was significantly greater in CA1 of AI rats relative to young adults, suggesting that the increase in Arc protein relative to mRNA may be mediated in part by an increase in the rate of elongation. We also found that eIF4A3 - a protein known to regulate Arc mRNA stability - is significantly increased in CA3 and CA1 after behavioral testing in young and AU rats, but not among AI animals. Decreased eIF4A3 could directly contribute to the increase in Arc protein relative to mRNA seen in the AI hippocampus by allowing additional rounds of translation to occur before the mRNA transcript is degraded. These findings indicate that compensatory changes in translational regulation result in maintained behavioral induction of Arc protein in AI rats that are nonetheless insufficient to support normal learning and memory. By this view impairment may arise from a decrement in the fine spatial and temporal regulation of Arc translation that is critical for its proper function in synaptic plasticity.

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Poster

919. Learning and Memory: Systems and Aging

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Program#/Poster#:919.22/EEE13

Topic: F.02. Animal Cognition and Behavior

Title: Behavioral effects of progranulin loss on a transgenic mouse model through aging

Authors: *C. VOLLERT[1], K. NGO[1], H. POURGARZHAM[1], G. L. GERTON[2], J. ERIKSEN[1]
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Abstract: Frontotemporal dementia (FTD) is the second most common cause of pre-senile dementia in patients under the age of 65. Recent evidence has shown that mutations in the human GRN gene are associated with frontotemporal lobar dementia with ubiquitinated TDP-43 inclusions, resulting in only one functional allele. This evidence suggests that a global reduction in eitherexpressed or secreted levels of progranulin (PGRN) is important in the etiology of dementia. Despite growing interest in the role of pgrn in dementia and the central nervous system, there have been few studies characterizing the behavioral effects of PGRN haploinsufficiency. In this study, 6, 12, and 24 month old PGRN haploinsufficientmice were subjected to a battery of behavioral tests and evaluated for the effect of PGRN loss on activity, anxiety, motor performance, and memory.

Disclosures: C. Vollert: None. **K. Ngo:** None. **H. Pourgarzham:** None. **G.L. Gerton:** None. **J. Eriksen:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant AG16765

Title: Hormone treatment and fine motor skill learning in middle-aged rats

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Abstract: During menopause, decreasing levels of estradiol have been shown to affect numerous neurobiological functions, but relatively little is known about motor functions. Estrogen receptors are distributed in the motor cortex and in cells projecting to the motor cortex. Dramatic changes of estradiol in menopause could therefore change synaptic plasticity in the motor cortex and the motor cortical network, and have detrimental effects on motor skill learning ability. Our study sought to evaluate the effects of hormone treatment on fine motor skill learning. Using a forearm reaching motor skill task in which rats are trained to reach through a narrow slot to retrieve a food pellet, we measured the fine motor skill learning ability in middle-aged Sprague-Dawley female rats after estradiol treatment. The potential cognitive, motivational and bodyweight changes related to aging and estradiol treatment were also monitored. All rats were

ovariectomized (OVX) and received estradiol or vehicle capsule during the surgery at 11 months-old. After 3 months, capsules were either exchanged, removed or continued to accomplish different timings and durations of hormone treatment. This resulted in four groups, described from the time of OVX: 1) 3 months estradiol followed by vehicle; 2) 3 months vehicle followed by estradiol; 3) continuous estradiol; 4) continuous vehicle. Forearm reaching motor learning tests were performed at 15 months of age. A total of 30 sugar pellets were given in one session of forearm reaching test per day for three weeks. We found that at early stages of motor skill learning, rats with continuous vehicle treatment (group 4) had the lowest success rates, while rats given estradiol capsules, whether constantly, introduced later, or removed later, had comparable success rates. At later learning stages, the success rate between groups converged. We also noticed that a high percentage of rats would not participate the training with vehicle treatment either constantly or introduced later even though they eat all pellets in their home cage and from the floor of training cage. In addition, we found dramatic effects of timing and duration of estradiol treatment on bodyweight changes. Our data suggest that 1) estradiol treatment facilitates early stage of fine motor skill learning. 2) With continuous motor skill training, rats can reach comparable success rates with or without estradiol treatment. 3) Motivation of fine motor skill learning decreased without estradiol treatment.

Disclosures: N. Dang: None. **A.N. Garcia:** None. **T.A. Jones:** None. **A.C. Gore:** None. **W. Yin:** None.

Poster

920. Learning and Memory: Physiology I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:920.01/EEE15

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant NS036999

Title: Learning generalization within and across sensory modalities in a mouse model

Authors: *A. T. POPESCU, A. KANG, J. TAO, M. R. ZHOU, H. E. TAN, M.-M. POO
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Abstract: Although the phenomenon of learning transfer has been described and characterized in different forms, the neural circuit basis remains largely unknown. Here, we attempt to uncover the neural mechanisms in a mouse model of stimulus discrimination. Specifically, we compared learning transfer between consecutive tasks using stimuli within or across sensory modalities. Mice were trained to discriminate two stimuli (either two different sounds or two visual stimuli,

one second in duration), and learned that licking right after one of them (conditioned stimulus - CS+) resulted in water delivery while the other stimulus had no effect (non-conditioned stimulus - CS-). Performance on this task was measured as percent of CS+ presentations followed by successful reward delivery. A discrimination score was also calculated based on anticipatory licking during the stimuli presentations. Both performance and stimulus discrimination increased consistently over the ten days of training, reaching stable values within five sessions. After ten days, animals started a similar discrimination task with stimuli either in the same or a different sensory modality. We observed different forms of learning generalization: the new stimulus-response acquisition for same modality tasks was almost instantaneous, leading to a significant difference between the two tasks (p<0.0001, n=11). Similarly, discrimination between the new stimuli was acquired faster compared to the first set (p=0.001, n=11). These effects were not observed when the new stimuli were in a different sensory modality (visual-to-auditory, or auditory-to-visual): stimulus-response acquisition occurred at a similar rate (p=0.15, n=8), and discrimination for the second set of stimuli was in fact impaired (p=0.022, n=8). Dopamine D1 receptor antagonist SCH-23390 injected in the medial prefrontal cortex (mPFC) impaired discrimination learning, but not stimulus-response acquisition. Animals that received SCH-23390 injections in mPFC during training on the first task (n=6) learned to discriminate the second, cross-modal set of stimuli much better than controls (p=0.016). This indicates that learning stimulus-discrimination in one sensory modality hinders learning the same task in a different modality, and that dopamine signaling in mPFC plays a crucial part in this process. Ongoing voltametry recordings and optogenetic experiments suggest that dopamine fluctuations in the mPFC are important for acquiring, but not performing the stimulus discrimination.

Disclosures: A.T. Popescu: None. **A. Kang:** None. **J. Tao:** None. **M.R. Zhou:** None. **H.E. Tan:** None. **M. Poo:** None.

Poster

920. Learning and Memory: Physiology I

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Program#/Poster#:920.02/EEE16

Topic: F.02. Animal Cognition and Behavior

Title: CA1 specific deletion of P/Q type calcium channel alters spatial representation of place cells.

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Abstract: Voltage-gated Calcium Channels, VGCCs, play a key role in transmitting signals between neurons, involving in different cellular functions such as cell to cell communications, and the regulation of certain genes etc. Specifically, it has been implicated with modulatory effect on spatial memory in the hippocampal pyramidal neurons. Although P/Q type calcium channel has been well studied on modulating signal transmission and synaptic plasticity in the cerebellum, neither cellular mechanism nor effect on the hippocampal place cell activity has yet been investigated. The hippocampal pyramidal cells are characterized by its increased firing rate at a specific location and its burst spike known as complex spiking. In addition, it recently has been reported that the temporal properties of spiking pattern of the pyramidal cells modulate the place cell behavior in relation with spatial memory. Therefore, we examined place cell properties of CA1 pyramidal cells in hippocampus of mice with CA1 selective deletion of Cav2.1 P/Q type calcium channel to investigate its role for spatial representation of place cells. We conducted single unit recordings of CA1 pyramidal cells in an open-field arena with a local cue for 2 consecutive sessions, interrupted by a 30-minute interval. The result showed that similarity of the place fields between the two sessions tended to be reduced in the conditional knock-out mice compared to the control mice whereas other spatial information including selectivity and coherence was not significantly different. Moreover, the place fields formed during the first session were remapped in the second session more often in the knock-out mice group, suggesting that spatial representation of place cells of the knock-out mice were less stable compared to the control group.

Disclosures: J. Cho: None. **D. Jung:** None. **H. Shin:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Netherlands Organization for Scientific Research–VICI Grant 918.46.609

EU FP7-ICT grant 217148

Title: Phase-Amplitude coupling in rat orbitofrontal cortex discriminates between stimuli predicting reward and aversive outcome during associative learning

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Abstract: The orbitofrontal cortex (OFC) plays a key role in the adjustment of ongoing behavior guided by stimuli in the external world and an animals' current motivational state. It is thought to fulfill this role, at least in part, by representing the outcome associated with single cues or cue configurations. However, lacking efferents to primary motor structures, OFC can only indirectly influence ongoing behavior. It is hypothesized that OFC transmits outcome-predicting value information to target cortical and subcortical structures where it could be used to modify stimulus-outcome, action-outcome or stimulus-action mappings.
Efficient cross-structural communication requires a mechanism by which the excitability of structures involved in the transfer of information is synchronized. Rhythmic mass activity as observed in the extracellularly recorded local field potentials (LFP) exhibits oscillatory patterns in different frequency bands that covary with neuronal excitability and can become coherent with LFP rhythms in remote structures, especially in low-frequency bands. Interactions between lower and higher oscillatory frequencies have been found in the form of phase-amplitude coupling (PAC) where the phase of slower oscillations, reflecting general excitability of networks, modulates the amplitude of high-frequent oscillations.
Here, we present data indicating that PAC occurs between theta and gamma band frequencies in LFPs recorded from rat OFC. Preferentially during the odor sampling phase of a 2-odor Go/NoGo task, PAC magnitude reached peak values, both for stimuli predicting positive outcome (S+) and negative outcome (S-). However, PAC magnitude on S+ trials was significantly higher than on False Alarm trials (S- Go trials), but not Correct Rejections (S- NoGo trials). When examined over trials within sessions, we found that PAC magnitude correlated with learning in both S+ and S- trials, with PAC magnitude on S+ trials leading S- trials. Importantly, the increase of PAC magnitude was not confounded with theta power, which did not systematically increase with trial number.
These results suggest that PAC could function as a general mechanism for retrieving stimulus-outcome associations in OFC during stimulus sampling and possibly in disseminating the associative value of cues to target structures.

Disclosures: M. van Wingerden: None. **R. van der Meij:** None. **E. Maris:** None. **C.M.A. Pennartz:** None.

Poster

920. Learning and Memory: Physiology I

Location: Hall F-J

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Program#/Poster#:920.04/EEE18

Topic: F.02. Animal Cognition and Behavior

Title: How emotional conditions modulate ventral and dorsal hippocampal place cell firing?

Authors: *E. G. WANN[1], T. J. DU[1], S. STEAD[2], I. MUZZIO[1]
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Abstract: Lesion and neuroanatomical studies indicate that the dorsal hippocampus specializes in spatial processing while the ventral hippocampus is more involved in emotion and olfaction. Despite these dissimilarities, it is currently unclear how ventral hippocampal neurons respond to different kinds of stimuli, including stimuli of high emotional valance. In vivo studies from ventral area CA3 demonstrate that the scaling of place fields increases as a continuum from the dorsal to the ventral pole of the hippocampus (Kjelstrup et al., 2008; Royer et al., 2010). However, it remains unclear how sensitive these large, non-sparse ventral fields are to visuospatial changes in the environment. Moreover, with the exception of visuospatial cues, cellular responses to the different sensory modalities comprising space have not been thoroughly studied. To characterize the firing patterns along the longitudinal axis, we conducted chronic single unit recordings from area CA1 in the dorsal proximal, dorsal distal, or ventral hippocampus while mice were exposed to a variety of visual and olfactory stimuli, a subset of which varied in emotional valence. Consistent with previous observations, we report that ventral cell representations are larger than those of the dorsal area. Importantly, we also found that a subset of these large ventral fields is very sensitive to visuospatial changes in the environment. In anxiogenic contexts, the in-field firing rates of dorsal cells increase, whereas those of ventral cells decrease. Because olfactory inputs are differentially distributed along the longitudinal axis, we also tested cellular responses to odorants of different emotional valence. Ventral cells display more global remapping than dorsal cells in the presence of an odor, especially when the odor presented has negative valence. In addition to remapping, ventral fields are reduced while dorsal fields expanded in the presence of an aversive odor. These results indicate that cells in the ventral hippocampus are heterogeneous in their sensitivity to spatial cues but most cells are dramatically altered in aversive conditions regardless of whether they are spatially tuned or not.

Disclosures: E.G. Wann: None. **T.J. Du:** None. **S. Stead:** None. **I. Muzzio:** None.

Poster

920. Learning and Memory: Physiology I

Location: Hall F-J

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Program#/Poster#:920.05/EEE19

Topic: F.02. Animal Cognition and Behavior

Title: Hippocampal CA1 place cell properties and high-frequency oscillations during spatial navigation in Arc/Arg3.1 knockout-mice

Authors: *H. A. MALKKI[1], P. MERTENS[1], F. VAN SCHALKWIJK[1], L. A. B. DONGA[1], J. LANKELMA[1], F. P. BATTAGLIA[1], C. MAHLKE[2], D. KUHL[3], C. M. A. PENNARTZ[1]
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Abstract: Arc/Arg3.1 is an immediate early gene that has a dual role as a regulator of AMPA receptor trafficking and actin polymerization. In humans, increased Arc mRNA levels have been linked to Alzheimer's disease, Angelman syndrome and Prader-Willi syndrome, whereas decreased levels are present in certain heritable forms of autism. Furthermore, Arc mRNA levels are altered in mouse models of Alzheimer's disease and Fragile X.
Arc/Arg3.1 has been shown to be crucial for maintenance of long-term synaptic plasticity. Interestingly, short term memory of Arc/Arg3.1 knock-out mice appears intact, but they have severe deficits in long-term memory, suggesting Arc/Arg3.1 to be essential for memory consolidation.
Previous studies have shown that Arc/Arg3.1 is enriched in hippocampal pyramidal neurons and it is rapidly expressed upon exploration of novel environments. Furthermore, loss of Arc/Arg3.1 function impairs acquisition of spatial learning. Together, these findings suggest Arc/Arg3.1 to play a pivotal role in spatial coding, although the mechanism by which it is involved in spatial representation is elusive.
To study the involvement of Arc/Arg3.1 in these processes, we recorded hippocampal CA1 ensembles and local field potentials in four wildtype (WT) and three Arc/Arg3.1 knock-out (KO) mice using a 24-channel "Lantern" microdrive while they ran a circular maze motivated by sucrose pellets. The circular maze task was conducted in identically designed circular mazes placed in two different environments (different odor and visual cues, lighting and noise levels). Each mouse was recorded for four sessions in each condition, yielding 104 cells in WT mice and 101 cells in KO mice.
Consistent with previous behavioral studies, Arc KO animals expressed no difference in baseline behavior such as movement speed. We also found the sleep architecture of the Arc KO animals to be indistinguishable from that of the WT animals.
Analysis of place cell activity did not indicate robust changes in firing rates of putative pyramidal cells. However, place fields of KO mice appeared to have a lower degree of coverage of the arena (i.e., smaller place field size). Furthermore, KO mice had a specific attenuation in high frequency bands, particularly in beta2 (23-30Hz) and gamma (35-100 Hz) range. These results suggest that Arc/Arg3.1 is important for the constitution of spatial representations and types of fast temporal processing implied in spatial coding and memory formation.

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Poster

920. Learning and Memory: Physiology I

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Program#/Poster#:920.06/EEE20

Topic: F.02. Animal Cognition and Behavior

Support: Sigrid Juselius Foundation, Finland

Title: Hippocampal replay events in the context of a spatial working memory task

Authors: *H. TANILA[1,2], S. KU[2], F. KLOOSTERMAN[2,3,4], M. A. WILSON[2]
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Abstract: In exploration, during pauses characterized by sharp-wave-ripple events in hippocampal EEG, ensembles of place cells re-express firing sequences that correspond to recent spatial experience. Such ‘replays’ often start or end at the current location of the animal, but may also represent a remote location. Selective disruption of sharp-wave-ripples has been shown to impair spatial learning, but evidence is still lacking to link replay events directly to memory formation.
To this end, we recorded ensembles of 40 to 80 CA1 place cells while the rat was running in a complex symmetric maze (180 cm x 140 cm) with high walls following a start left - turn right (or vice versa) nonmatch rule. A correct path choice was rewarded with 4 minipellets, while a wrong choice led to a blind alley and finally to a reduced reward size after the rat had returned and taken the correct path. The choice accuracy varied greatly during the 10-30 recording sessions in each of 4 rats, which enabled us to compare the contents of replay events during correct vs. erroneous choices. We used neural decoding to analyze replayed trajectories in detail. Instances of replay of trajectories toward goal or start box were found while the rat stopped in the middle of the maze. We are currently analyzing the relation between replayed trajectories, animal's behavior, and task performance.

Disclosures: H. Tanila: None. **S. Ku:** None. **F. Kloosterman:** None. **M.A. Wilson:** None.

Poster

920. Learning and Memory: Physiology I

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Topic: F.02. Animal Cognition and Behavior

Support: Global COE

Title: Involvement of increase in Zn^{2+} signal in hippocampal CA1 pyramidal neurons in stress-mediated memory deficit.

Authors: *M. NAKAMURA[1], S. TAKADA[1], M. SUZUKI[2], N. OKU[2], A. TAKEDA[1]
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Abstract: Glucocorticoid-glutamatergic interactions have been proposed as a potential model to explain stress-mediated impairment of cognition. However, it is unknown whether glucocorticoid-zincergic interactions are involved in this impairment. Zn^{2+} is co-released with glutamate from zincergic neurons. In the present study, involvement of synaptic Zn^{2+} in stress-induced attenuation of CA1 LTP was examined in hippocampal slices prepared from young rats after exposure to tail suspension stress for 30 s, which significantly increased serum corticosterone. Stress-induced attenuation of CA1 LTP was ameliorated by administration of clioquinol, a membrane permeable zinc chelator, to rats prior to exposure to stress, implying that the reduction of synaptic Zn^{2+} by clioquinol participates in this amelioration. To pursue the involvement of corticosterone-mediated Zn^{2+} signal in the attenuated CA1 LTP by stress, dynamics of synaptic Zn^{2+} was checked in hippocampal slices exposed to corticosterone. Corticosterone increased extracellular Zn^{2+} levels measured with ZnAF-2 dose-dependently, as well as the intracellular Ca^{2+} levels measured with calcium orange AM, suggesting that corticosterone excites zincergic neurons in the hippocampus and increases Zn^{2+} release from the neuron terminals. Intracellular Zn^{2+} levels measured with ZnAF-2DA were also increased dose-dependently, but not in the coexistence of CaEDTA, a membrane-impermeable zinc chelator, suggesting that intracellular Zn^{2+} levels is increased by the influx of extracellular Zn^{2+}. Furthermore, corticosterone-induced attenuation of CA1 LTP was abolished in the coexistence of CaEDTA. These results suggest that corticosterone-mediated increase in postsynaptic Zn^{2+} signal in the cytosolic compartment is involved in the attenuation of CA1 LTP after exposure to acute stress. We examined the idea that the transient increase in Zn^{2+} signal in the cytosolic compartment affect on memory. Local injection of $ZnCl_2$ into hippocampal CA1 region increased intracellular Zn^{2+} levels in CA1 region, followed by object recognition memory deficit. In the case of the cytosolic increase in endogenous Zn^{2+} in the CA1 induced by KCl (100 mM 1

[[Unsupported Character - Symbol Font ]]l), furthermore, object recognition memory was also transiently impaired, while ameliorated by co-injection of 1 mM CaEDTA to block the increase in cytosolic Zn^{2+}. The present study indicates that the transient increase in cytosolic Zn^{2+} in CA1 pyramidal neurons may be involved in acute stress-mediated impairments of CA1 LTP induction and object recognition memory.

Disclosures: M. Nakamura: None. **S. Takada:** None. **M. Suzuki:** None. **N. Oku:** None. **A. Takeda:** None.

Poster

920. Learning and Memory: Physiology I

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Conte MH086400

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NSF IOB 0645886

Title: Effects of high and low microstimulation in the nucleus accumbens in associative learning

Authors: *C. MARTINEZ-RUBIO[1], S. R. PATEL[1], S. C. FRY[2], E. N. ESKANDAR[1]
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Abstract: The nucleus accumbens is a collection of neurons found in the ventral striatum. This structure is thought to play an important role in reward-oriented behaviors and may underlie neurological disorders such as major depression, obsessive compulsive disorder, and drug addiction. Recent findings suggest that the nucleus accumbens encodes reward expectation and thus predetermines, in part, the motivation to perform behavioral tasks. In this investigation, two non-human primates were trained to perform a simple visual-motor association task. During this task, the animal was required to learn, by trail and error, to associate four images with a specific saccade direction; in each block, two images are novel and two are familiar. High and low microstimulation was applied during three different epochs of the task (initial fixation, stimulus,

and feedback) to one of the novel images in each block. Learning performance and reaction times were evaluated during each of these stimulated epochs. Our preliminary results show an improvement in the learning rate using low frequency stimulation during the fixation epoch of the task. Moreover, low frequency stimulation seems to reduce reaction times during initial fixation and stimulus presentation. Interestingly, application of high frequency stimulation has only shown an effect during the stimulus presentation. These results increase our understanding of the role of the nucleus accumbens in associating learning and the possibilities of a potential new treatment of various disorders related to mood and motivation.

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Poster

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Program#/Poster#:920.09/EEE23

Topic: F.02. Animal Cognition and Behavior

Support: ANR-07-Neuro-019-01

Title: Social presence modulates visuomotor learning in macaque monkeys

Authors: *M. DEMOLLIENS[1], F. ISBAINE[1], A. BROVELLI[2], D. BOUSSAOUD[1], P. HUGUET[3]
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Abstract: Interest in the way the presence of conspecifics affects individual behavior has a long history in social psychology, with a myriad of experiments involving species as diverse as humans and cockroaches. This presence was generally found to facilitate performance on simple or well-learned tasks, and to deteriorate performance on difficult or poorly learned tasks (compared with performance in isolation context). These phenomena, known as social facilitation/inhibition (SFI) effects, received much attention, but their boundary conditions and neural correlates remain poorly understood. Here, we addressed these important issues in non-human primates. Two macaques learned to perform simple or difficult visuo-motor tasks involving arbitrary associations between abstract images and spatial targets (white squares) on a

touch screen. They initiated a trial by putting the hand on a lever (white rectangle) located near their chest. Then a visual cue appeared together with 2 (easy task) or 4 (difficult task) targets. Pressing the correct target led to a success feedback (green ring) followed by a drop of fruit juice, whereas incorrect responses led to error feedback (red ring) with no reward. Each animal acted as “actor” or “observer”, i.e., performed new tasks in isolation and in presence of the other animal, or observed the other animal performing such tasks. Whereas half the targets were located in the actor’s personal space, the other half were located in the observers’ personal space, so as to test whether entering the observers’ personal space can moderate SFI effects _ an important but neglected point in past research. Finally, single neurons were recorded from the dorsolateral prefrontal cortex (DLPF) of one of the two monkeys, so as to determine the neural correlates of these effects. The behavioral findings offer preliminary evidence that SFI effects depend on whether task performance does or does not imply for the actor to enter the observer’s personal space. More importantly, the neural findings provide first evidence that the presence of conspecifics modulates learning-related activity of a significant proportion of single DLPF cells, with cells whose firing rate was stronger when the actor monkey learned in presence of the observer, compared to isolation, and cells with the opposite pattern; a neural pattern revealing new facets of the so-called social brain in primates. Thus, our findings reveal that entering the observer’s personal space matters in SFI effects, which helps specify the boundary conditions for these effects, and also make a significant first step toward a better understanding of the neurophysiological bases of such effects in primates.

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Poster

920. Learning and Memory: Physiology I

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Program#/Poster#:920.10/EEE24

Topic: F.02. Animal Cognition and Behavior

Title: Oxytocin-based neuromodulation of mammalian social behavior

Authors: *B. J. JONES, R. C. FROEMKE
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Abstract: Social interactions are essential for normative development and lifelong physical and mental health. The neuromodulator oxytocin is believed to be important for some forms of social behavior, specifically parent-child bonding and maternal behavior. Here we investigate a

fundamental form of rodent social behavior that depends on both social cues and modification of neural substrates: pup retrieval.
To initiate pup retrieval behavior, mouse pups produce ultrasonic vocalizations (USVs) when separated from the nest. Dams and other experienced caregivers use these acoustic signals to locate and retrieve isolated pups. Importantly, virgin female mice can also learn a variety of maternal behaviors, including pup retrieval, and we hypothesize that oxytocin plays a major role in this form of learned social behavior (Pedersen et al., Science 1982). However, it is unclear how oxytocin impacts the activity of the cortex, leading to the robust increase in maternal behavior.
We confirmed that virgins can also learn to retrieve pups. Furthermore, learning is accelerated after either systemic or cortical application of oxytocin. Virgins were first tested for initial retrieval abilities. Virgins were then co-housed with a dam and her pups for up to three days, with some animals receiving oxytocin and other animals receiving saline. After 12 hours of co-housing, 16/31 (~50%) animals injected with oxytocin learned to retrieve pups, but only 6/24 (25%) control saline-injected virgins retrieved pups. Similarly, 7/9 animals receiving oxytocin infusion into left auditory cortex via cannula retrieved pups within 12 hours, but 0/4 cannulated virgins receiving saline infusion retrieved pups. Finally, infusing the GABA-A receptor agonist muscimol into left auditory cortex, to inactivate local circuitry, reduced or fully prevented pup retrieval in 6/9 experienced animals. Infusion into right auditory cortex did not seem to significantly affect retrieval. This demonstrates that left auditory cortex is required for USVs to engage pup retrieval. Furthermore, these data suggest that oxytocin-based neuromodulation, paired with acoustic stimuli, can modify auditory cortex to enable or improve learned social behavior.

Disclosures: B.J. Jones: None. **R.C. Froemke:** None.

Poster

920. Learning and Memory: Physiology I

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Topic: F.02. Animal Cognition and Behavior

Support: CNRS (young investigator ATIP grant)

Fondation pour la Recherche sur le Cerveau (FRC)

Région Rhône-Alpes (CIBLE program)

Title: Memory and forgetting : Implication of sleep.

Authors: *R. PARMENTIER, L. PRAST, M. YOUSSEF, J. COMTE, P. A. LIBOUREL, P. H. LUPPI, P. SALIN, G. MALLERET
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Abstract: Our memory is our ability to retain and use knowledge or acquired information. Understanding the mechanisms underlying memory functions is essential.
Moreover, it is generally accepted that sleep is a process that promotes these memory processes. Given the differences between slow
wave sleep (SWS) and paradoxical sleep (PS), these two sleep phases must surely have different roles. The 'sequential function of sleep hypothesis' (Giuditta et al., 1995) suggests that these roles are interdependent. A first processing would result in a reduction of "non-essential" memory traces during SWS, while PS would allow the consolidation of the remaining relevant information. In other words, we can say that SWS would promote forgetting while PS would facilitate the consolidation of information. We tested this hypothesis by coupling in the rat an electrophysiological approach to sophisticated behavioral tasks aimed to assess three distinct forms of memory in a radial maze (Malleret et al., 2010): 1) a working memory (WM) task with high level of proactive interference (HIWM) during which storage of variable information is required but forgetting of previous trials is preferable for the animal not to be overloaded with irrelevant information, 2) a working memory task with low level of interference (LIWM) during which such forgetting is not required, and 3) a reference memory (RM) task requiring the long-term consolidation of invariable information. Each day, post-learning sleep EEG/EMG oscillatory activity was recorded.
We observed a transient increase in the amount of PS (+40%) the day the animal has learned the RM rule. This day was determined by a performance above 50% of correct responses that is maintained the day after. In HIWM, no quantitative changes in SWS or PS were noted during the ten days of learning. However, a significant positive correlation is seen between performance on day n+1 and the amount of SWS (but not PS) shown by the animal the day before (day n). This result suggests that the more time spent in SWS, the better the animal deals with interferences. In contrast, in LIWM, no notable change exists in the amounts of SWS or PS or in the correlations between SWS or PS and WM performance.
In conclusion, our results suggest that PS is transitorily involved in the long-term storage of information whereas SWS would be required for proper treatment of memory interference and therefore forgetting of irrelevant information required for working memory.

Disclosures: R. Parmentier: Research Grant; Young Investigator ATIP Grant, Fondation pour la Recheche sur le Cerveau (FRC), Région Rhône-Alpes (CIBLE program). **L. Prast:** None. **M. Youssef:** None. **J. Comte:** None. **P.A. Libourel:** None. **P.H. Luppi:** None. **P. Salin:** None. **G. Malleret:** None.

Poster

920. Learning and Memory: Physiology I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:920.12/EEE26

Topic: F.02. Animal Cognition and Behavior

Support: R01DC009318

Title: Excitotoxic lesions of striatum spare head direction cell function within the anterodorsal thalamus.

Authors: *S. S. WINTER, S. VALERIO, J. S. TAUBE
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Abstract: Maintaining spatial orientation is a complex process that involves the coordination of multiple brain regions involved in processing selective components of spatial information. For example, head direction (HD) cells activate dependent upon the heading orientation of an animal within their environment functioning like a compass. The classic HD circuit projects from dorsal tegmental nuclei → lateral mammillary nuclei → anterodorsal thalamus → postsubiculum and retrosplenial cortex → entorhinal cortex. Information is processed serially through this circuit with integration of self-movement and environmental cues in distinct regions. Interestingly, HD cells have been located in areas outside of the classic HD circuit (i.e., striatum and medial agranular cortex), but their functional relationship to the known circuitry remains unknown. The current study examined the effect of excitotoxic striatal lesions upon the function of HD cells within the anterodorsal thalamus. Lesions of the striatum spared anterodorsal thalamic HD cell firing characteristics (peak firing rate, directional firing range), and control of HD firing by both visual landmarks and self-movement cues. These results provide evidence that striatal HD cells do not provide significant influence over thalamic HD cells, and suggest that striatal HD cells are generated from output of the classic HD circuit, most likely via subicular efferents or through its connections with the interpeduncular nucleus/habenula system, which has reciprocal connections with the dorsal tegmental nuclei.

Disclosures: S.S. Winter: None. **S. Valerio:** None. **J.S. Taube:** None.

Poster

920. Learning and Memory: Physiology I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:920.13/EEE27

Topic: F.02. Animal Cognition and Behavior

Support: NINDS Grant R01NS053907

Title: The nucleus prepositus contributes to head direction cell stability in rats

Authors: *W. N. BUTLER, J. S. TAUBE
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Abstract: The rat limbic system contains a unique population of cells that convey information about the animal's head direction (HD) in the horizontal plane. Previous studies have shown the importance of the vestibular system in generating the HD signal, but the roles of different nuclei within the ascending vestibular pathway to the HD cell circuit are largely unknown. Previous studies of the HD circuit have also shown a continuum of instability in HD cells following different lesions. Lesions of the retrosplenial cortex (Clark et al., 2010) or postsubiculum (Goodridge & Taube, 1997) led to HD cells whose preferred firing directions (PFD) drifted gradually as the animal moved about the environment, while lesions associated with the vestibular system led to cells which only fired in extended bursts of activity (Stackman & Taube, 1997; Muir et al., 2009; Yoder & Taube, 2009). Vestibular information is projected to the HD cell circuit via the nucleus prepositus hypoglossi (NPH) and supragenual nucleus (SGN). While SGN lesions disrupt direction-specific firing in the anterodorsal thalamus (ADN) (Clark et al., 2012), it has also been hypothesized that the adjacent NPH also plays a critical role in generating the HD signal.

To test this hypothesis, we made small bilateral electrolytic lesions of the NPH and recorded cell activity in the ADN in rats, an area that contains a high percentage of HD cells. Cells were monitored as rats foraged for food pellets dropped randomly onto the floor of a 76 cm diameter cylinder that contained a single prominent visual cue. The properties of cells recorded in the ADN of lesioned rats (n=6) were compared to the firing properties of HD cells in non-lesioned controls. Lesioned rats did not display stable HD-related activity; rather, they either had cells whose PFD drifted significantly at different rates throughout the recording session (n=5), or had cells whose PFD was highly unstable and fired in bursts (n=1). The number of these cells found in each lesioned rat was comparable to the number of HD cells found in controls. These results stand in contrast to findings when the SGN is lesioned, which led to the complete loss of direction-specific firing without any cells being recognizable as 'drifty' HD cells. These findings provide evidence that the NPH plays an important role in integrating vestibular cues with other inputs in order to maintain a stable HD cell signal.

Disclosures: W.N. Butler: None. **J.S. Taube:** None.

Poster

920. Learning and Memory: Physiology I

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:920.14/EEE28

Topic: F.02. Animal Cognition and Behavior

Support: BFU2011-29089

P07-CVI-02487

BFU2011-29286

P07-CVI-02686

EU FP7 DEVANX-201714

Title: Observational learning in mice can be prevented by medial prefrontal cortex stimulation and enhanced by nucleus accumbens stimulation

Authors: *A. GRUART, M. JURADO-PARRAS, J. CARPONCY, J. DELGADO-GARCÍA
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Abstract: The neural structures involved in ongoing appetitive and/or observational learning behaviors remain largely unknown. Ongoing behaviors are the result of underlying neural and cognitive processes that determine the sequence of motor activities and the proper analysis of the successive results. It can be assumed that electrical stimulation of the brain structures putatively involved in those processes affect only behavioral sequences present at the precise moment of the stimulation. We decided to study the putative modification of ongoing behaviors, and of the acquisition of new cognitive abilities by the electrical stimulation of selected brain sites in alert behaving wild-type mice. Operant conditioning and observational learning were evoked and recorded in mice with the help of a modified Skinner box provided with an on-line video recording system. Animals were implanted with chronic EEG recording and/or stimulating electrodes in selected hippocampal, prefrontal, and/or accumbens nucleus sites. Animals were firstly trained for the acquisition of a fixed (1:1) ratio schedule and then switched to different experimental protocols. Naïve mice improved their acquisition of a simple operant conditioning task by observational learning; that is, observing the proper performance of a demonstrator during the instrumental conditioning task. Electrical stimulation of the observer's medial prefrontal cortex (mPFC) at a key moment of the demonstration - that is, when the demonstrator presses a lever in order to obtain a reward - cancels out the benefits of observation. In contrast, electrical stimulation of the observer's nucleus accumbens (NAc) enhances observational learning. Ongoing cognitive processes in the demonstrator could also be driven by the electrical stimulation of these two (mPFC and NAc) structures, preventing the proper execution of the ongoing instrumental task (mPFC) or stopping pellet intake (NAc). Long-term potentiation

(LTP) evoked in these two structures did not prevent the acquisition or retrieval process - namely, mPFC and/or NAc stimulation only prevented, or modified, the ongoing behavioral process. EEG recorded at both mPFC and NAc was modified during the acquisition process and the observational period. The dorsal hippocampus was not involved in any of these two behavioral processes. In addition, LTP evoked in the dorsal hippocampus did not prevent the acquisition process and/or its proper performance. Thus, both ongoing observational learning and performance of an instrumental task require of the active contribution of the mPFC and the NAc nucleus.

Disclosures: A. Gruart: None. **M. Jurado-Parras:** None. **J. Carponcy:** None. **J. Delgado-García:** None.

Poster

921. Learning and Memory: Physiology II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.01/EEE29

Topic: F.02. Animal Cognition and Behavior

Support: Clark Foundation

Title: Effects of insulin on CA1 pyramidal neurons in the trim vs. obese rat hippocampus

Authors: *E. UNDERWOOD[1,2], L. T. THOMPSON[2]
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Abstract: Insulin has been shown to affect not only glucose utilization but also synaptic transmission in the CA1 region of the hippocampus, a region critically involved in learning new tasks and in consolidation of declarative memories. Learning-dependent plasticity of CA1 post-burst AHPs and spike-frequency accommodation have been reported by our own lab and many others following task acquisition, in multiple tasks and multiple species. Preliminary work from our lab has shown that insulin increases intrinsic excitability of CA1 pyramidal neurons. Obese rat models have been shown to have decreased performance in tests of spatial learning and decreased measures in excitability. The current study assessed excitability effects across a range of physiological concentrations in average weight (trim) and obese rats, preliminary to learning and memory-related studies. Young adult male rats fed either a control diet or a high-fat high carbohydrate diet for 6 weeks were anesthetized, and 400 µm brain slices including ventral and dorsal CA1 or horizontal slices including only ventral CA1 were prepared for recording. Sharp electrodes filled with 3M KCl were used for intracellular recordings from submerged slices held at 30°C in oxygenated aCSF using standard protocols. Measures of excitability (including

mAHP, sAHP, and AHP peak amplitudes, AHP duration and area, and of spike-frequency accommodation), input resistance, and sag were assessed from neurons held at -67 ± 2 mV. Vehicle (aCSF) or various concentrations of insulin (0.0 - 50 µM) were then bath applied and these measures were again assessed. Insulin increased excitability of CA1 pyramidal neurons in a concentration-dependent manner. All AHP measures were reduced, along with decreased accommodation (increased spike firing to a sustained input). U-shaped dose-response curves typical of other nootropic drugs enhancing hippocampal excitability were observed. Comparisons of intrinsic excitability before and after insulin perfusion between neurons from trim and obese rats were consistent with impairments associated with glucose and insulin dysregulation. The results have implications both for future learning and memory studies and for diabetics and others managing blood glucose levels with insulin treatments.

Disclosures: E. Underwood: None. **L.T. Thompson:** None.

Poster

921. Learning and Memory: Physiology II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.02/EEE30

Topic: F.02. Animal Cognition and Behavior

Support: Clark Foundation

Title: Expression of novelty in the hippocampus: Reductions in post-burst AHPs of CA1 pyramidal neurons after single novel experiences

Authors: *E. S. LOVITZ, J. P. ELLIS, E. D. VERA, A. S. FIRMAN, L. T. THOMPSON
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Abstract: Novel experiences are a trigger for the initiation of learning, in which the brain distinguishes between something familiar and something new, whether a difference in environment, task, or cue. Novelty can activate multiple brain regions involved in reward and memory, but responses quickly diminish after reexposure. Dopamine (DA) release from the substantia nigra (SN) and ventral tegmental area (VTA) is triggered by novelty; Steinfels et al. (1982) reported enhanced firing rates by DA neurons in the SN and VTA (Horvitz et al. 1998) during presentations of novel unconditioned clicks or light stimuli. fMRI studies show BOLD signal changes in these same regions during novel cue learning tasks (Schott et al. 2004; Wittman et al. 2005; Bunzeck & Duzel 2006). fMRI and EEG studies show the hippocampus and parahippocampal areas, which receive DA inputs, are active during presentation of novel stimuli. In rats, LTP is enhanced after neonatal exposure to a novel environment (Tang & Zou 2002).

Jeewajee et al. (2008) found exposure to novel environments reduces theta frequency in the hippocampus, and exposure to an unexpected environment leads to a greater reduction in theta frequency than novelty alone. Our lab (Farmer et al., 2012) found reductions in ventral CA1 (vCA1) pyramidal neuron post-burst afterhyperpolarization (AHP) amplitudes following exposure to a novel inhibitory avoidance shuttle box, lasting up to 1 hr post exposure. The current study extends these findings, using two different novel experiences.
Male LE rats (2 mo) were handled daily for 5 min over 3 d to habituate them to handling, then exposed the next day once to a novel open field environment, or trained on a novel object detection task. Rats were sacrificed 1 hr post-exposure or post-training, and intracellular recordings were made from 400 µm slices containing vCA1. Sharp electrodes filled with 3M KCl were used for intracellular recordings from pyramidal neurons in submerged slices at 30°C using standard protocols. RMP, IR, sag, and multiple measures of excitability (AHP peak amplitude and duration, measures of mAHP and sAHP, and of accommodation) were assessed from neurons held at -67±3 mV.
We found reductions in AHPs after exposure to a novel open field similar to those reported after exposure to a novel IA shuttle box. These findings suggest novelty is encoded by vCA1 pyramidal neurons via AHP plasticity, and may initiate learning of more salient information.

Disclosures: E.S. Lovitz: None. **J.P. Ellis:** None. **E.D. Vera:** None. **A.S. Firman:** None. **L.T. Thompson:** None.

Poster

921. Learning and Memory: Physiology II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.03/EEE31

Topic: F.02. Animal Cognition and Behavior

Support: American Tinnitus Association

Title: Hippocampal place cells and interneurons in tinnitus: Time course of behavioral and single-unit plasticity

Authors: *M. R. KAPOLOWICZ, S. B. TEMPLET, S. YAN, L. T. THOMPSON
Behavioral and Brain Sci., The Univ. of Texas at Dallas, Richardson, TX

Abstract: Møller (2004) and Eggermont & Roberts (2004) speculate that tinnitus-related plasticity occurring in the limbic system is due to the activation of the extralemniscal auditory pathway which is not typically active in healthy adults (Møller & Rollins, 2002). By way of the dorsal-medial thalamus, the non-classical ascending auditory pathway is afferent to the limbic

system, and it can trigger plasticity that contributes to the pathology of tinnitus. Goble *et al*. (2009) showed that place-fields of neurons in the CA1 region of the hippocampus destabilize within 15 min of high intensity noise exposure in an experimental tinnitus model, with plasticity continuing across at least the next 24 hr. The aim of the current study is to characterize both the nature and long-term time course underlying this place-field plasticity by comparing CA1 excitatory (complex-spike) pyramidal neuron activity and inhibitory (theta) interneuron activity with behavioral signs of tinnitus. Adult male Long-Evans rats were chronically implanted with individually drivable multi-tetrode bundles into dorsal CA1 and trained to search for 0.1 mL chocolate milk rewards at the ends of the arms of a radial maze for identification and assessment of interneuron activity and of place cell place-field stability. Rats were pre-tested for absence of tinnitus using a gap detection startle paradigm adapted from Turner *et al*. (2006). A day later, rats were randomly assigned as controls (placed in the sound apparatus without auditory stimulation) or for tinnitus induction (bilaterally exposed to a 16 kHz tone at 115 dB SPL for 1 hr). Immediately following this exposure, 10 min maze recording sessions were repeated multiple times daily for up to 1 mo. Single-unit activity and spatial location were monitored in each session using a Plexon Inst. MAP system to quantify neuronal activity and characterize place-fields on several dimensions. Rats were examined weekly for behavioral evidence of tinnitus using the gap detection startle paradigm. Single-unit activity and behavioral data from controls and noise-exposed rats were compared and assessed at time intervals throughout the post-exposure period. Behavioral evidence of tinnitus developed much more slowly than plasticity in place-fields within the hippocampus. The relationship between place cell plasticity and interneuron plasticity was also compared across these intervals. The early onset of hippocampal plasticity prior to evidence of behavioral plasticity (i.e. ringing in the ears associated with tinnitus) then serves as a basis for testing the effectiveness of therapies designed to reduce or eliminate both forms of plasticity.

Disclosures: M.R. Kapolowicz: None. **S.B. Templet:** None. **S. Yan:** None. **L.T. Thompson:** None.

Poster

921. Learning and Memory: Physiology II

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Program#/Poster#:921.04/EEE32

Topic: F.02. Animal Cognition and Behavior

Support: Clark Foundation

American Tinnitus Association

Title: BLA modulation of CA1 place field plasticity when an auditory CS is specifically paired with place cell firing

Authors: *E. J. DONZIS, L. T. THOMPSON
Univ. of Texas at Dallas, Richardson, TX

Abstract: The basolateral complex of the amygdala (BLA) is involved in emotional learning and spatial memory and modulates plasticity in multiple brain regions, including the hippocampus during spatial learning. Pyramidal cells in the CA1 region of the hippocampus fire preferentially when a rat is in one specific location of an environment. These cells are known as place cells and the area in which they preferentially fire is the place field. Changes seen in a cell's place field indicate a change in that cell's activity. Changes in the physical components of an environment typically result in place field plasticity. Nonphysical changes, such as fear conditioning a rat in an environment, also result in generalized place field plasticity. It is unknown what happens to place fields when a fear conditioned stimulus (CS) is associated with only one particular location in an environment, nor the physiological mechanisms for fear-conditioning-related place field plasticity. Hebbian learning suggests that CS presentation in a specific place will result in plasticity of cells firing during CS presentation (i.e. within their place field) and a lack of changes in cells not firing (i.e. outside their place field). This plasticity is likely due to BLA modulation of hippocampal activity.
Rats were implanted with 4 individually drivable tetrodes in the CA1 of the dorsal hippocampus and a guide cannula into the BLA. Rats were trained to explore all 8 arms of a radial-arm maze until stable unit activity was established using a Plexon MAP system. Rats were placed in a completely separate environment and presented a tone CS (70 dB, 5 kHz, 5 sec) paired 3 times (ITI 4 min ± 30 sec) with a footshock (0.5 mA, 1 sec). 24 h later, the rats were returned to the radial-arm maze and the CS was presented. Immediately after CS presentation, lidocaine or saline was infused into the BLA. Unit activity was recorded at intervals 0.5 to 24 h post-CS presentation and place field maps constructed for each neuron for each recording session. Place field firing plasticity was compared before and after CS presentation between lidocaine and saline infused rats. Freezing responses to CS presentation was also compared to rats that received pseudoconditioning (3 tone CS presentation and 3 footshocks unpaired).
Auditory fear conditioning resulted in increased freezing to the CS compared to pseudoconditioning. Place field stability increased when the CS was presented out-of-field (i.e. when a place cell was not firing) whereas place fields were disrupted when the CS was presented in-field (when the place cell was firing). Infusion of lidocaine into the BLA immediately post CS presentation in-field attenuated this place field plasticity.

Disclosures: E.J. Donzis: None. **L.T. Thompson:** None.

Poster

921. Learning and Memory: Physiology II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.05/EEE33

Topic: F.02. Animal Cognition and Behavior

Title: Firing rate dynamics underlying stress-induced impaired spatial encoding in the hippocampal network

Authors: *A. TOMAR[1], D. POLYGALOV[2], S. CHATTARJI[1], T. J. MCHUGH[2]
[1]Natl. Ctr. For Biol. Sci., Bangalore, India; [2]Lab. for Circuit and Behavioral Physiol., RIKEN Brain Sci. Inst., Wako City, Saitama, Japan

Abstract: Repeated and uncontrollable stress has debilitating effects on hippocamapal structure and function. Rodent models of stress exhibit deficits in spatial learning tasks, while at the cellular level, dendritic atrophy and impaired synaptic plasticity is observed across hippocampus in these animals. Surprisingly, the neuronal ensemble responses, that can provide an insight into stress-induced spatial memory deficits, either during stress or afterwards, remains poorly studied. Therefore we monitored hippocampal ensemble activity (dorsal CA1) during repeated stressful experiences (Immobilization: 2h/d for 10d) and also when mice explored different linear tracks or rested. Here we show that the first exposure of immobilization stress (D1) initially caused muted neuronal firing (first 30 min). This stress-induced deficit in neuronal firing recovered at later stage of stress (last 30 min). However, the effects of stress grew stronger with repeated applications, as on day 6 the initial firing was higher than that of D1. In fact, by the last day of stress (D10), neuronal firing rate remained abnormally high throughout the 2 h of immobilization episode. Consequently, one day after termination of chronic stress (D11), stress group showed enhanced neuronal excitability during rest/sleep state . To investigate the impact of chronic stress on spatial representations, we recorded place cell activity while mice explored familiar and novel linear tracks. On day 6, stressed animals showed impaired spatial representations during exploration of a familiar track. However, novel track representations were found intact as no difference was observed in either place field size or firing rates between control and stress group. Later, one day after termination of chronic stress (D11), stress group exhibited impaired spatial representations, as these animals failed to show novelty-induced enhancement of firing rate. Together, these data suggest that repeated stress interferes with neuronal excitability of CA1 neuron and induces firing rate rigidity as stressed animals failed to change their firing rates across different context and reveals differential effects of the chronic versus transient impact of stress.

Disclosures: A. Tomar: None. **D. Polygalov:** None. **S. Chattarji:** None. **T.J. McHugh:** None.

Poster

921. Learning and Memory: Physiology II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.06/EEE34

Topic: F.02. Animal Cognition and Behavior

Title: The altered spiking pattern of CA1 pyramidal cells of mice with CA1-selective deletion of Cav2.1 P/Q-type Ca^{2+} channel.

Authors: *D. JUNG[1,2], H. SHIN[1], J. CHO[1,2]
[1]Seongbok-Gu, Korea Institude of Sci. and Technology(Kist), Seoul, Korea, Republic of; [2]Univ. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Voltage-gated Calcium channels (VGCC) mediate Ca^{2+} flux in response to neural signals. Various subtypes of these channels are involved in Ca^{2+}-dependent processes including neurotransmitter release and synaptic plasticity through diverse neuronal mechanisms. Although Cav2.1 P/Q type calcium channel, a subtype of the VGCCs, is highly expressed in the hippocampus, its role for spatial memory has rarely been studied compared to other families of VGCCs. Hippocampal pyramidal neurons are known for location-specific discharges, showing 2 distinct spiking patterns: single spike and complex spiking burst. The recent study showed the role of temporal spiking characteristics of CA1 place cells in relation with spatial learning/memory. However, factors affecting the spiking properties of the pyramidal cell and learning/memory behavior are still unknown. Therefore, using mice with CA1 selective deletion of Cav2.1 P/Q type Ca^{2+} channel, we conducted single unit recordings of the pyramidal cells to elucidate the role of P/Q type Ca^{2+} channel on the structure of CA1 pyramidal spiking. Our study showed the conditional knock-out mice had tendency of reduced burst frequency and increased single spike frequency, resulting in decrease of the burst ratio while the overall spike number was not different. Moreover, temporal properties of bursting were also altered in terms of prolonged intra-burst interval and temporal burst length. These results suggest that P/Q type calcium channels could be involved in shaping the spiking pattern of CA1 place cells.

Disclosures: D. Jung: None. **H. Shin:** None. **J. Cho:** None.

Poster

921. Learning and Memory: Physiology II

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: HHMI

Title: Creation of hippocampal place fields via single-cell manipulation of excitability during spatial exploration

Authors: *D. LEE, B.-J. LIN, A. K. LEE
Howard Hughes Med. Institute, Janelia Farm Res. Campus, ASHBURN, VA

Abstract: The mechanism underlying hippocampal place cell firing is not known. For CA1 pyramidal neurons, the spatiotemporal summation of converging inputs from other spatially-tuned neurons in CA3 and the entorhinal cortex is considered to play a pivotal role in determining the spatial code, i.e., whether each cell is a place or silent cell in a given environment. Employing the whole-cell recording technique in freely moving rats, we show that manipulating a single CA1 pyramidal neuron's excitability without affecting its presynaptic activity reversibly converts the cell between place and silent modes during spatial exploration. A spatially-uniform depolarization of the somatic membrane potential (V_m) of a silent cell caused not only place field spiking but also an underlying spatially-tuned subthreshold response to appear. Conversely, a spatially-uniform hyperpolarization of a place cell prevented both the spatially-tuned subthreshold response and spiking. Graded manipulation of V_m by applying multiple levels of current injection revealed that the subthreshold response suddenly occurs above a cell-specific threshold. When no current was injected into silent cells, no difference in mean V_m, input resistance, or Vm fluctuations was found between the location of the eventual place field and the region outside of this field, implying that spatially-tuned synaptic inputs do not reach the soma at the resting V_m level. These results suggest that gating of inputs into the soma by cellular excitability may be a decisive factor for the spatial code.

Disclosures: D. Lee: None. **B. Lin:** None. **A.K. Lee:** None.

Poster

921. Learning and Memory: Physiology II

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Topic: F.02. Animal Cognition and Behavior

Support: Wellcome Trust

Title: Electrophysiological characterization of spatial memory cells in the anteromedial thalamic nuclei

Authors: *E. D. TRAN[1,2,3], J. PASSECKER[2], S. O'MARA[2]
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Abstract: The Papez circuit appears to play a crucial role in the formation of episodic memory. In particular, the interconnections between the hippocampus and the anterior thalamus seem to be vital for an animal's mnemonic ability to orientate itself to and navigate through its environment. This is further supported by electrophysiological studies in freely moving rats, which have discovered neurons that encode for location (place cells) in the hippocampus and directional heading (head direction cells) in the anterodorsal and anteroventral nuclei of the thalamus. Due to the relative inaccessibility of the anteromedial nucleus of the thalamus, its electrophysiological properties are not well-characterised, and its role in spatial processing and cognition still remains elusive. In this study, we were able to successfully record from a population of head direction (20%) and theta rhythmic neurons (15%) in the anteromedial thalamic nucleus, suggesting that this structure plays an important role in the temporal-diencephalic-cortical pathways that regulate spatial processing.

Disclosures: E.D. Tran: None. **J. Passecker:** None. **S. O'Mara:** None.

Poster

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Program#/Poster#:921.09/EEE37

Topic: F.02. Animal Cognition and Behavior

Support: NIH CA141549

NIH NS057105

Title: Hippocampal dopamine deficiency underlies spatial learning deficits in Neurofibromatosis-1 mutant mice

Authors: *K. A. DIGGS-ANDREWS, K. TOKUDA, Y. IZUMI, C. F. ZORUMSKI, D. F. WOZNIAK, D. H. GUTMANN
Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Children with the cancer predisposition syndrome Neurofibromatosis type 1 (NF1) are prone to learning and behavioral abnormalities, including problems with spatial learning and attention. Studies in *Nf1* genetically-engineered mice (*Nf1* CKO) have uncovered defects in neuronal morphology and function along with cognitive defects, yet the molecular mechanism underlying these deficits remains unclear. Using primary neuronal culture, electrophysiology, and behavioral testing, we demonstrate that a primary defect influencing hippocampal neuron function and spatial learning/memory in CKO mice is impaired dopamine signaling. Direct measurements of hippocampal dopamine levels (20%) and its downstream effector DARPP32 (60%) show that dopamine signaling is significantly reduced in CKO mice relative to controls. In cultured neurons, *Nf1* loss results in shorter neuronal processes and growth cones. These hippocampal neuron defects are completely rescued by either dopamine (100uM) or dopamine D1 receptor agonist (SKF38393, 1 uM) treatment *in vitro*. Functionally, long-term potentiation (LTP) of CKO slices is diminished (128.8+ 7.6%, n=8) in response to high frequency stimulation (HFS) (control, 165.2 + 6.8%, n=8). This response is completely restored by a dopamine D1 receptor agonist (SKF38393; 158.5+7.7%, n=8, $p<0.01$ vs. untreated). Further, dopamine (L-DOPA) treatment ameliorates the spatial learning abnormalities in *Nf1* CKO mice *in vivo*. Specifically, untreated CKO mice show no spatial bias in the Morris Water Maze. Following L-DOPA treatment, CKO mice exhibit significant spatial bias comparable to controls ($p<0.002$ for target vs all others). Together, these results firmly establish defective dopaminergic function as a primary abnormality underlying both spatial learning/memory and hippocampal function in *Nf1* mutant mice.

Disclosures: K.A. Diggs-Andrews: None. **K. Tokuda:** None. **Y. Izumi:** None. **C.F. Zorumski:** None. **D.F. Wozniak:** None. **D.H. Gutmann:** None.

Poster

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Support: ERC grant GABAcellsAndMemory grant 250047

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Innovation fond Frontier 0075.3.10

Title: Identification and characterization of parvalbumin-expressing interneurons in the medial entorhinal cortex of behaving mice

Authors: *C. BUETFERING, A. KEVIN, W. KELSCH, H. MONYER
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Abstract: A comprehensive understanding of how the medial entorhinal cortex (MEC) processes spatial information will require a cell-type specific dissection of its local circuitry. A major cell type carrying spatial information in the MEC are grid cells that fire only at the vertices of a grid of equilateral triangles. Inhibitory interneurons in the MEC may control grid cell activity via local inhibitory feedback. However, the interactions between grid cells and interneurons are largely unknown. Here we studied the function of parvalbumin-expressing (PV) interneurons by combining tetrode recordings and cell-type specific photostimulation. Cre-dependent viral expression of Channelrhodopsin-2 in the MEC of parvalbuminCre mice allowed regionally selective photostimulation of PV interneurons in behaving mice. Brief pulses of light (10 ms, 6 Hz, 473 nm) were used to unambiguously identify PV interneurons. PV interneurons and simultaneously recorded grid cells represented two distinct neuronal populations. In comparison to grid cells, PV interneurons had higher mean firing rates, lower grid scores and their firing was less spatially selective. The firing rate of both cell types was modulated by theta oscillations, with the peak firing of most grid cells occurring at the descending phase and preceding the peak firing of PV interneurons. Spike-time crosscorrelation analysis revealed the presence of putative monosynaptic excitatory connections between grid cells and PV interneurons. Conversely, photoactivation of PV interneurons led to a strong inhibition of grid cell activity. These results are consistent with the existence of an inhibitory feedback circuit between PV interneurons and grid cells. We are currently examining the functional involvement of this connectivity for the spatial firing of grid cells.

Disclosures: C. Buetfering: None. **A. Kevin:** None. **W. Kelsch:** None. **H. Monyer:** None.

Poster

921. Learning and Memory: Physiology II

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.11/EEE39

Topic: F.02. Animal Cognition and Behavior

Support: Tamop grant 4.2.1

Title: Involvement of neurotransmitters in the action of apelin.13 on passive avoidance learning

Authors: *G. TELEGDY[1], A. ADAMIK[2], M. JASZBERENYI[2]
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Abstract: Involvement of neurotransmitter in the action of apelin-13 on passive avoidance learning in rats.
Gyula Telegdy [a,b], Agnes Adamik[a], Miklós Jászberényi[a].
Department of Pathophysiology[a], Neuroscience Research Group of the Hungarian Academy of Sciences[b], University of Szeged, Szeged Hungary,
The action of apelin-13 on passive avoidance learning has been tested in rats. Apelin-13 facilitated the consolidation of passive avoidance learning in a dose-dependent manner, however was ineffective on retrieval processes.
In order to asses the possible involvement of transmitters in this action, the animals were pretreated with receptor blockers in doses with itself did not influence the behavioral paradigm. The following receptor blockers were used: a nonselective α-adrenergic receptor antagonist, phenoxybenzamine, a β-adrenergic receptor antagonist, propranolol, a nonselective 5-HT2 serotonergic receptor antagonist, cyproheptadine, a nonselective muscarinic acetylcholine receptor antagonist, atropine, a D2, D3, D4 dopamine receptor antagonist, haloperidol, a γ-aminobutyric acid subunit **A** (GABA-A) receptor antagonist, bicuculline, and a nonselective opioid receptor antagonist, naloxone.
Phenoxybenzamine, , cyproheptadine, atropine, haloperidol, bicuculline prevented the action of apelin 13 on the consolidateion of passive avouidance learning. Propranolon and naloxone were ineffective.
The data suggest that apeling-13 elicits its action on passive avoidance consolidation via α-adrenergic, 5-HT2 serotonergic, cholinergic, dopaminergic and GABA-A-ergic mediation.

Disclosures: G. Telegdy: None. **A. Adamik:** None. **M. Jaszberenyi:** None.

Poster

921. Learning and Memory: Physiology II

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The American Health and Assistance Foundation

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Title: Role of ITSN-1 in learning and memory

Authors: *N. MALAKOOTI[1], H. A. COLEMAN[2], X. M. LIM[2], A. CORLETT[3], D. I. FINKELSTEIN[4], H. C. PARKINGTON[2], M. A. PRITCHARD[3]
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Abstract: Intersectin1 (ITSN1) is present on Chromosome 21 and over-expressed in Down syndrome (DS). All DS individuals exhibit learning and memory impairment. ITSN1 functions as a scaffolding protein involved in endocytosis and exocytosis and hence contributes to pre- and post-synaptic plasticity. In this study, we examined the role of ITSN1 in pre-synaptic plasticity using paired pulse facilitation (PPF) and post-synaptic plasticity via long term potentiation (LTP). A Morris water maze test was also used to examine spatial learning and long-term memory. Itsn1 long isoform-specific null (Itsn1-LKO) and wild type (WT) male mice were used for both studies.
In hippocampal brain slices (300 µm) a recording electrode was placed in CA1 stratum radiatum and a stimulating electrode in the Schaffer collaterals. PPF was recorded at 500, 160, 70 and 25 ms interpulse intervals. For LTP, field excitatory synaptic potentials (fEPSPs) were evoked by stimulation every 20 s for 20 minutes followed by 4 bursts of 100 Hz for 1 s, at 20 s intervals. Then the routine of a single stimulation every 20 s was resumed for 3 hours.
PPF was significantly enhanced from 1.65± 0.06 (n= 12) to 1.99± 0.12 (n=20) fold at 70 ms (p=0.01) in LKO mice. LTP was also significantly enhanced from 1.8 ± 0.1 (n=8) fold in WT slices to 2.9 ± 0.3 fold (n=13) (p=0.001) in Itsn1-LKO animals. While LTP had completely declined to basal level by 2 h in WT slices, 1.6 ± 0.2 fold remained at 2 h in KO slices. This result is interpreted in terms of slowing of endocytosis-driven removal of post-synaptic receptors in the dendritic spines.
For the water maze test, 5-6 month old mice were used (n=11 per genotype cohort). In a 150 cm diameter circular pool, an escape platform was placed in a fixed position (NW quadrant, 25cm from the wall). The pool was filled with tepid water (23 degrees C), and non-toxic paint was added to the water to create an opaque appearance. Over the 6 days of place discrimination training to learn the position of the submerged platform, Itsn1-LKO mice (mean of 84.6 seconds) showed slower escape latencies than WT (mean of 37.6 seconds) with a P value of 0.0058. These data indicate that ITSN1 is involved in synaptic plasticity and spatial learning and long term memory. Therefore when over-expressed, ITSN1 may contribute to the learning and memory deficits characteristic of DS.

Disclosures: N. Malakooti: None. **H.A. Coleman:** None. **X.M. Lim:** None. **A. Corlett:** None. **D.I. Finkelstein:** None. **H.C. Parkington:** None. **M.A. Pritchard:** None.

Poster

921. Learning and Memory: Physiology II

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Program#/Poster#:921.13/EEE41

Topic: F.02. Animal Cognition and Behavior

Support: The Wellcome Trust

MRC

Title: Depletion of perineuronal nets enhances cognition

Authors: *C. ROMBERG[1,2], S. YANG[3], M. R. ANDREWS[3], A. E. HORNER[2], T. J. BUSSEY[2,4], J. W. FAWCETT[3], T. PIZZORUSSO[5,6], L. M. SAKSIDA[2,4]
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Abstract: Perineuronal nets (PNNs) are extracellular matrix structures that form around the synapses on the cell soma and proximal dendrites of neurons. Previously, it was shown that genetic or pharmacological removal of PNNs reactivates plasticity in the adult CNS. Therefore, we hypothesised that manipulation of PNNs may also enhance cognitive processes requiring synaptic plasticity, such as declarative/explicit learning and memory. Expression of the link protein Crtl1/Hapln1 in neurons has recently been identified as the key event triggering the formation of PNNs. Here we tested adult mice lacking the Crtl1 gene in the CNS on an object recognition paradigm and showed that the attenuation of PNNs dramatically enhances long-term object recognition memory. Virtually identical prolongation of memory followed digestion of PNNs in the perirhinal cortex with chondroitinase ABC. Moreover, we performed extracellular field recordings in the perirhinal cortex of Crtl1 knock out mice and found that depleted PNNs facilitated Long Term Depression, a synaptic plasticity mechanism thought to underlie the encoding of this type of memory. Our results suggest that removal of PNNs may be used to enhance cognition, and reveal a previously unknown mechanism regulating synaptic plasticity processes important for learning and memory.

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Poster

921. Learning and Memory: Physiology II

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Program#/Poster#:921.14/EEE42

Topic: F.02. Animal Cognition and Behavior

Support: R01MH080007

R01MH093807

RR00165

Title: Spatial representation in the macaque entorhinal cortex

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Abstract: Place-modulated activity of neurons in the hippocampal formation presents a means to organize contextual information in the service of memory formation and recall (Moser et al., 2008). In particular, grid cells and border cells have been observed in the entorhinal cortex (EC) of rats and bats (Hafting et al., 2005, Yartsev et al., 2011), but have yet to be described in single units in primates. Here, we examined spatial representations in the EC of head-fixed monkeys performing a free-viewing visual memory task (Jutras and Buffalo, 2010). Individual neurons were identified in the primate EC that emitted action potentials when the monkey fixated multiple discrete locations in the visual field across the presentation of up to hundreds of novel images. These firing fields possessed spatial periodicity that was reflected in a hexagonal pattern in the spatial autocorrelation. Analyses of 60° symmetry in the autocorrelation revealed a significant proportion of EC neurons could be classified as grid cells, based on accepted methods used to analyze rodent grid cells (12%, 23/193, $p<0.05$; Sargolini et al., 2006). A separate significant population of neurons in the EC preferentially fired when part of the stimulus bounds were viewed, analogous to rat border cells (9%, 18/193, $p<0.05$; Solstad et al., 2008). One prominent model of the generation of the grid structure requires theta-band modulation of grid cell spiking. In support of this model, it was recently demonstrated that, in rodents, disruption of theta oscillations disrupts grid cell firing patterns (Brandon et al., 2011; Koenig et al., 2011). However, the necessity of this relationship was contested by Yartsev et al., 2011, who reported that grid cells in bats do not require theta oscillations, and that theta-band activity in bats occurs in discrete bouts in contrast to the continuous theta that is observed in rats. In the present study, 48% of EC neurons that passed the threshold to be considered grid cells (11/23) demonstrated

theta-band (3-12 Hz) oscillatory activity in the autocorrelogram (theta index > 5) with preferred spiking at the trough of the theta-band in the simultaneously recorded LFP. This modulation occurred in the presence of intermittent theta oscillations (bouts from 3-12 Hz with a median duration of 0.96 sec and a median inter-bout interval of 0.87 sec). Taken together, these data suggest that EC neurons in the primate display spatial representations that are in many ways analogous to grid cells and border cells that have been described in the rat. These spatial representations may provide a spatial and temporal framework to anchor the encoding of stimulus content in a complex visual scene.

Disclosures: N.J. Killian: None. **M.J. Jutras:** None. **E. Buffalo:** None.

Poster

921. Learning and Memory: Physiology II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.15/EEE43

Topic: F.02. Animal Cognition and Behavior

Support: Global COE

Title: Loss of Zn2+ signal in the dentate gyrus affects object recognition memory without changing Ca2+ signal

Authors: *H. FUJII[1], S. TAKADA[1], M. NAKAMURA[1], N. OKU[2], A. TAKEDA[1]
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Abstract: The hippocampus plays an important role in learning, memory and recognition of novelty. The hippocampus is required for memory storage for a limited period of time after learning. The physiological role of histochemically reactive (chelatable) zinc in the hippocampus, which serves as a signal factor (Zn^{2+} signal), at glutamatergic (zincergic) synapses is poorly understood. The role of Zn^{2+} signal in learning and memory can be evaluated by the transient loss of Zn^{2+} signal by zinc chelators. In this case, it is required that the chelator does not interfere with the tightly bound zinc pool, such as zinc fingers and numerous catalytic enzymes, which are essential for cellular functions. Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline; CQ) forms lipophilic chelates with cations such as zinc and copper and is a candidate for by the transient loss of synaptic Zn^{2+} signal. Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline; CQ) has a relatively weak affinity for zinc (K_d, approximately 1×10^{-7} M). N,N,N',N'-Tetrakis-(2-pyridylmethyl) ethylendediamine (TPEN: $K_d = 2.6 \times 10^{-16}$ M) also forms a lipophilic chelate with zinc and has a higher affinity for zinc. Both chelators have the potential as scientific tools to

evaluate the role of synaptic Zn^{2+} signal in normal animals. Here we analyzed the effects of the transient loss of Zn^{2+} signal in the dentate gyrus by zinc chelators to evaluate the role of Zn^{2+} signal in the dentate gyrus in object recognition memory. The induction of dentate gyrus long-term potentiation (LTP) under conscious condition, which were maintained more than for 6 days, were observed as an index of memory acquisition. The transient loss of Zn^{2+} signal in the dentate gyrus was induced by intraperitoneal injection of zinc chelators or local injection of zinc chelators into the dentate gyrus. The transient loss of Zn^{2+} signal in the dentate gyrus, which was determined by ZnAF-2 and ZnAF-2DA, a membrane-impermeable and membrane-permeable indicator, respectively, was induced 6 h after both injections At this time, the induction of dentate gyrus LTP and object recognition memory were affected. The loss of Zn^{2+} signal did not significantly affect intracellular Ca^{2+} signal determined by calcium orange AM in the dentate gyrus. When Zn^{2+} signal in the dentate gyrus was returned to the control level, dentate gyrus LTP and object recognition memory were observed normally. The present study demonstrates that synaptic Zn^{2+} signal in the dentate gyrus may be required for object recognition memory.

Disclosures: H. Fujii: None. **S. Takada:** None. **M. Nakamura:** None. **N. Oku:** None. **A. Takeda:** None.

Poster

921. Learning and Memory: Physiology II

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.16/EEE44

Topic: F.02. Animal Cognition and Behavior

Support: WELFund, University of Hartford

Title: Environmental enrichment mitigates cognitive deficits in weanling rats on a ketogenic diet

Authors: *J. P. HARNEY[1], J. SCICHILONE[2], D. BUTLER[2]
[2]Biol., [1]Univ. Hartford, WEST HARTFORD, CT

Abstract:
For decades the ketogenic diet (KD) has been an acceptable and moderately effective treatment of intractable epilepsy in children. Recent studies suggest that the KD, which commonly consists of a high fat, low carbohydrate and protein diet, has adverse effects on cognition in weanling rats. Rats reared in an enriched environment (EE) exhibit improved spatial learning, memory, and increased neurogenesis. Thus, the focus of the present study was to determine if weanling rats administered a KD experience altered learning, memory, anxiety or depression compared to rodent chow controls and if so would exposure to an EE reverse the effects of the KD. Weanling

rats were randomly assigned to either a standard rodent chow (RC) diet or a KD ad libitum, and housed in an EE or caged alone (ic) for 8 weeks. Weekly weights showed a steady increase in body mass for all groups with the RCic gaining the most weight. Blood ketones in rats fed a KD were significantly higher ($P<0.05$) in those raised in an EE (KDee) compared to those raised in isolation (KDic). Rats raised in an EE showed significantly improved learning ($P<0.05$) at both 4 and 8 weeks compared to those raised in isolation, however environment did not significantly impact memory retention. KD rats did show diminished memory at 8 weeks, but not at 4 weeks, compared to RC-fed controls. At 4, but not 8, weeks rats raised in an EE had less ($P<0.01$) anxiety than rats raised in isolation regardless of diet. Additionally, at 4 weeks RC fed rats were less anxious ($P<0.05$) than KD-fed rats. Interestingly, rats raised in isolation or fed KD were more mobile (thus less depressed; $P<0.05$) than EE or RC rats in the modified forced swim test. Hippocampal weights and BDNF levels were elevated in rats raised in an EE compared to those raised in isolation. In the present study we conclude that EE is a suitable means of retaining learning ability in weanling rats administered a KD. These findings suggest that environment should be an additional consideration for children receiving dietary therapies.

Disclosures: J.P. Harney: None. **J. Scichilone:** None. **D. Butler:** None.

Poster

921. Learning and Memory: Physiology II

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Program#/Poster#:921.17/EEE45

Topic: F.02. Animal Cognition and Behavior

Support: ARRA supplement to RR000168/OD011103

Title: Insulin resistance and abdominal fat are associated with cognitive deficits in non-diabetic, aged rhesus monkeys

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[1]NE Prim Res. Ctr/Harvard Med. Sch., Southboro, MA; [2]Joslin Diabetes Ctr., BOSTON, MA; [3]Beth Israel Deaconess Med. Ctr/Harvard Med. Sch., Boston, MA; [4]Stony Brook U Sch. Med, Winthrop U Hosp, Mineola, NY; [5]McLean Hosp/Harvard Med. Sch., Belmont, MA; [6]Div. Endocrinology, Brigham and Women's Hosp/Harvard Med. Sch., Boston, MA

Abstract: Dementia and type 2 diabetes mellitus (T2DM) are common disorders that are increasing in prevalence, have a major impact on public health in the United States, and may have common causal pathways. Insulin resistance, a hallmark of T2DM, has been proposed as a risk factor for development of dementia as well as milder forms of cognitive impairment in aging humans. Rhesus monkeys develop metabolic disturbances with age, making them an ideal model species for studying T2DM. Affected macaques, primarily those with increased adiposity and older than age 10, demonstrate a syndrome characterized in part by impaired glucose tolerance and decreased insulin sensitivity. In the present study, we examined the relationship between metabolic disturbances and cognitive performance, as measured with a novel object recognition (NOR) test, in rhesus monkeys. Gross morphological measures, body composition (via DEXA-Scan) and response to intravenous glucose tolerance tests (IVGTTs) were determined in fifteen aged (mean=18-years old, SD=3.1) non-diabetic female rhesus monkeys. Monkeys then were tested in a NOR procedure with escalating phases of difficulty (Easy: 24 h access to identical objects, test with a single novel object; Moderate: 24 h access to different objects, test with a single novel object; Difficult: 10 min access to different objects, test with single novel object). Our results showed that successful performance on the NOR task positively correlated ($R^2=0.55$ to 0.79, $p<0.05$) with measures of insulin sensitivity during the IVGTT (glucose k rate/mean insulin levels). In addition, successful performance on the NOR task negatively correlated with mean insulin levels, as well as caliper measures of abdominal fat. These results suggest that performance on the NOR task was impaired in monkeys that were more insulin resistant and had higher abdominal fat levels when compared with healthy animals. In general, these findings suggest that a pre-diabetic condition is associated with impairments in cognition, which in turn might predispose individuals with T2DM to exacerbated cognitive decline.

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Poster

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Topic: F.02. Animal Cognition and Behavior

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The Wilhelm and Martina Lundgren Foundation

Title: Sex-dependent differences in the response to cranial irradiation of the young mouse brain

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Abstract: Radiation therapy in the treatment of pediatric brain tumors may lead to difficulties in cognitive functions later in life. These so called late effects may be influenced by age, treatment and gender. For example, young girls suffer more severe late effects compared to young boys. Unfortunately, most studies in this field of research have been performed using male rodents, and possible gender differences have not been considered. Therefore, we wanted to investigate possible short or long-term sex differences in response to cranial irradiation (IR) in mice. To do so, male and female mice were irradiated with a single dose of 8 Gray to the whole brain on postnatal day 14. We observed pronounced cell death in the brains of irradiated animals, as judged by caspase-3 activity 6 h after IR. IR also increased the levels of the chemokines MIP-1 alpha and CCL2 (MCP-1), indicating an acute IR-induced inflammation in the brain. No sex differences in these acute responses were however observed. Basal neurogenesis in non-irradiated mice was higher in females but this was not apparent until the animals were adult. Proliferation in the subgranular zone of the dentate gyrus in the hippocampus, as judged by the number of phospho-histone H3-positive cells, was reduced by half already 6 h after IR, same in males and females. This resulted in hampered growth of the granule cell layer (GCL), reduced BrdU incorporation and severely reduced neurogenesis (doublecortin-positive cells) in adulthood (4 months after IR), and this reduction was more pronounced in females. Irradiated mice displayed reduced brain weight, more pronounced in females (4.4% in males, 10.4% in females). To study behavioral effects after cranial IR, we used the open field test and the elevated plus maze 2 months after IR. The open field test showed that irradiated mice spent less time in the center of the arena, more pronounced in females (29% less in IR males, 54% less in IR females), possibly indicating increased anxiety. This was confirmed in the elevated plus maze, where females subjected to IR made shorter visits to the open arms, a difference that was not observed in males after IR. In an IntelliCage test, place learning was impaired by IR in females but not

males. Taken together, our results demonstrate that the young female mouse brain is more susceptible to ionizing radiation.

Disclosures: K.E. Roughton: None. **M. Kalm:** None. **K. Blomgren:** None.

Poster

921. Learning and Memory: Physiology II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.19/EEE47

Topic: F.02. Animal Cognition and Behavior

Support: CIHR Grant (MOP-102624)

Title: Outside the olfactory bulb? An investigation into the role of the piriform cortex in early odor preference learning in rats.

Authors: *G. MORRISON[1,2], C. FONTAINE[2], C. HARLEY[2], Q. YUAN[2]
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Abstract: Early odor preference learning provides us with a simple model to study cellular and synaptic processes that underlie learning and memory. In this model, we use associative learning and pair an aversive odor (peppermint) with tactile stimulation to train rat pups to form a preference for peppermint. Memory encoding processes in the olfactory bulb (OB) are well-documented during early odor preference learning, however the role of the olfactory cortex remains elusive. One major projection from the OB, the lateral olfactory tract (LOT), occurs in the dendrites of the pyramidal cells in layer Ia of the piriform cortex (PC). In this work, we investigate the role of the PC in early odor preference memory.
To gain insight into the role of the PC in early odor preference learning, we infused lidocaine (LIDO) into the anterior PC (aPC) at varying times during training or testing phases. LIDO transiently silences neurons, which was verified using pCREB immunohistochemistry (IHC). LIDO infusion into the aPC 10 minutes prior to training produced a reduced preference for peppermint bedding compared to saline-infused counterparts. In addition, LIDO infusion into the aPC 10 minutes prior to testing produced a reduced preference for peppermint bedding compared to saline-infused rats. These results suggest the aPC may play a role in both the encoding and retrieval of early odor memories. To expand on this, we used unilateral nostril occlusion and examined pCREB staining in pyramidal cells of the PC 10 minutes following either odor only or odor plus stroking. Preliminary results showed more pCREB staining in the spared hemispheres compared to occluded hemispheres, and more staining in the odor plus stroking animals compared to odor only.

To explore the cellular mechanisms underlying memory processes at the LOT-pyramidal cell synapse in the aPC we conducted *in vitro* electrophysiology recordings of field EPSP in layer Ia of the aPC with LOT stimulation. We induced long-term potentiation (LTP) following 1 theta burst stimulation (TBS), 4 TBS, and 8 TBS protocols. We studied the input/output relationship before and following LTP induction and used the initial slope of field EPSP to measure the AMPA components of EPSP. The ratio of the change in the presynaptic volley to the AMPA slope was measured and was greater post-LTP versus pre-LTP, suggesting a postsynaptic expression mechanism involved in LOT-PC LTP. Finally, LTP at this synapse is NMDAR-dependent. Using an NMDAR antagonist (D-APV), we were unable to induce LTP, and once D-APV was washed out, LTP was inducible. Future *ex vivo* experiments will test the common properties between natural odor preference learning and *in vitro* LTP mechanisms.

Disclosures: G. Morrison: None. **C. Fontaine:** None. **C. Harley:** None. **Q. Yuan:** None.

Poster

921. Learning and Memory: Physiology II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.20/EEE48

Topic: F.02. Animal Cognition and Behavior

Title: Sleep-promoting doses of Diazepam, Eszopiclone and Zolpidem disrupt Rhesus macaque memory and attention as compared to DORA-22, a dual orexin receptor antagonist.

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Abstract: Approximately 10% of the adult population experiences insomnia, with 1/3 of sufferers seeking pharmacotherapy intervention in order to treat their symptoms. A novel mechanism is represented by the orexinergic system which plays a critical role in the regulation of sleep/wake cycles. In an effort to develop medications with improved tolerability we have identified a potent and selective dual orexin receptor antagonist, DORA-22. In the current study we have compared the effects of the nonbenzodiazepine GABA-A receptor modulators Eszopiclone and Zolpidem, and the benozodiazepine Diazepam, to DORA-22 on short term recognition memory (delayed match to sample [DMS]) and sustained spatial attention (serial

choice reaction time [SCRT]) in rhesus macaques, using a novel in-cage touchscreen system. The doses of compounds tested in cognition assays were selected based on those shown to reduce active wake, a measure of sleep promotion, established by polysomograpgy. In the absence of a decrease in active wake the compounds were assessed up to the maximum dose at which monkeys would consistently work in cognitive tasks. At these doses, Diazepam, Eszopiclone and Zolpidem resulted in a disruption in recognition memory in DMS that was independent of delay. In marked contrast recognition performance was unaffected by DORA-22 at doses up to 30X greater than the minimum effective dose that reduced active wake. Similarly, administration of Diazepam, Eszopiclone and Zolpidem, at doses at, or below, the minimum effective dose reducing active wake, resulted in disrupted performance in SCRT that was marked at short, but not long, cue durations, indicative of impaired attention. DORA-22 was without effect on SCRT performance at 30X greater than the minimum effective dose in active wake. These preclinical data provide further evidence that DORAs may represent an important new strategy in the treatment of insomnia. Clinical studies are currently in progress to further understand the safety and efficacy of dual orexin receptor antagonists.

Disclosures: S.J. Tye: None. **J.M. Uslaner:** None. **D.M. Eddins:** None. **A.T. Savitz:** None. **S.V. Fox:** None. **C.E. Cannon:** None. **J. Binns:** None. **S.L. Garson:** None. **J. Stevens:** None. **L. Yao:** None. **R. Hodgson:** None. **J. Brunner:** None. **T.P. McDonald:** None. **M.R. Bowlby:** None. **P.L. Tannenbaum:** None. **S.D. Kuduk:** None. **A.L. Gotter:** None. **C.J. Winrow:** None. **J.J. Renger:** None.

Poster

921. Learning and Memory: Physiology II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.21/EEE49

Topic: F.02. Animal Cognition and Behavior

Title: Sleep-promoting doses of diazepam, eszopiclone and zolpidem more negatively impact novel object recognition memory in rat as compared to dual orexin 1 and 2 receptor antagonism.

Authors: *J. M. USLANER, D. M. EDDINS, X. WANG, S. J. TYE, S. V. FOX, C. E. CANNON, A. T. SAVITZ, J. BINNS, S. L. GARSON, L. YAO, R. HODGSON, J. BRUNNER, T. MCDONALD, P. L. TANNENBAUM, A. L. GOTTER, S. D. KUDUK, P. J. COLEMAN, C. J. WINROW, J. J. RENGER
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Abstract: Approximately 10% of the general population suffers from chronic insomnia and 30% of insomniacs use pharmacotherapy. The current standards of care, including the nonbenzodiazepine GABA-A receptor modulators Eszopiclone and Zolpidem, may carry unwanted side-effects, including cognitive disruption. With the aim of developing better tolerated medications, we have identified dual orexin 1 and 2 receptor antagonists (DORAs), which promote sleep in preclinical species as well as in humans. Here we compared the effects of the GABA modulators Eszopiclone, Zolpidem, and the benzodiazepine Diazepam to a DORA on novel object recognition memory performance in rats. Doses of each compound tested were based on the ability to reduce active wake, as defined by electroencephalogram (EEG) and electromyogram (EMG). We report that in preclinical assays Eszopiclone, Zolpidem, and Diazepam disrupted recognition memory at doses well below those affecting active wake. In marked contrast, DORA-22 only disrupted novel object recognition performance at doses far exceeding those required to reduce wakefulness. Moreover, we found that the low doses of the GABA modulators that impacted recognition memory also decreased hippocampal ARC protein expression, which is involved in synaptic plasticity, whereas only very high doses of DORA-22 impacted ARC. These findings in two preclinical models suggest that DORAs may carry less risk associated with cognitive impairment than the current standards of care, which could have important implications for treating insomnia. Clinical studies are ongoing to understand the efficacy and safety profile of DORAs.

Disclosures: J.M. Uslaner: None. **D.M. Eddins:** None. **X. Wang:** None. **S.J. Tye:** None. **S.V. Fox:** None. **C.E. Cannon:** None. **A.T. Savitz:** None. **J. Binns:** None. **S.L. Garson:** None. **L. Yao:** None. **R. Hodgson:** None. **J. Brunner:** None. **T. Mcdonald:** None. **P.L. Tannenbaum:** None. **A.L. Gotter:** None. **S.D. Kuduk:** None. **P.J. Coleman:** None. **C.J. Winrow:** None. **J.J. Renger:** None.

Poster

921. Learning and Memory: Physiology II

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.22/EEE50

Topic: F.02. Animal Cognition and Behavior

Support: NIDA-IRP

Title: Dissociating accounts of risk vs. salience encoding in the orbitofrontal cortex

Authors: *G. ESBER[1], S. SHAPIRO[2], A. HERNANDEZ[3], M. OGAWA[4], J. TRAGESER[5], T. STALNAKER[1], G. SCHOENBAUM[1]

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Abstract: Several labs have reported that a subpopulation of OFC neurons responds more in anticipation of probabilistic than certain rewards. This finding has led to the proposal that OFC is involved in encoding of risk or uncertainty. We recently
investigated this hypothesis by recording the activity of OFC neurons while rats responded to four odor cues rewarded at 100%, 67%, 33%, or 0% probability. Consistent with prior reports, we found a population of neurons particularly
responsive in anticipation of uncertain reward (33% or 67% > 100% or 0%). However, these neurons also exhibited larger responses prior to certain reward than certain non-reward (100% > 0%) and prior to low than high probability
reward (33% > 67%). This asymmetrical pattern is poorly explained by risk or uncertainty accounts, for they predict that a similar neuronal response should have been observed in anticipation of the two certain outcomes (100% = 0%) as
well as the two probabilistic ones (33% = 67%). Interestingly, these results are precisely what would be expected by theoretical accounts of acquired salience. To further explore the contrasting predictions of risk vs. salience, here we conducted a variation of the above study in which activity of OFC neurons was recorded across two blocks of trials. In the first block, intended to replicate our previous findings, rats were trained to respond to five odor cues rewarded at 100%, 75%, 50%, 25%, and 0% probability. In the second, critical block, another set of five odors signaled the presentation of either of two equally valued rewards at 100%, 75%, 50%, 25%, and 0% probability. Notably, the cues in the second block should have the same salience as their counterparts in the first block, but equal risk. An analysis of the data will be presented that will dissociate risk- vs. salience-based hypotheses.

Disclosures: G. Esber: None. **S. Shapiro:** None. **A. Hernandez:** None. **M. Ogawa:** None. **J. Trageser:** None. **T. Stalnaker:** None. **G. Schoenbaum:** None.

Poster

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Program#/Poster#:921.23/EEE51

Topic: F.02. Animal Cognition and Behavior

Support: NIDA Grant DA017949

NIDA Grant DA024787

NCI Grant CA143187

NIDA Training Grant T32DA007237

Title: Thyroid receptor beta is critically involved in the cognitive enhancing effects of nicotine, while thyroid receptor alpha1 is not

Authors: *P. T. LEACH[1], J. W. KENNEY[2], T. J. GOULD[1]
[1]Psychology/Neuroscience, Temple Univ., Philadelphia, PA; [2]Univ. of Southampton, Southampton, United Kingdom

Abstract: Cigarette smoking is common despite its adverse effects on health, including cardiovascular disease and stroke. Nicotine, the main psychoactive component of cigarette smoke, may contribute to the development of addiction by enhancing maladaptive drug-context associations. Nicotine may also have long-lasting detrimental effects on the endocrine system. Specifically, nicotine may increase thyroid hormone secretion, metabolism, and receptor activation. Low thyroid hormone levels in adulthood can confer memory deficits. Animal models of disrupted thyroid function suggest nicotine reverses the memory deficits associated with low hormone levels. Initial examination with Protein/DNA arrays (Panomics) identified hippocampal thyroid receptor activation during nicotine enhancement of hippocampus-dependent learning. The present work evaluates the contribution of thyroid hormone receptor β (TRβ) and thyroid hormone receptor α1 (TRα1) in the effect of nicotine on hippocampus-dependent memory using a contextual fear conditioning paradigm. It was hypothesized that TR would be important for the acute effects of nicotine on contextual fear conditioning, but it was unclear which receptor type would be critical. To determine the role of TRβ and TRα1 in the effects of nicotine on memory, mice lacking the TRβ and TRα1 gene (KOs) and their wildtype littermates (WTs) were administered acute nicotine prior to training and testing on contextual fear conditioning. Twenty-four hours after training, mice were returned to the training context and evaluated for freezing, a species-typical fear response, for 5 minutes and this was used as a measure of contextual fear memory. For TRβ mutants, WTs receiving nicotine froze significantly more than WTs receiving vehicle alone, while KOs receiving nicotine did not freeze more than those receiving saline. This demonstrates a critical role for TRβ in the acute effect of nicotine on hippocampus-dependent memory. For TRα1 mutants, WTs and KOs receiving nicotine froze significantly more than those receiving vehicle alone. The present study may inform novel treatment approaches to smoking cessation therapies. Future directions include the evaluation of the role for TRα1 and TRβ on the cognitive deficit associated with nicotine withdrawal.

Disclosures: P.T. Leach: Research Grant; National Institute on Drug Abuse, DA017949, National Institute on Drug Abuse, DA024787, National Cancer Institute, CA143187. Other Research Support; National Institute on Drug Abuse Training Grant, T32DA007237. **J.W.**

Kenney: None. **T.J. Gould:** Research Grant; National Institute on Drug Abuse, DA017949, National Institute on Drug Abuse, DA024787, National Cancer Institute, CA143187.

Poster

921. Learning and Memory: Physiology II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:921.24/EEE52

Topic: F.02. Animal Cognition and Behavior

Support: ANR-07-Neuro-019-01

Title: Neuronal activity of the monkey prefrontal cortex during observational learning

Authors: *F. ISBAINE[1], A. BELMALIH[2], M. DEMOLLIENS[2], A. BROVELLI[3], M. MEUNIER[4], D. BOUSSAOUD[2]
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Abstract: We have been investigating neuronal mechanisms of observational learning in monkeys. Two animals sitting in primate chairs, facing each other, executed a visuomotor task involving arbitrary associations between images and spatial targets (white squares) on a touch screen. Only one monkey had access to the touch screen at a time (actor). The other could only (observer). The monkeys alternated between the actor and observer role. The actor initiated a trial by placing his hand on a white rectangle located near his chest. Then a visual cue appeared together with 2 or 4 targets. Pressing the correct target led to a success signal (green ring), which was followed by a reward (a drop of fruit juice) after a delay. Incorrect response led to an error signal (red ring) and no reward was given. Single neurons and local field potentials (LFPs) were recorded from the dorsolateral prefrontal cortex (DLPF) of one monkey while he observed the other monkey performing two tasks: 1) learning by trial-and-error arbitrary visuomotor association; 2) learning after having previously observed the correct and incorrect associations made by the actor. In a previous abstract (Belmalih & al, 2010), we have shown that feedback-related activity of DLPF single neurons varied across experimental conditions: observation, learning after observation (LeO), trial-and-error learning or execution of a familiar task. Here, we analyzed the LFPs from 61 recording sessions to determine variations of neural activity across conditions at the population level. Event related potentials (ERP) and event related

spectral perturbations (ERSP), aligned on the presentation of correct and incorrect visual feedbacks were analyzed. We found that the ERP and ERSP signals were modulated depending on the conditions. In particular, the temporal and spectral specificity of these signals was significantly different between the two main conditions, i.e. TE and LeO. These results provide additional evidence supporting the involvement of DLPF in observational learning.
Ref.: A.BELMALIH , D. THURA, F. ISBAINE, A. BROVELLI, M. DEMOLLIENS, M. L. MEUNIER, D. BOUSSAOUD. Neural correlates of observational learning in the macaque monkey. Program No. 404.19. 2010. Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online.

Disclosures: F. Isbaine: None. **A. Belmalih:** None. **M. Demolliens:** None. **A. Brovelli:** None. **M. Meunier:** None. **D. Boussaoud:** None.

Poster

921. Learning and Memory: Physiology II

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Program#/Poster#:921.25/EEE53

Topic: F.02. Animal Cognition and Behavior

Support: NIDA Grant DA017949

NIDA Grant DA024787

NCI Grant CA143187

Title: Relationship between tolerance to the cognitive enhancing effect of nicotine and nicotine withdrawal-related impairments in contextual conditioning

Authors: *T. J. GOULD, D. S. WILKINSON, M. D. ADOFF
Dept Psychol, Temple Univ., PHILADELPHIA, PA

Abstract: Nicotine addiction is a multifaceted disease that can be influenced by several factors. Nicotine modulates various types of learning and memory and the ability of nicotine to alter cognitive processes may contribute to its addictive liability. Acute nicotine enhances contextual conditioning in mice, tolerance develops to this effect with chronic administration, and withdrawal from chronic nicotine impairs contextual conditioning. While tolerance and withdrawal-induced learning impairments both occur following chronic nicotine administration, it is unknown if they share similar or separate mechanisms. The present series of experiments were designed to provide evidence that tolerance and withdrawal are dissociable. C57BL/6J mice

were implanted with osmotic minipumps that delivered constant nicotine or saline for various durations and then were trained and tested in contextual conditioning either during chronic nicotine administration or 24 hours after pump removal. Chronic nicotine enhanced contextual conditioning in a dose- and time-dependent manner. Tolerance developed quickly to the enhancing effect of chronic nicotine. Furthermore, the duration of chronic nicotine treatment required to produce cognitive deficits upon cessation of treatment differed than that required to produce tolerance, which suggests that tolerance and withdrawal are mediated by separate mechanisms. The current findings add to the growing body of literature dissociating tolerance from withdrawal.

Disclosures: T.J. Gould: Research Grant; NIDA DA017949, NIDA DA024787, NCI CA143187. **D.S. Wilkinson:** None. **M.D. Adoff:** None.

Poster

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Program#/Poster#:921.26/EEE54

Topic: F.02. Animal Cognition and Behavior

Support: BMBF 01GQ0440

Title: Complex virtual environments for mongolian gerbils.

Authors: *C. LEIBOLD, J. HENKE, A. WÄTZIG, B. LUDWIG, J. HERMANN, C. TATARAU, K. THURLEY
Univ. of Munich, Planegg-Martinsried, Germany

Abstract: Virtual reality (VR) environments are becoming increasingly available to study spatial navigation in rodents. In many approaches so far spatial navigation did not go beyond simple open fields or linear tracks. How to make rodents learn to navigate in more complex environments is hardly investigated. We use a VR setup in which a spherical treadmill is placed inside a 360 degree toroidal screen onto which the virtual environment is projected. This design permits a fixation of the animal such that it can freely rotate around its vertical body axis. We trained gerbils (Meriones unguiculatus) to perform spatial tasks in virtual mazes of different complexity. First the animals learned to run back and forth between the two ends of a virtual linear track. At the ends they received a food reward. They had to learn to orient with only visual information and to avoid contact with the virtual walls. In this way they optimized the path from one reward location to the next. Performance increased within about 10 training sessions to almost optimal values: The animals avoided virtual walls and ran straight paths from one track

end to the other. Running speed was consistent with what is typically observed in real environments. When afterwards presenting more complex mazes such as U- or 8-shaped arenas the animals were able to transfer their previously acquired skills to the new tasks after only a few trials. Our behavioral setup can be straight-forwardly combined with extracellular recording techniques such as tetrodes.

Disclosures: C. Leibold: None. **J. Henke:** None. **A. Wätzig:** None. **B. Ludwig:** None. **J. Hermann:** None. **C. Tatarau:** None. **K. Thurley:** None.

Poster

921. Learning and Memory: Physiology II

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Program#/Poster#:921.27/EEE55

Topic: F.02. Animal Cognition and Behavior

Support: R01MH084964

R01MH086563

Title: Cells in the primate hippocampus provide a timing signal for particular trial contexts.

Authors: *J. J. SAKON[1], S. WIRTH[2], Y. NAYA[1], W. A. SUZUKI[1]
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Abstract: Recent work from our lab has implicated the hippocampus in keeping track of time through incremental changes in neuronal firing rates during a temporal order memory task (Naya & Suzuki, *Science* **333** August 2011). This incremental timing signal is characterized by gradual increases or decreases in firing rate across a delay period between task-essential encoding events. Here we ask if incremental timing can be found during an object-place association task that is not contingent on temporal order memory. In this task (S. Wirth *et al*, *Neuron* **61** March 2009), monkeys were presented with one of two novel objects shown in one of two possible places on a computer monitor. Following a 700 ms delay period with only a fixation point on the screen, the subject was cued to release a bar during the presentation of either an orange circle (early release), or 500 ms later, a green circle (late release). All trials required visual fixation on a central fixation dot. Each day, the subjects learned that two of the four possible object-place combinations were associated with the early bar release while the remaining two were associated with the late bar release. Correct responses were always followed by juice reward. We analyzed 137 isolated hippocampal neurons with a firing rate greater than 1 Hz averaged across the delay interval of this task. We find a large group of cells (N=79) that display a gradual increase or

decrease in firing rate across the delay, indicating that incremental timing signal is present during this object-place task. These 79 cells are separated into two groups. Similar to the incremental timing signal initially described in the temporal order memory task, some of these cells (N=34/79) signal timing similarly for both early and late release trial types. A second group of cells (N=45/79) provide timing signals selective to particular trial contexts. Specifically, these latter cells show incremental timing signals selective to either early or late release trials during the delay. Similar contextual control of a hippocampal timing signal was also reported in rodents during the performance of an object-odor association task (MacDonald *et al, Neuron* **71** August 2011). Such a context-dependent incremental timing signal could contribute to differentiating between distinct episodic memories.

Disclosures: J.J. Sakon: None. **S. Wirth:** None. **Y. Naya:** None. **W.A. Suzuki:** None.

Poster

922. Learning and Memory Invertebrates II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:922.01/EEE56

Topic: F.04. Neuroethology

Support: FP7 BIOTACT ICT-215910

Title: Toward a fusion model of feature and spatial tactile memory in the Australian crayfish Cherax destructor

Authors: *F. W. GRASSO[1], M. EVANS[2], J. A. BASIL, X[1], T. J. PRESCOTT[2]
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Abstract: Australian crayfish Cherax destructor (ACCD) use their haptic sense to navigate in enclosed spaces in the absence of visual input, use their mobile antennae to explore novel objects and remember, in some manner, the configuration of the surfaces in their environment. We report on parallel biological and robotic simulation studies intended to advance our understanding of the memory systems underlying this ability. We hypothesize that the Medial Protocerebrum (MPC), an unpaired, high-order central brain structure that receives highly processed perceptual information from all the cerebral sensory modalities and from the thoractic ganglia is a site for spatial memory processing. The two key streams of information useful for spatial mapping in ACCD, tactile input from the antennae and proprioceptive information from the legs (‘step-counting’ odometry) converge in the MPC. The control architecture of our CrayTouchBot (CTBR) robot models this neurobiological system. In previous cybernetic

research we demonstrated the plausibility of an interrupt-based cross-correlation (IBCC) mechanism using sensors mechanically fixed to the robot to account for some of the ACCD's spatial memory abilities: learning an arena's configuration by abstracting and recording series of wall contacts during thigmotaxis (wall-following) behavior. We extended those results using a pair of movable mechanical touch-sensors to inform both the IBCC and a second memory module to capture surface exploration ACCD behavior. Numerical correlation between the current sensor sweeps or 'antennation' sensation patterns (ASP) and stored previously experienced ASPs permit the classification of the current location as familiar or novel. In the CTBR control system the IBCC and the ASP memory systems share information to enhance each other's performance in its primary function: the positional confidence of the IBCC biases the ASP recall process and vice versa to arrive at combined recall readouts that use both forms of stored information and recognition. These in turn inform steering decisions in the CTBR during exploration. The combination of the ASP and IBCC memory systems is a novel computational approach that is constrained by the MPC connectivity. We report on animal and robot studies aimed at determining whether the behavior of the CTBR endowed with these interacting memory systems reproduces ACCD exploration behavior: thigmotaxis punctuated by episodes of antennation at surfaces containing tactilely inhomogeneous structures. Understanding such haptic memory systems might also be useful designing robotics applications where GPS and vision are not available for spatial guidance.

Disclosures: F.W. Grasso: None. **M. Evans:** None. **J.A. Basil:** None. **T.J. Prescott:** None.

Poster

922. Learning and Memory Invertebrates II

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:922.02/EEE57

Topic: F.02. Animal Cognition and Behavior

Support: Facultad de Medicina, UNAM

Title: Memory and social interactions in crayfish

Authors: N. JIMÉNEZ-MORALES[1], A. R. ESPINOZA[2], K. MENDOZA-ANGELES[1], G. ROLDÁN[2], *J. HERNANDEZ-FALCON[2]
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Abstract: In crustaceans smell is the most important sensory cue for agonistic interactions and it has been proposed that memory is essential to maintain the hierarchical organization within a

given group. However, this kind of memory (which we will call agonistic memory) has not been characterized. How long this memory lasts? How is it consolidated? Is it related with sleep? These are the main questions that we approached in this work. Our first goal was to characterize this agonistic memory, but memory tests for crustaceans are scarce. Therefore we designed a set of experiments in order to investigate forgetting.
Hierarchy in crustaceans is stable in a given group as measured in repeated trials which may imply a clear role for memory. In these conditions, the intensity of interactions diminishes on a daily fashion, in a well described pattern. On these bases we performed experiments that disturb this progression in memory consolidation.
We used male adult crayfish, Procambarus Clarkii, triads in intermolt to evaluate social interactions. Agonistic interactions were videotaped for one hour in a circular arena for 5 consecutive days. We had two experimental groups. Group 1 animals received an injection of scopolamine (100 ng/g) after the fist interaction. Group 2 animals were cold anesthetized. A third group was injected with saline solution finally; a fourth group was a non manipulated control. Results show that scopolamine impairs long-term hierarchical organization in subsequent encounters. This is expressed as an increase in positive contacts, especially from the dominant subject, although, scopolamine did not modify the hierarchical organization established in the first encounter. Cold anesthesia also impaired the hierarchical organization in a similar way as scopolamine, though with less intensity. Surprisingly saline injection also seemed to produce forgetting, but this effect was slight and of short duration. These results suggest that the social status is learned and can be forgotten.

Disclosures: N. Jiménez-Morales: None. **A.R. Espinoza:** None. **K. Mendoza-Angeles:** None. **G. Roldán:** None. **J. Hernandez-Falcon:** None.

Poster

922. Learning and Memory Invertebrates II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:922.03/EEE58

Topic: F.02. Animal Cognition and Behavior

Title: Short term memory in the leg muscle control system of the stick insect Cuniculina impigra

Authors: *E. BERG, J. SCHMIDT, A. BÜSCHGES
Univ. of Cologne, Cologne, Germany

Abstract: Walking stick insects that do not find a foothold perform searching movements (SMs) with the respective leg (Bässler, 1993; Dürr, 2001). SMs are rather stereotypic, cyclic

movements. Here we explore changes in space and time in the SMs upon a one-time disturbance by an obstacle. Experiments were performed on stick insects (Cuniculina impigra) with a single intact foreleg that was restrained to move in the vertical plane. A stick was moved into the leg's movement path such that it was touched one time by the distal tibia. After touching, animals modified their SMs (i) by shifting them towards the position of disturbance and (ii) by significantly decreasing SM amplitude. These changes were independent of where in the leg's trajectory the stick was touched. Only slowly the movements shifted back to the original position accompanied by a simultaneous increase in amplitude. This targeted response outlasting a single stimulus for several seconds expresses properties of a sort of short term memory of the insect leg muscle control system.

We investigated the role of leg sense organs for the successful execution of the targeted motor response by means of ablation experiments: (i) no influence on SMs was detected following replacement of the tibia by a peg leg, i.e. following removal of sensory signals distal to the femur. (ii) SMs were impaired when proprioceptors reporting positions of femur and tibia were ablated.

In order to study the underlying changes in motor output leg muscle activity was monitored by EMG recordings from levator and depressor trochanteris. The ratio between the integrals of their muscle activity changed upon disturbance and was related to the direction of shift of SMs. Duty cycles and phase relations did, however, not change. Currently, we further explore the mechanism underlying the targeted response by intracellularly recording leg motor and premotor interneurons.

Disclosures: E. Berg: None. **J. Schmidt:** None. **A. Büschges:** None.

Poster

922. Learning and Memory Invertebrates II

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Program#/Poster#:922.04/EEE59

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant NS052351

Title: Distinct traces for appetitive versus aversive olfactory memories in DPM neurons of *Drosophila*

Authors: *I. CERVANTES-SANDOVAL, R. L. DAVIS
Neurosci., The Scripps Res. Inst., Jupiter, FL

Abstract: The general logic used by the brain for differentially encoding positive and negative memories remains unknown along with how such experiences are represented by collections of memory traces at the cellular level. Here we compare the cellular memory traces that form in the dorsal paired medial (DPM) neurons of Drosophila after conditioning flies with odors associated with negative or positive unconditioned stimuli (US). Our results show that the appetitive DPM neuron trace is distinguished from the aversive trace in three fundamental features: (1) The DPM neurons do not respond to an appetitive US of sucrose by itself, in contrast to their robust response to an aversive US of electric shock. (2) The appetitive trace persists for twice as long as the aversive trace. (3) The appetitive trace is expressed in both neurite branches of the neuron, rather than being confined to a single neurite branch like the aversive trace. Aditionally, we demonstrate that training flies with non-nutritive sugars that elicit a behavioral memory that decays within 24 hr generates, like aversive conditioning, a short-lived and branch-restricted memory trace, whereas training with nutrient sugars generates non-decaying behavioral memory and the more persistent and cell-wide memory trace. These results indicate that the persistence and breadth of the DPM neuron memory trace influences the duration of behavioral memory.

Disclosures: I. Cervantes-Sandoval: None. **R.L. Davis:** None.

Poster

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Program#/Poster#:922.05/EEE60

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant NS19904

Title: Dopamine is required for learning and forgetting in *Drosophila*

Authors: *J. A. BERRY[1,2], I. CERVANTES-SANDOVAL[1], E. P. NICHOLAS[1], R. L. DAVIS[1]
[1]The Scripps Res. Inst. Florida, Jupiter, FL; [2]Developmental Biol., Baylor Col. of Med., Houston, TX

Abstract: Psychological studies in humans and behavioral studies of model organisms suggest that forgetting is a common and biologically regulated process, but the molecular, cellular, and circuit mechanisms underlying forgetting are poorly understood. Here we show that the bidirectional modulation of a small subset of dopamine neurons (DANs) after olfactory learning regulates the rate of forgetting of both punishing (aversive) and rewarding (appetitive) memories. Two of these DANs, MP1 and MV1, exhibit synchronized ongoing activity in the mushroom body neuropil in alive and awake flies before and after learning as revealed by functional cellular

imaging. Furthermore, while the mushroom body-expressed dDA1 dopamine receptor is essential for the acquisition of memory, we show that the dopamine receptor DAMB, also highly expressed in mushroom body neurons, is required for forgetting. We propose a dual role for dopamine: memory acquisition through dDA1 signaling and forgetting through DAMB signaling in the mushroom body neurons.

Disclosures: J.A. Berry: None. **I. Cervantes-Sandoval:** None. **E.P. Nicholas:** None. **R.L. Davis:** None.

Poster

922. Learning and Memory Invertebrates II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH K99 MH092294

NIH R01 NS19904

Title: Uniform activation of postsynaptic cAMP responses by dopaminergic neurons in the *Drosophila* mushroom body

Authors: *S. M. TOMCHIK, R. L. DAVIS
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Abstract: Changes in cAMP are critical for olfactory associative learning, and cAMP signaling in the mushroom body (MB) is necessary for learning in *Drosophila*. Dopamine is believed to relay punishment signals to the mushroom body during olfactory classical conditioning, elevating cAMP to generate the plasticity underlying learning. In order to understand how dopamine activates cAMP, we imaged dopamine responses with a genetically encoded cAMP reporter in the *Drosophila* mushroom bodies *in vivo*. The thermosensitive TRPA1 channel was expressed in different subsets of afferent dopaminergic neurons that innervate the mushroom bodies. When stimulating each of these subsets of neurons, we observed increases in cAMP. These increases were observed across the vertical and medial mushroom body lobes. These data suggest that dopamine elevates cAMP in the mushroom body lobes in a uniform manner. Further experiments are needed to decipher how these responses are differentiated from those recruited during classical conditioning, where neuronal activity encoding the conditioned stimulus precedes or overlaps activation of dopaminergic pathways that signal the unconditioned (aversive) stimulus.

Disclosures: S.M. Tomchik: None. **R.L. Davis:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

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NSFC Grant 31130027

Title: Cross-modal memory transfer in *Drosophila*

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Abstract: In animals, cross-modal plasticity happens universally when the inter-modal state meets the changing environment. Here, by using the sensory preconditioning (SP) paradigm, we investigated the cross-modal memory transfer in *Drosophila*. It was reported that the conditioned response to the reinforced olfactory/visual stimulus (S1) can be transferred to the unreinforced visual/olfactory stimulus (S2) [1]. Typically, the behavioral expression of memory retrieval was thought via S1-S2 association [2]. However, whether other associations to S2 exist in the SP is largely unknown.

The mushroom bodies (MBs) and the fan-shaped body (FB) of *Drosophila* are thought to form and reserve the short-term olfactory and visual memory, respectively. Firstly, we proved that the 247>*shibire* specifically impaired the olfactory memory retrieval, but left the visual elevation memory retrieval intact. Besides, c205>*shibire* specifically impaired the elevation memory retrieval but not the olfactory memory. It was indicated that the output of α/β lobes of MBs and the layer 5 of FB were indispensable for uni-modal olfactory and visual memory retrieval, respectively. Next, we investigated cross-modal memory transfer in both olfactory-visual (O-V) and visual-olfactory (V-O) directions.

In the O-V direction, the olfactory cues were reinforced by heat punishment (US) after unreinforced exposure to paired olfactory and visual stimuli. The response to visual cues was

then tested. We found that the conditioned response to visual stimuli cannot be impaired by blocking MBs, which was contradictory to the S2-S1-US assumption, because the memory recall through S1-US should be blocked here. Therefore, the S1-S2 association may be not the only way for S2 memory retrieval. The existence of S2-(US)-Response can be further supported by the fact that blocking FB left the memory retrieval in the V-O direction intact. On the other hand, MBs blocked impaired the conditioned response in the V-O direction. However, FB blocked left the O-V direction's memory retrieval intact. The asymmetric function of MBs and FB in the process of cross-modal memory transfer strongly suggested that the MBs may reserve the inter-modal associations. It can be supported by the results that dysfunction of MBs and FB simultaneously blocked the memory retrieval in both O-V and V-O directions.
In sum, we found the conditioned response to unreinforced cues can be recalled from parallel pathways rather than S1-S2 association; we also speculated the multi-function role played by MBs in the cross-modal memory transfer processing.
1. Guo J, Guo A (2005). Science 309: 307-310
2. Gewirtz JC, Davis M (2000). Learning & Memory 7: 257-266

Disclosures: X. Zhang: None. **Q. Ren:** None. **A. Guo:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: BBSRC (BB/G008973/1)

Wellcome PhD program (083361)

Title: The role of CASK and CaMKII autophosphorylation in regulation of synaptic growth, calcium signalling and associative learning

Authors: *J. J. HODGE, B. R. MALIK, J. M. GILLESPIE
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Abstract: CaMKII autophosphorylation at threonine 287 results in a kinase that is constitutively active and calcium independent. Synaptic mechanisms regulating CaMKII autophosphorylation are therefore central to neural plasticity and memory formation with disregulation of autophosphorylation being associated with a range of pathological states. One such CaMKII-regulatory molecule is CASK which when mutated in human causes a number of neurological

defects including mental retardation. CASK knock-out mice are lethal due to a cleft palate phenotype. *Drosophila* however has a highly conserved homologue of the single human CASK gene which when deleted is viable. We show removal of the CASK isoform that contains the CaMK-like and L27 domains (CASK-ß) results in larva with more numerous but smaller synapses at their neuromuscular junctions. These CASK-ß mutant synapses also had increased levels of CaMKII T287 autophosphorylation. Direct motorneuron expression of phospho-mimic CaMKII T287D also caused a similar increase in synapse number as the CASK-ß mutant with CASK overexpression returning the synapse number to wild-type levels. CASK mutants showed reduced activity dependent calcium (Ca^{2+}) signalling in motorneuron cell bodies and synaptic terminals accompanied by a reduction in vesicle recycling. Reduction in CASK and T287D overexpression both gave a similar reduction in Ca^{2+} signalling with CASK overexpression partially returning Ca^{2+} signalling to normal levels consistent with CASK and CaMKII acting in a common pathway controlling activity dependent Ca^{2+} signalling and synaptic growth. We also show CASK mutant larvae have reduced olfactory associative learning, which localised to the mushroom body with targeted reduction of CASK in these neurons causing a reduction in odour induced Ca^{2+} response. In adults, CASK mutation removes short- and long-term memory, with CASK function in the mushroom body α' and ß' neurons being required for its role in memory. Likewise directly changing the levels of CaMKII autophosphorylation in these neurons similarly reduced short- and long-term memory. Therefore CASK regulates CaMKII autophosphorylation in a pathway required for memory formation involving activity dependent changes in Ca^{2+} signalling in the mushroom body α' and ß' neurons.

Disclosures: J.J. Hodge: Employment; University of Bristol. Research Grant; BBSRC (BB/G008973/1). Other Research Support; Wellcome PhD program (083361). **B.R. Malik:** None. **J.M. Gillespie:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH/NIGMS T32GM007507

Title: Nuclear gating of *Drosophila* CREB in response to environmental stimuli

Authors: *R. A. FROPF[1], T. C. TUBON, Jr[2], J. C. P. YIN[1]
[1]Genet., Univ. of Wisconsin Madison, Madison, WI; [2]Biotech., Madison Area Tech. Col., Madison, WI

Abstract: CREB (cAMP Response Element Binding protein) is a crucial transcriptional regulator of numerous nervous system processes. The Drosophila homolog of CREB, dCREB-2, has well-described roles in sleep, homeostasis, memory formation, and neurodegenerative diseases. Additionally, different protein isoforms of the dCREB2 gene have been shown to have differential effects on these processes. Here, we generated antibodies against specific dCREB2 isoforms to investigate the activity and subcellular localization of these different protein species. We found that these antibodies reveal dramatically different cellular localization of protein isoforms in both fly brains and cultured Drosophila Schneider cells. While at baseline, the majority of dCREB2 protein is located outside the nucleus, relevant stimuli, such as serum starvation in cells and memory formation in flies, can change these localizations. These results indicate that dCREB2 localization is an important component to an organism's response to its environment. Understanding this process may provide a more mechanistic picture for how these responses are mediated and regulated during dCREB-2's participation in adaptive responses.

Disclosures: R.A. Fropf: None. **T.C. Tubon:** None. **J.C.P. Yin:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Program 973 2006CB500806

Program 973 2009CB941301

Title: Two subsets of mushroom body neurons are required to retrieve olfactory memory in *Drosophila*.

Authors: *Z. XIE[1], Y. ZHONG[2]
[1]Beijing, China; [2]Cold Spring Harbor Lab., New York, NY

Abstract: The identification of neural ensembles involved in memory retrieval is crucial for understanding the mechanism of memory processing, and memory deficiency displayed in many neurological diseases. Mushroom body is a core place for olfactory memory processing. To

further identify the mushroom body neural ensembles involved in memory retrieval, we examined the roles of various subsets of mushroom body neurons in olfactory 3h memory retrieval. Our results revealed that the retrieval of aversive and appetitive 3h memories required the synaptic outputs from two subsets of mushroom body neurons. Thus, in *Drosophila* mushroom body, both aversive and appetitive 3h memories are retrieved through two routes. Using at least two routes to retrieve memory may serve as an evolutionary conserved strategy during memory processing.

Disclosures: Z. Xie: None. **Y. Zhong:** None.

Poster

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Program#/Poster#:922.11/EEE66

Topic: F.02. Animal Cognition and Behavior

Support: DFG Research Group 1363

Title: Octopamine plays a role in the proboscis extension response (PER) of honeybees (Apis mellifera)

Authors: C. BUCKEMÜLLER, R. ZEUMER, *D. EISENHARDT
Bio, Chem, Pharm, Freie Univ. Berlin, Berlin, Germany

Abstract: Octopamine, a biogenic amine, is most likely to play an important role as a neurotransmitter, a neuromodulator and a neurohormone in invertebrates. It is important for different behaviors, i.e. aggression, egg-laying and walking. It also plays a crucial role in appetitive learning in Drosophila and honeybees. However, the precise mechanisms are unknown. Therefore, we here aim towards understanding the dependency of learning and memory formation and octopamine in honeybees.
We present data on the role of octopamine in eliciting the proboscis extension response (PER). During classical appetitive conditioning of harnessed honeybees, the PER serves as the unconditioned response (UR). Therefore it is crucial to understand the role of octopamine in eliciting the PER before asking about its impact on olfactory conditioning in honeybees.
In our experiments we predominantly used epinastine, which has been demonstrated to antagonize octopamine receptors in honeybees (Roeder et al., Eur J Pharmacol, 1998, 349, 171-177). We first determined the median lethal dose (LD 50) of systemically injected epinastine to find out about the appropriate epinastine concentration to work with.
Following this analysis we studied the impact of octopamine on the PER. First we asked if

epinastine interferes with the PER depending on the concentration of sucrose used to elicit the response. Three different concentrations of sucrose were used to elicit the PER and the impact of systemically injected epinastin on the PER rate was tested. In a second experiment we asked if epinastin effects the animals' motivation to elicit their proboscis. We compared hungry and satiated bees that were injected with epinastine.
Our experiments suggest that in honeybees octopamine modulates the proboscis extension response according to the animals' motivational status. Further experiments will reveal if this octopamine-dependent modulation of the PER plays a role in learning and memory formation.

Disclosures: C. Buckemüller: None. **R. Zeumer:** None. **D. Eisenhardt:** None.

Poster

922. Learning and Memory Invertebrates II

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Program#/Poster#:922.12/EEE67

Topic: F.02. Animal Cognition and Behavior

Support: Federal Ministry of Education and Research, Germany

Title: How are reward experiences translated into memory?

Authors: *K. MARTER, D. EISENHARDT
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Abstract: In appetitive classical conditioning the presentation of a conditioned stimulus (CS) precedes the presentation of an unconditioned stimulus (US), i.e. the reward. An animal learns the association between the previously neutral CS and the reward and reacts with a conditioned response (CR) when the CS is presented. Thus, an animal learns that the previously neutral CS predicts the occurrence of the reward. The term associative strength describes the extent to which the CS predicts the reward and it is generally accepted that the associative strength is mirrored in the CR during acquisition (Rescorla & Wagner, 1972).
A recent study in honeybees demonstrates a correlation between the reward duration during classical conditioning and the susceptibility of a long-term extinction memory for protein synthesis inhibition (Stollhoff & Eisenhardt, 2009). Rescorla and Wagner (1972) proposed an impact of the reward magnitude on the associative strength, i.e. the CR. Although the reward duration might be interpreted as an aspect of the reward magnitude, a "Rescorla & Wagner like" correlation between the CR during acquisition and the reward duration was not observed by Stollhoff & Eisenhardt (2009). A reason for the contradiction between this previous result and the proposed outcome might be the fact, that only the occurrence of the PER was recorded,

resulting in dichotomous data.
Therefore, we here aim to further elucidate the impact of the reward duration on the associative strength gained during acquisition but also on long-term memory (LTM) formation.
We study this problem in classical olfactory conditioning of the proboscis extension response (PER) in harnessed honeybees (Apis mellifera) by analyzing the animals behavior and by quantifying the activity of the proboscis extending muscle M17. Moreover behavioral pharmacology is used to study the impact of the reward duration on the biochemical mechanisms underlying the formation of LTM.
Our analyses demonstrate that the reward duration has no impact on the CR during acquisition and long-term memory retention 24 h later. However, the resistance to extinction observed 24 h after acquisition differs after conditioning with different reward durations. In addition, the sensitivity of the LTM to protein synthesis inhibition is affected by the reward duration. This suggests a dependency of the memories strength and the molecular mechanisms underlying memory formation on the duration of the reward presented during acquisition, although the CR during acquisition is not affected.

Disclosures: K. Marter: None. **D. Eisenhardt:** None.

Poster

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Program#/Poster#:922.13/EEE68

Topic: F.02. Animal Cognition and Behavior

Title: A role of protein degradation in memory consolidation after initial learning and extinction learning in the honeybee (Apis mellifera)

Authors: *J. FELSENBERG[1], V. DOMBROWSKI[2], D. EISENHARDT[1]
[1]Neurobio., [2]Biochem., Freie Univ. Berlin, Berlin, Germany

Abstract: In classical conditioning animals learn the association between a conditioned stimulus (CS) and an unconditioned stimulus (US) and form a memory about this association. Retrieving a CS-US memory multiple times results in a decrease of the CR, which is termed extinction. During extinction learning the animals learn that the previously learned CS-US association no longer holds true. Accordingly, an extinction memory is formed about a CS-noUS association. Extinction memories undergo, like the initial memory, a consolidation process resulting in a long-lasting and stable extinction memory. The formation of this consolidated extinction memory has recently been shown to dependent on protein degradation and it has been proposed

that protein degradation is necessary to destabilize the extinguished CS-US memory to enable the formation of the CS-noUS memory. Here we test this hypothesis to further understand the mechanisms involved in extinction memory formation.
Using a classical appetitive conditioning paradigm in the honeybee (Apis mellifera) we demonstrate that an inhibitor of protein degradation, MG132, interferes with long-term memory formation after extinction learning, thereby supporting previous findings in vertebrates. However, we also show that the application of MG132 enhances long-term memory formation after initial learning, i.e. in a situation were no pre-existing memory has to be destabilized. These results support the idea that MG132 inhibits protein degradation during formation of a long-term memory rather than destabilizing an existing memory trace. In addition, we demonstrate that the sensitivity of memory formation after initial learning and extinction learning to MG132 depends in the same way on the number of CS-US trials and the inter-trial interval applied during initial learning. We conclude from these results that the learning parameters during acquisition are critical for memory formation after extinction. Thus protein degradation in both learning processes might be functionally linked.
We next asked what protein degradation system might be responsible for the behavioural effects. We demonstrate that MG132 blocks the proteasome in honeybee brain tissue as does a specific inhibitor of the ubiquitin-proteasome system, clasto-Lactacystin β-lactone (β-lac). β-lac injection after initial learning enhances memory formation in the same way as MG132 does. These results support the notion that proteasome-mediated protein degradation is inhibited by MG132 and restricts long-term memory formation in honeybees.

Disclosures: J. Felsenberg: None. **V. Dombrowski:** None. **D. Eisenhardt:** None.

Poster

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Topic: F.04. Neuroethology

Support: NIH Pioneer Award DP1 OD006416

NIH Training Grant HD007333

Title: Mapping reward pathways in the honey bee brain by gene induction

Authors: *M. MCNEILL[1,2], A. BROCKMANN[1,2], T. MCGILL[1], G. E. ROBINSON[1,2,3]
[1]Dept. of Entomology, Univ. of Illinois At Urbana-Champaign, Urbana, IL; [2]Neurosci. Program, [3]Inst. for Genomic Biol., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Circuits that mediate perception of reward, collectively known as the reward system, add salience to some of the essentials of life: food and reproduction. Solitary insects collect rewarding foods to benefit their own nutritional and reproductive needs, while eusocial insects, such as honey bee foragers (*Apis mellifera*), collect rewarding food resources to share with their nest mates. Thus, the altered behavioral response to rewarding food sources may indicate that the reward system has been co-opted during the evolution of social behavior. To begin to understand how the reward system may have been co-opted by the transition from “me to we,” we first wanted to identify neurons responsive to reward. To identify those neurons, we either allowed bees to fly to and collect a food reward, or we provided them with a food reward in a laboratory setting. We collected brains from several hundred individuals immediately after they had received the reward and at several subsequent time points. We processed the brains for fluorescent mRNA *in situ* hybridization targeting the broadly expressed immediate early gene, *c-jun*, which served as a marker for neuronal activation. We then imaged whole brains using confocal microscopy, and registered brain images onto a standardized honey bee brain model in 3D space so that the location of a voxel (3D pixel) in one image stack represented a voxel in every subsequent image stack. Significant changes in voxel intensity across conditions and time ponts represented differential neuronal activation. We detected changes in *c-jun* expression in a pair of cells in the dorsal posterior protocerebrum, which co-express a marker for dopamine synthesis, *tyrosine hydroxylase*. We also detected *c-jun* expression level changes in sub-populations of intrinsic and extrinsic mushroom body Kenyon cells. Our results provide a framework for future studies on the neurobiological and molecular basis of reward-based behavior in honey bees.

Disclosures: M. McNeill: None. **A. Brockmann:** None. **T. McGill:** None. **G.E. Robinson:** None.

Poster

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Program#/Poster#:922.15/EEE70

Topic: F.02. Animal Cognition and Behavior

Support: DFG Me 365/34-1

Title: Learning to navigate: Exploratory orientation flights of young honeybees

Authors: *J. DEGEN[1], A. KIRBACH[1], K. LEHMANN[1], R. MENZEL[1,2]
[1]Dept. of Neurobio., Freie Univ. Berlin, Berlin, Germany; [2]Bernstein Ctr. for Computat. Neurosci. (BCCN), Berlin, Germany

Abstract: Honeybees (Apis mellifera) perform exploratory orientation flights to learn how to return safely to their hives. Path integration and learning of landmarks are thought to contribute to a safe return but the role of landmark learning is still not known. In order to investigate whether landmarks are learned in spatial relation to the hive we analysed the patterns of consecutive orientation flights applying the harmonic radar technique for tracking the complete flight paths of individually marked bees. A total of 266 orientation flights of 167 bees were analysed. Usually bees explore different regions around the hive in consecutive orientation flights but bees may also deviate from this pattern. Consequently the first foraging flight may not lead into an area they had explored before. To investigate the role of landmark learning we excluded the use of path integration by displacing the bees after their very first orientation flight and found that bees displaced into the explored area returned back to the hive significantly faster than when displaced into an unexplored area. In control experiments we proved that bees were unable to return to the hive if they did not perform at least one orientation flight. We conclude that bees learn the spatial relations of landmarks relative to the hive during their very first exploratory orientation flights, and odors emanating from the hive can be excluded as a guiding signal.

Disclosures: J. Degen: None. **A. Kirbach:** None. **K. Lehmann:** None. **R. Menzel:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: DFG Me 365/41-1

Title: Searching for neural correlates of decision making in honeybees

Authors: *H. ZWAKA[1], R. MENZEL[1,2]
[1]Dept. of Neurobio., Freie Univ. Berlin, Berlin, Germany; [2]Bernstein Ctr. for Computat. Neurosci. (BCCN), Berlin, Germany

Abstract: The focus of our work is the search for neural correlates of decision making in honeybees learning to navigate in a virtual environment. We record multiple units of mushroom

body (MB) extrinsic neurons in behaving animals.
The MB, a higher-order integration center in the insect brain, is known to be involved in the consolidation of memory and retrieving memory. MB extrinsic neurons are expected to recode the sensory input according to its value, and thus we expect neural read-out from the MB to be involved in decision making after operant learning.
We achieve long lasting extracellular recordings from multiple MB extrinsic neurons in animals controlling their visual environment by the movement of an air supported ball. The visual environment consists of differently colored objects whose virtual distance is indicated by their different parallax. In addition the ground structure below the animal and the pattern of an UV illuminated polarization pattern above it provides the honeybee with information about its movements. Animals are trained in the virtual environment (VE) to choose selected objects or routes. Depending on the particular organization of the virtual environment the animal can e.g. solve simple learned tasks or more complex, navigation-like tasks. In some of our experiments we use bees that were first trained in free flight and than introduced into the VE presenting corresponding visual patterns. We find preceding neural activities related to behavioral decisions.

Disclosures: H. Zwaka: None. **R. Menzel:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: BMBF/DLR Bernstein Fokus-Lernen 01GQ0941

Title: In search of the transmitters of Kenyon cells in the mushroom body of the honey bee

Authors: *R. BARTELS[1], S. VOIGT[2], R. MENZEL[1,3]
[1]Neurobiologie, AG Menzel, Freie Univ. Berlin, Berlin, Germany; [2]Univ. Potsdam, D14469 Potsdam, Germany; [3]Bernstein Ctr. for Computat. Neurosci., Berlin, Germany

Abstract: The insect brain is widely used to study neural circuits. Honey bees in particular are able to integrate and store a wide range of sensory information. The mushroom body (MB) is a central neuropil structure and crucial for the neural networks underlying these capabilities. A major limitation in analyzing the function of the MB is the lack of knowledge about the transmitters of MB intrinsic neurons, the Kenyon cells. Immunocytochemistry revealed a range of small peptides and glutamate. Furthermore, immunoreactivity was found against taurin.

Kenyon cells converge on extrinsic neurons (EN). We search for the transmitters of Kenyon cells by extra- and intracellular recordings from ENs combined with pharmacological manipulations. We found increases and decreases of spontaneous activity when manipulating NMDA-like receptors. In addition we saw changes of spike amplitude and stimulus dependent changes. Currently we perform experiments in which Kenyon cells are stimulated under various pharmacological treatments.

Disclosures: R. Bartels: None. **S. Voigt:** None. **R. Menzel:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: PhD fellowship Stiftung Polytechnische Gesellschaft

Title: Ionic current modulation of honeybee mushroom body and antennal lobe neurons

Authors: *S. HIMMELREICH, B. GRÜNEWALD
Goethe Univ., Frankfurt, Germany

Abstract: The honeybee (*Apis mellifera*) is a model organism for the study of learning and memory formation and its underlying cellular mechanisms. The neuronal pathway for associative olfactory learning includes two neuropils: the antennal lobes and the mushroom bodies. Here, the excitatory cholinergic pathway and the octopaminergic reward pathway converge. For learning-related plasticity, the coincident occurrence of the conditioned stimulus (CS) and the reward (unconditioned, US) has to be detected by the nervous system.
We performed patch-clamp recordings and cell calcium (Ca^{2+})-imaging to investigate the cellular interactions of the nicotinic acetylcholine receptor (nAChR) system and the octopamine receptor system of antennal lobe neurons and mushroom body intrinsic Kenyon cells *in vitro*. Patch-clamp experiments showed ACh-induced excitatory currents in antennal lobe and Kenyon cells. Co-application of ACh and biogenic amines (10µM octopamine or 10µM serotonin) revealed reversibly decreased currents. Also currents in antennal lobe neurons were reduced by co-applications of ACh and the adenylyl cyclase activator forskolin (10µM). This indicates involvement of a cAMP-dependent signaling cascade.The Ca^{2+}-imaging experiments have shown that biogenic amines(octopamine and serotonin, both 1µM) evoked Ca^{2+}-transients in antennal lobe neurons and Kenyon cells. Octopamine-induced transients were blocked by the antagonist mianserin (100µM). Co-applications of ACh (100µM) and the two biogenic amines octopamine

and serotonin (both 1µM) reduced Ca^{2+}-signals. The same was observed during co-applications of ACh and forskolin (10µM).Due to these findings, we postulate two possible cellular coincidence detectors between the cholinergic CS pathway and the reward (US pathway) during olfactory learning. These detectors may modulate further the incoming stimuli: (1) The activation of a calcium (Ca^{2+}) -dependent kinase which may be activated by an elevated intracellular Ca^{2+}-concentration induced by the coincident activation of the nAChR and an α-adrenergic-like octopamine receptor, AmOA1. (2) The possible phosphorylation of the nAChR by a cAMP-dependent protein kinase (PKA) triggered by β-adrenergic-like octopamine receptor activation. Both assumptions would lead to modulations of ACh-induced ionic currents as observed in our experiments. They may indicate coincidence mechanisms during olfactory learning. However, further experiments to unravel the cellular bases of learning related synaptic plasticity are in progress.

Disclosures: S. Himmelreich: None. **B. Grünewald:** Research Grant; Stiftung Polytechnische Gesellschaft.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: The effects of maturation and foraging experience on behavioural flexibility and the mushroom bodies of bumblebees (Bombus impatiens)

Authors: *C. G. STRANG, A. P. NGO, D. F. SHERRY
Dept. of Psychology, Univ. of Western Ontario, London, ON, Canada

Abstract: Bees' impressive ability to learn about their environment and form associations has been extensively studied. Much less is known about how bees adapt their behaviour to a changing environment. Bumblebees use a flexible sampling strategy at the outset of foraging, but eventually establish 'flower constancy'. Coincident with this change in natural foraging behaviour, the mushroom bodies - a structure in the invertebrate brain linked to learning - increase in volume. Both age and experience are thought to influence total mushroom body size. We tested laboratory housed bumblebees (Bombus impatiens) of varying ages and foraging experience on a serial reversal task to assess the relationship between maturation, experience,

and behavioural flexibility. We predicted that increased age and experience would both decrease behavioural flexibility. The serial reversal task required bees to learn a colour discrimination and repeatedly reverse their pattern of responding when reward contingencies were reversed. Bees completed an initial discrimination session and three reversals. Foraging bees were classified at the time of testing as either low experience (0-3 days foraging) or high experience (5-8 days foraging). Brains were collected immediately following testing and the volume of the mushroom bodies correlated with serial reversal performance, allowing us to directly measure the relationship between mushroom body size and behavioural flexibility. Older bees (with larger mushroom bodies) were less behaviourally flexible than younger bees and age was found to influence behavioural flexibility more than foraging experience. These findings are consistent with our predictions based on the natural foraging behaviour of bumblebees.

Disclosures: C.G. Strang: None. **A.P. Ngo:** None. **D.F. Sherry:** None.

Poster

922. Learning and Memory Invertebrates II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:922.20/EEE75

Topic: F.04. Neuroethology

Support: NIH Grant OD006416

NIH Training Grant HD007333

Title: Neuroplasticity and mushroom body egr expression in honey bees

Authors: *C. C. LUTZ[1], E. E. MURDOCH[2], G. E. ROBINSON[1,3,4]
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Abstract: Honey bees (Apis mellifera) learn to navigate in the environment surrounding the hive by performing “orientation” flights for several days before they begin to forage. Honey bee mushroom bodies (a paired region of the insect brain involved in learning) show both experience-expectant and experience-dependent changes in neuropil volume, coincident with the increased cognitive demands of orientation flights and foraging behavior. We previously reported that orientation flights trigger in the mushroom bodies a rapid and transient upregulation of egr, a homolog of egr-1, an immediate-early gene well known to respond to novel experience. Egr-1 also exhibits higher basal levels in brain regions undergoing development or remodeling in vertebrates; we therefore hypothesized that an increase in basal egr mushroom body expression

occurs at the age when bees take orientation flights. Laboratory-reared bees showed increased mushroom body basal egr expression at 4-6 days in comparison with earlier and later time points. This timing is consistent with the possibility that egr supports pre-foraging mushroom body neuropil growth. In addition, comparison of these results with egr expression dynamics under natural conditions suggested an association between egr expression and onset of foraging behavior, again consistent with a role for this gene in mushroom body neuropil expansion. These findings suggest that egr is involved in the acquisition and consolidation of spatial memory. Further studies of honey bees have the potential to extend understanding of the functional role of egr-related pathways in adult neuroplasticity.

Disclosures: C.C. Lutz: None. **E.E. Murdoch:** None. **G.E. Robinson:** None.

Poster

922. Learning and Memory Invertebrates II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:922.21/EEE76

Topic: F.02. Animal Cognition and Behavior

Support: Villanova University Faculty Summer Research Grant

Title: Identity discrimation in bumblebee colonies

Authors: *M. F. BROWN, J. M. SAYDE
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Abstract: Four colonies of bumblebees (*Bumbus impatiens*) were trained to respond differentially to visual displays consisting of either two identical stimuli or two different stimuli. The stimuli were either two colors (two colonies) or two line orientations (two colonies). Discriminative responses were defined by flying to an area on either the left or right side of the display, with one side correct during a "same" display and the other correct during a "different" display. A correct choice resulted in access to sugar solution. Following training, the colonies were tested with the alternative (novel) stimulus set, under conditions of non-differential reinforcement. All four colonies showed transfer of the discrimination in a manner consistent with control by a same/different concept.

Disclosures: M.F. Brown: None. **J.M. Sayde:** None.

Poster

923. Dopamine and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:923.01/EEE77

Topic: F.03. Motivation and Emotion

Support: NIDA Grant U01DA016194

NIMH Grant MH078023

NIMH Grant MH094966

Title: Dissociable effects of dopamine antagonists and cannabinoid CB1 antagonists: studies using the concurrent progressive ratio/chow feeding task

Authors: *P. A. RANDALL[1], E. J. NUNES[1], M. PARDO[2], B. KHAN[1], P. SHAH[1], C. A. LEE[1], V. K. VEMURI[3], A. MAKRIYANNIS[3], Y. BAQI[4], C. E. MÜLLER[4], M. CORREA[1,2], J. D. SALAMONE[1]
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Abstract: A number of behavioral tests have been developed to assess effort-based choice behavior in rats, including a T-maze barrier choice task, discounting tasks, and operant procedures. One widely employed paradigm is the concurrent fixed-ratio 5 (FR5)/chow feeding choice task, in which rats can either lever press on a FR5 schedule for a preferred high-carbohydrate food, or approach and consume a less-preferred chow that was freely available in the chamber. Manipulations that interfere with dopamine (DA) transmission cause animals trained on this task to shift their behavior away from the effortful task of lever pressing and towards the consumption of the freely available chow. More recently, a new task has been developed in which the FR5 work requirement has been replaced with a progressive ratio (PROG) lever pressing requirement. Initial studies showed that the DA antagonist haloperidol decreased PROG lever pressing and maximum ratio achieved, but did not decrease chow consumption. This differed from the effects seen with the cannabinoid CB1 antagonist/inverse agonist and putative appetite suppressant AM251, which decreased both lever pressing and chow intake. This demonstrated that the effects of DA antagonism are not the result of a decrease in appetite or food motivation. The present work studied several additional manipulations, including more selective DA antagonists and additional CB1 antagonists/inverse agonists, using the PROG/chow feeding choice procedure. The first set of experiments studied the effects of the DA D1 antagonist ecopipam and the D2 antagonist eticlopride. Both drugs decreased number of lever presses and maximum ratio achieved while leaving chow consumption unaffected. Moreover, the adenosine A2A antagonist MSX-3 reversed the effects of eticlopride on PROG responding. Additional experiments studied the effects of cannabinoid CB1 antagonist/inverse

agonists. Unlike DA antagonists, these compounds produced profound reductions in chow intake at doses at or below those that suppressed lever pressing. These results indicate that an adenosine A2A antagonist is capable of reversing the impairments induced by a DA D2 antagonist on this procedure. Moreover, the effects seen with DA D1 and D2 selective antagonists differ substantially from those seen with appetite suppressants and pre-feeding, further demonstrating that the decrease in PROG lever pressing produced by DA antagonism is not the result of a decrease in appetite, primary food motivation or the unconditioned reinforcing properties of food.

Disclosures: P.A. Randall: None. **E.J. Nunes:** None. **M. Pardo:** None. **B. Khan:** None. **P. Shah:** None. **C.A. Lee:** None. **V.K. Vemuri:** None. **A. Makriyannis:** None. **Y. Baqi:** None. **C.E. Müller:** None. **M. Correa:** None. **J.D. Salamone:** None.

Poster

923. Dopamine and Behavior

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Program#/Poster#:923.02/EEE78

Topic: F.03. Motivation and Emotion

Support: NIMH: MH078023

MH094966

NIDA: U01DA016194

Title: Development of rodent models of the motivational symptoms of depression: The catecholamine depleting agent tetrabenazine affects effort-related decision making in the concurrent FR5/chow choice task.

Authors: *E. J. NUNES[1], P. A. RANDALL[1], C. FREELAND[1], E. HART[1], C. E. MÜLLER[2], Y. BAQI[2], M. CORREA[1,3], J. D. SALAMONE[1]
[1]Psychology, Neurosci. Grad. Program, Storrs mansfield, CT; [2]Pharmazeutische Chemie, Univ. Bonn, Bonn, Germany; [3]Univ. of Jaume I, Castelló, Spain

Abstract: Mesolimbic dopamine (DA), particularly within the nucleus accumbens, has been shown to be a critical component of the brain circuitry regulating behavioral activation and effort-related processes. Interference with accumbens DA alters response allocation in choice procedures that involve cost/benefit evaluations of reinforcement value and response costs. Several behavioral tests have been developed to assess effort-based choice behavior in rats,

including maze procedures and operant tasks. The concurrent FR5/chow feeding choice procedure developed by our laboratory is a frequently used task. With this task, rats can lever press for a more preferred food or consume the less palatable freely available laboratory chow. Under baseline or control conditions, rats spend most of their time lever pressing for the more preferred food and eat very little of the chow. Accumbens DA depletions or administration of DA D1 or D2 family antagonists produce a shift in choice behavior, decreasing lever pressing and increasing chow intake. It has been suggested that these drug-induced shifts in effort-based choice that are seen in animals could be analogous to symptoms such as psychomotor retardation, anergia, and fatigue, which can be observed in individuals with depression, parkinsonism, and other disorders. For that reason, the current studies investigated the effects of the catecholamine depleting agent tetrabenazine (TBZ) on effort-related choice behavior using the concurrent FR5/chow feeding choice procedure. TBZ inhibits the vesicular monoamine transporter 2 (VMAT-2), and is used to treat Huntington's disease. Considerable evidence indicates that the vast majority of VMAT-2 binding in nucleus accumbens and striatum is on DA terminals, and that TBZ depletes DA. Moreover, TBZ has been shown to produce depressive side effects in some patients, including motivational symptoms such as psychomotor slowing, anergia, and fatigue. In rats tested on the concurrent FR5/chow procedure, TBZ (0.25-1.0 mg/kg IP) decreased lever pressing and increased chow consumption. Initial evidence indicates that these effects of TBZ on effort-related choice behavior can be reversed by co-administration of the adenosine A2A antagonist MSX-3 as well as the well established antidepressant bupropion. Taken together, these results demonstrate that administration of low doses of TBZ can alter effort-related choice behavior, biasing animals towards low effort alternatives. The present research may contribute to the development of novel treatments for effort-related motivational symptoms in humans.

Disclosures: E.J. Nunes: None. **P.A. Randall:** None. **C. Freeland:** None. **E. Hart:** None. **C.E. Müller:** None. **Y. Baqi:** None. **M. Correa:** None. **J.D. Salamone:** None.

Poster

923. Dopamine and Behavior

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Program#/Poster#:923.03/EEE79

Topic: F.03. Motivation and Emotion

Support: 078865/Z/05/Z

Title: Dissecting the role of dopamine in behavioral control with pharmacological and genetic tools in healthy humans

Authors: *M. GUITART MASIP[1], M. FRANK[2], M. ECONOMIDES[1], Q. HUYS[3], A. RICHTER[4], R. CHOWDHURY[1], P. DAYAN[3], B. SCHOTT[4], E. DUZEL[5], R. DOLAN[1]
[1]Wellcome Trust Ctr. For Neuroimaging, UCL, London, United Kingdom; [2]Brown Univ., Providence, RI; [3]Gatsby Computat. Unit, UCL, London, United Kingdom; [4]Leibniz Inst. for Neurobio., Magdeburg, Germany; [5]Deutsches Zentrum fur Neurodegenreative Erkrannkungen, Magdeburg, Germany

Abstract: Dopamine plays a dual role in decision-making: on one hand, it is widely observed to signal anticipation of future rewards and reward prediction errors, but on the other, it is associated with the invigoration of actions that lead to a reward. This dual involvement of dopamine in signalling valence and action may lie at the heart of behavioural anomalies such as are observed when action and valence are orthogonally manipulated during learning. We saw that healthy human participants found it more difficult to learn to withhold an action to obtain a reward (so called no-go-to-win) than to emit an action to obtain a reward (so called go-to-win), or to withhold the action to avoid punishment (so called no-go-to-avoid losing). Here we assessed pharmacological and genetic basis of this anomaly, using levodopa to increase dopamine levels, and looking at well-described single nucleotide polymorphisms that selectively affect dopamine function in the prefrontal cortex (COMT) or the indirect pathway in the striatum (DRD2). Contrary to expectation, we found that boosting dopamine levels improved performance in the no-go-to-win condition. This effect was also expressed by variability in the COMT gene: participants carrying the met allele, which is associated with higher dopamine levels in the prefrontal cortex, performed better in the no-go-to-win condition. Finally, whereas subjects with different DRD2 genotypes performed equally well on average, we detected an interaction between COMT and DRD2 genotypes such that dopamine signalling in the indirect pathway in the striatum is counterbalanced by dopamine signalling in the prefrontal cortex. These results show that the dominant effect of dopamine during learning is to facilitate a decoupling between action and valence which is likely to depend on the prefrontal cortex. On the other hand, we provide evidence that dopamine in the striatum may have the opposite effect. These data help refine our understanding of the role of dopamine in behavioral control.

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Poster

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Topic: F.03. Motivation and Emotion

Support: NIH grant R01-DA027858

DFG grant Schw 559/8-3

Title: Phasic dopamine release in the nucleus accumbens in response to ultrasonic vocalizations serving a pro-social communicative function in rats

Authors: *M. WÖHR[1], A. TOSE[2], M. J. WANAT,[2], A. S. HART[2], N. G. HOLLON[2], P. E. M. PHILLIPS[2], R. K. W. SCHWARTING[1], I. WILLUHN[2]
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Abstract: Rats emit distinct types of ultrasonic vocalizations (USVs), which serve as situation-dependent affective signals. Juvenile and adult rats produce 50-kHz USVs in appetitive situations such as rough-and-tumble play (Knutson et al., 1998), whereas 22-kHz USVs occur in aversive situations such as social defeat (Kroes et al., 2007). While 50-kHz USVs induce social approach behavior in the recipient, supporting the notion that they serve as social contact calls, 22-kHz USVs lead to freezing behavior, indicating an alarming function (Wöhr & Schwarting, 2007). These opposite behavioral responses are paralleled by distinct patterns of brain activation; with 22-kHz USVs inducing activation in amygdala and periaqueductal gray, both implicated in the regulation of anxiety and fear, whereas 50-kHz USVs evoke neuronal activation in the nucleus accumbens (NAcc; Sadananda et al., 2008). The NAcc is strongly implicated in appetitive behavior, which is critically modulated by dopaminergic input that encodes reward and reward-predicting stimuli. Therefore, we hypothesized that NAcc dopamine signaling is involved in social approach towards pro-social 50-kHz USVs and tested whether 50-kHz USVs elicit phasic dopamine release in NAcc using fast-scan cyclic voltammetry. Rats were exposed to playback of four acoustic stimuli: 1) 50-kHz USVs; 2) 22-kHz USVs; 3) time- and amplitude-matched white noise; 4) background noise. At the beginning of the experiment, all rats were exposed to white noise to reduce novelty. Then, rats were repeatedly exposed to sequences of all four stimuli in random order. Consistent with previous studies, social approach behavior and ultrasonic calling were evoked exclusively in response to 50-kHz USVs. Paralleling the behavioral effects, phasic dopamine release was only observed during playback of 50-kHz USVs. These effects of 50-kHz USVs on both dopamine signaling and pro-social behavior were most pronounced in response to the first playback, but then declined rapidly with subsequent presentations, indicating a close relationship between the two measures. The finding that phasic dopamine release in NAcc encodes pro-social 50-kHz but not alarming 22-kHz USVs, further supports the notion that the two call-types are processed by distinct brain areas, involved in regulating approach and avoidance, respectively. Importantly, stimulating dopamine neurotransmission by microinfusion of amphetamine into the NAcc elicits 50-kHz but not 22-kHz USVs (Thompson et al., 2006). Therefore, the NAcc may serve as the functional link

between detection and production of 50-kHz USVs, which is particularly relevant in social reciprocal communicatory signals.

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Poster

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Support: NIMH MH094966

Fundació Bancaixa-UJI (P1.1B2010-43)

Fundació Bancaixa-UJI (PREDOC-2007-43)

Title: Development of rodent models of the motivational symptoms of depression: Comparison of the effects of the monoamine depleting agents reserpine and tetrabenazine on effort-related decision making.

Authors: *J. D. SALAMONE[1], E. J. NUNES[1], P. A. RANDALL[1], M. HUIZENGA[1], V. NOWAK[1], M. PARDO[2], M. CORREA[2]
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Abstract: Nucleus accumbens dopamine (DA) is a critical component of the brain circuitry regulating behavioral activation and effort-related processes. Interference with accumbens DA alters response allocation in cost/benefit choice procedures that offer animals a choice between high reward/high effort vs. low reward/low effort options. Several behavioral tests have been developed to assess effort-based choice behavior in rats, including maze procedures and operant tasks. Accumbens DA depletions or injection of DA D1 or D2 family antagonists produce a shift in the behavior, decreasing selection of the high reward/high effort option and increasing choice of the low reward/low effort option. Procedures involving effort-related choice behavior could be useful as animal models of psychiatric symptoms such as psychomotor retardation, anergia, and fatigue, which are seen in patients with depression, parkinsonism, and other disorders. For that reason, the current studies investigated the effects of the monoamine depleting agents reserpine (RES) and tetrabenazine (TBZ) on effort-related choice behavior in rats. RES is an irreversible

inhibitor of the vesicular monoamine transporters 1 and 2 (VMAT-1,2), while tetrabenazine is a reversible inhibitor of VMAT-2. RES and TBZ produce depressive side effects in some patients, including motivational symptoms such as psychomotor slowing, anergia, and fatigue. Rats were tested on a concurrent fixed ratio 5 lever pressing/chow feeding choice procedure. With this task, rats can lever press for a more preferred food (high carbohydrate pellets) or consume a less preferred but freely available laboratory chow. Administration of RES and TBZ both decreased lever pressing, but substantially increased chow intake. However, with repeated administration, RES produced severe motor impairments that led to a general disruption of all behavior, and also induced catalepsy. In contrast, TBZ (0.5-1.0 mg/kg) produced consistent effects on choice behavior over several weeks of injections. TBZ also affected effort-related choice in rats tested on a progressive ratio/chow feeding choice procedure, and a task in which rats could choose between lever pressing for a higher concentration of sucrose vs. consumption of a less preferred concentration. Taken together, these results demonstrate that RES is a more difficult drug to work with than TBZ. TBZ injections altered effort-related choice behavior, biasing animals towards low effort alternatives in a consistent and reproducible manner, across multiple tasks. The present research may contribute to the development of novel treatments for effort-related motivational symptoms in humans.

Disclosures: J.D. Salamone: None. **E.J. Nunes:** None. **P.A. Randall:** None. **M. Huizenga:** None. **V. Nowak:** None. **M. Pardo:** None. **M. Correa:** None.

Poster

923. Dopamine and Behavior

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Topic: F.03. Motivation and Emotion

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Ministerio de Educación, España PND (2010I024)

NIMH MH078023

Title: Impact of dopamine D2 receptor antagonism on the activational effects produced by conditioned stimuli and on the preference for primary reinforcers based on their effort requirements.

Authors: *M. CORREA[1], M. PARDO[1], L. LOPEZ-CRUZ[1], T. DOÑATE[1], M. CARBÓ-GAS[1], L. MONFERRER[2], J. D. SALAMONE[3]
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Abstract: Mesolimbic dopamine (DA) is a critical component of the brain circuitry regulating behavioral activation and effort-related processes. In the present experiments, the DA D2 receptor antagonist haloperidol was used to study DAergic involvement in the activational and directional components of motivated behaviors in Swiss male mice. Haloperidol (0.1 and 0.2 mg/kg) decreases spontaneous locomotion in an open field (OF) and a running wheel (RW). Presentation of an olfactory conditioned stimulus (CS+) associated with a sucrose solution was able to enhance motor activity in both paradigms. Haloperidol (0.1 mg/kg) did not suppress free intake of sucrose, or sucrose preference relative to water, but did block the activational effects of the CS+ at a dose (0.05 mg/kg) that did not affect spontaneous locomotion. In another experiment, a T-maze task was developed for the assessment of preference for two different types of rewards: physical activity (i.e., running in a RW) in one arm and sucrose access in the other. Under normal conditions sucrose consumption was less preferred. Haloperidol (0.1 mg/kg) produced a shift in arm preference, reducing time spent on the RW, but increasing selection of sucrose. Pre-exposing animals to both reinforcers did not produce this shift. Thus, D2 antagonism reduced the invigorating properties of primary and conditioned reinforcers, with no effect on free access to reinforcers that could be obtained with low effort. In summary, the present results implicate DA D2 receptors in the regulation of behavioral activation and effort-related functions. This research allows for a better understanding of psychiatric symptoms such as psychomotor slowing, fatigue or anergia that can be observed in pathologies like depression. Moreover, it demonstrates that DA systems regulate preference for engaging in physical activities relative to other reinforcing conditions.

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Poster

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Topic: F.03. Motivation and Emotion

Support: Wellcome Trust Research Training Fellowship WT088286MA

Title: Dopaminergic modulation of decision-making in older age

Authors: *R. CHOWDHURY[1,2], M. GUITART-MASIP[2], P. DAYAN[3], E. DUZEL[1,4], R. J. DOLAN[2]
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Abstract: Background: Reinforcement learning is impaired in older age. This may be related to a decline of dopamine neurons which occurs as part of normal aging. Abnormal signalling of reward anticipation and risk-taking in the ventral striatum has been reported in older adults. However the basis of prediction error signalling in older adults has yet to be elucidated.
Objective: To study the effects of pharmacological manipulation with the dopamine precursor levodopa (L-DOPA) on reward-based decision-making and prediction errors in healthy older adults.
Methods: This was a double-blind placebo controlled within-subject study. 32 healthy older adults performed a functional MRI 'two-arm bandit' task, on and off L-DOPA. Participants were required to choose between two stimuli, whereby the outcome was binary (win or no win). The probability of obtaining a reward for each stimulus were independent of each other and varied on a trial-to-trial basis according to a Gaussian random walk distribution. We used a standard reinforcement learning model to fit participants' behaviour, generating a learning rate (α) on placebo and on L-DOPA. We also modelled a choice perseveration parameter. The actual reward (R) and expected value (Qc) were used as parametric modulators of the outcome in the fMRI analysis. Thus the prediction error here represented the difference between the received reward and the expected reward given the choice on that trial.
Results: The percentage of times the optimal stimulus was chosen was similar on placebo and L-DOPA. The learning rate was significantly higher on L-DOPA than placebo, whereas choice perseveration was unaffected. Using a functional ROI approach we defined voxels in the ventral striatum that responded more to the rewards than to the Qc. However, this signal was only fully compatible with a prediction error signal (positive correlation with R and negative correlation with Qc) when participants received L-DOPA.
Conclusions: We show that dopamine increases the learning rate and boosted the prediction error signalling in the ventral striatum in healthy older adults performing a probabilistic reward-based learning task. Importantly, the pattern of prediction errors in the ventral striatum which has often been described in younger adults was only observed in our cohort of older adults following L-DOPA. These results suggest that older adults have abnormal neural representations of expected value which can be normalised by enhancing dopamine.

Disclosures: R. Chowdhury: None. **M. Guitart-Masip:** None. **P. Dayan:** None. **E. Duzel:** None. **R.J. Dolan:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: R01 DA029706

R01 MH085499

R21 DA032986

R01 AA018734

T32 DA007315

Title: Simultaneous *in vivo* imaging of changes in volume transmission of dopamine and noradrenaline in mouse cortex during learning

Authors: *A. MULLER[1], V. JOSEPH[4,2], N. TAYLOR[4], P. SLESINGER[4,5,2], D. KLEINFELD[1,2,3]
[1]Physics Dept., [2]Neurosciences graduate program, [3]Neurobio. Dept., Univ. of California San Diego, San Diego, CA; [4]Peptide Biol. Labs., The Salk Inst. for Biol. Studies, La Jolla, CA; [5]Mount Sinai Sch. of Med., New York, NY

Abstract: Volume transmission plays a fundamental role in neuromodulation in the normal and diseased brain. We developed implantable cell–based neurotransmitter fluorescent engineered reporters (CNiFERs) to study the volume conduction of neurotransmitter released in vivo. CNiFERs have a higher spatial resolution (100 µm) and a faster temporal resolution (~ 2 s) than microdialysis and differentiate among monoamines, unlike voltammetry. The first CNiFER was created to detect acetylcholine (ACh). We engineering HEK293 cells to express the M1 muscarinic G protein-coupled receptor (GPCR) and a FRET-based Ca2+ sensor. We have now constructed a new generation of CNiFERs, including a noradrenaline (NA) CNiFER using the Gq coupled alpha-1a adrenergic receptor and a dopamine (DA) CNiFER using the Gi/o coupled D2 type dopaminergic receptor.
The alpha1a-CNiFER exhibited nanoMolar sensitivity for NA (EC50 ~ 7 nM), with no significant cross reactivity at physiological concentrations of other neurotransmitters. In vivo

two-photon microscopy was used to assay alpha1a-CNiFERs chronically implanted in mouse frontal cortex. Alpha1a-CNiFERs responded robustly with an ~30 % increase in FRET to electrical stimulation of the locus coeruleus. The FRET response was blocked with the alpha1a receptor antagonist WB4101 (2 mg/kg, IP).
The D2-CNiFER exhibited nanoMolar sensitivity for DA (EC50 ~ 3 nM), also with no significant cross reactivity. In an ex vivo preparation that included the ventral tegmental area (VTA), D2-CNiFERs measured DA release induced by high [K+]. In vivo two-photon imaging with implanted D2-CNiFERS revealed a robust FRET change following electrical stimulation of the VTA. Response of the D2-CNiFERs to VTA stimulation was substantially enhanced by systemic injection of cocaine (15 mg/kg, IP) or methamphetamine (2 mg/kg, IP) and blocked by the D2 receptor antagonist eticlopride (0.1mg/kg, IP).
We are currently using the alpha1a- and D2-CNiFERs to simultaneously resolve the spatial and temporal dynamics of monoamine concentrations in cortex during Pavlovian and operant conditioning. With a tone-based paradigm using sugar water as a reward, the release of DA across multiple trials is observed to shift from the time of the reward toward that of the conditioning stimulus. In contrast, the release of NA appears to be timed to the reward.

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Poster

923. Dopamine and Behavior

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ETHZ Postdoctoral Fellowship

Title: Neurofeedback-mediated self-regulation of substantia nigra

Authors: *J. S. SULZER[1], R. SITARAM[2], M. BLEFARI[1], S. KOLLIAS[3], N. BIRBAUMER[4], K. STEPHAN[1], A. LUFT[3], R. GASSERT[1]
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Abstract: The dopaminergic system has been shown to play a role in fine motor skills, behavioral choice and reinforcement learning. It has also been shown to be highly involved in neurological diseases such as Parkinson's disease. Current approaches that aim at enhancing dopaminergic neurotransmission include different pharmacological agents. The goal of this study is to examine whether one can gain control of own dopamine production through guided mental imagery in the form of neurofeedback. To this extent, we used real-time functional magnetic resonance imaging (rtfMRI) neurofeedback, supplying online visual feedback of the blood oxygenation level dependent (BOLD) signal of substantia nigra and ventral tegmental area complex (SN/VTA). We examined two groups of subjects receiving contingent neurofeedback, one group receiving visual feedback positively proportional to SN/VTA BOLD signal (up-regulation), the other group receiving negatively proportional feedback (down-regulation). We recorded skin conductance response (SCR) during neurofeedback as a measure of attention to the task. We found that after one session, both groups significantly activated SN/VTA. However, the groups differed in the correlations between SN/VTA BOLD signal and SCR. Specifically, the SN/VTA activity was positively correlated to SCR in the up-regulation group, whereas the down-regulation group had a negative trend, significantly smaller than the up-regulation group . This result suggests that the ability to self-regulate SN/VTA BOLD signal is modulated by attention to the task. In addition to significant increases in SN/VTA in the up-regulation group, whole brain random effects analysis and psychophysiological interaction show a strong role of nucleus accumbens and hippocampus, indicating the possible role of the mesolimbic dopaminergic pathway. This is the first reporting of voluntary control of the midbrain dopamine pathways and represents a first step towards endogenous upregulation of dopaminergic transmission.

Disclosures: J.S. Sulzer: None. **R. Sitaram:** None. **M. Blefari:** None. **S. Kollias:** None. **N. Birbaumer:** None. **K. Stephan:** None. **A. Luft:** None. **R. Gassert:** None.

Poster

923. Dopamine and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:923.10/FFF2

Topic: F.03. Motivation and Emotion

Support: NSERC

Title: Acquisition and expression of catalepsy sensitization to haloperidol is conditional on the interaction between the drug and test environment

Authors: *T. J. BANASIKOWSKI[1], R. J. BENINGER[2]
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Abstract: Dopamine (DA) plays a critical role in our ability to move and in some forms of learning. DA drugs used to treat Parkinson's disease and schizophrenia produce progressively greater effects on behavior with repeated exposures (i.e., behavioral sensitization). Low doses of the DA D2-receptor preferring antagonist haloperidol (0.25 mg/kg, i.p.), repeatedly injected in a particular environment, produce day-to-day increases in catalepsy, quantified by time spent on a horizontal bar without active movement. In experiment 1, we observed that expression of catalepsy sensitization is environment-specific, as it is not observed when testing is performed in different environments despite usual haloperidol treatment. Furthermore, places associated with repeated haloperidol injections and catalepsy tests also elicit catalepsy, now conditioned. This was observed when saline was substituted for haloperidol and rats were tested in their regular, but not in a novel test environment. Following repeated saline-environment pairings, conditioned catalepsy was extinguished. Post-extinction challenge with haloperidol produced a small catalepsy response that was now independent of the test environment, suggesting that the haloperidol drug cue alone can elicit conditioned catalepsy. In experiment 2, we show that expression of the sensitization response is dependent on a drug-environment interaction, as animals with a previous history of drug treatment in a different environment failed to exhibit significant catalepsy when given haloperidol in the test environment. In experiment 3, we show for the first time that habituation to the test environment prior to commencing haloperidol treatment significantly enhanced the rate of catalepsy sensitization. This was not observed when habituation was performed in a different environment. Thus, acquisition and expression of catalepsy sensitization to haloperidol is conditional on the interaction between the drug and test environment. Moreover, it appears that the sensitization effect is due to a progressive loss of exploratory behaviors as a consequence of decreased engagement with environmental stimuli, which eventually manifests as catalepsy. Results suggest that the therapeutic effects of antipsychotic drugs depend on the environment associated with their use.

Disclosures: T.J. Banasikowski: None. **R.J. Beninger:** None.

Poster

923. Dopamine and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:923.11/FFF3

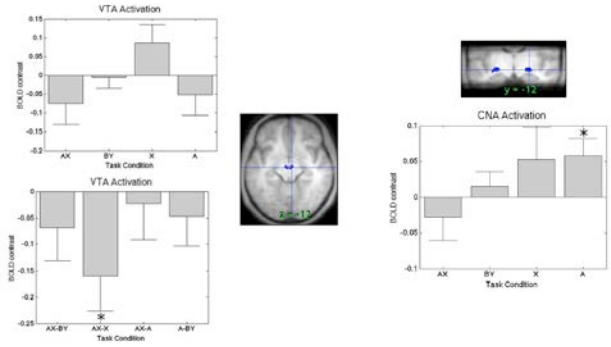
Topic: F.03. Motivation and Emotion

Support: NIH IBSC center 1-P50-MH079485

Title: Conditioned inhibition and the learning of negative values

Authors: *J. MOLLICK[1], R. O'REILLY[1], T. WAGER[1], G. FRANK[2]
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Abstract:



While the dopamine system in reward prediction is often modeled with the temporal-differences algorithm, we use a more biologically plausible model that considers the distinct computations of different brain areas. In our model, the central nucleus of the amygdala learns an association of a stimulus with reward, the ventral striatum inhibits the dopamine burst for a predicted reward, and the lateral habenula learns about negative valence rewards and the omission of positive rewards, and can inhibit the VTA to cause a dopamine dip for an omitted or negative valence reward.

To test our model's prediction that the learned value signal in the amygdala can be dissociated from the mechanism that inhibits dopamine firing at the time of reward, we ran a conditioned inhibition task, where subjects are conditioned on the pairing of a stimulus (A) with orange juice, and the pairing of a control stimulus with a neutral solution.

This is followed by four blocks of conditioned inhibition, which include the conditioned stimulus (A) and control from the earlier block. In these blocks, we also introduce a conditioned inhibitor (X) that predicts receipt of a neutral solution when paired with the reward-predictive stimulus. The inhibitor is shown alone and paired with the conditioned stimulus. These blocks also include the non-rewarded control, and a non-rewarded pairing of the control with another stimulus.

A preliminary analysis (n=12) found that the average activity for the conditioned stimulus was significantly greater than zero in the central nucleus of the amygdala. There was also significantly more central amygdala activity for the inhibitor (viewed alone) than to the control stimulus, which could be due to the salience of the inhibitor.

In the ventral tegmental area, we saw a significant decrease in activity for the CS-Inhibitor pair compared to the Inhibitor alone, which supports the idea that dopamine may also code for

salience. In the habenula region, we saw activity for the CS-Inhibitor pair compared to the control stimulus, indicating that it may be involved in the cancellation of an expected reward.

Disclosures: J. Mollick: None. **R. O'Reilly:** None. **T. Wager:** None. **G. Frank:** None.

Poster

923. Dopamine and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:923.12/FFF4

Topic: F.03. Motivation and Emotion

Support: NIMH: MH078023

MH: 094966

Title: Development of rodent models of the motivational symptoms of depression: The catecholamine depleting agent tetrabenazine affects effort-related decision making in the T-maze barrier choice task.

Authors: *S. E. YOHN[1], E. J. NUNES[1], P. A. RANDALL[1], Y. BAQI[2], C. E. MÜLLER[2], M. CORREA[1,3], J. D. SALAMONE[1]
[1]Psychology, Univ. of Connecticut, Storrs, CT; [2]Univ. Bonn, Bonn, Germany; [3]Univ. of Jaume I, Castello, Spain

Abstract: Mesolimbic dopamine (DA), particularly within the nucleus accumbens, has been shown to be a critical component of brain circuitry regulating behavioral activation and effort-related processes. Previous studies have shown that interference with accumbens DA alters response allocation in choice procedures that involve evaluations of reinforcement value and response costs. Several behavioral tests have been developed to assess effort-based choice behavior in rats, including maze procedures and operant tasks. The T-maze choice procedure developed by our laboratory is a frequently used task, in which the two arms of the maze contain different reinforcement densities (four vs. two or four vs. zero) and under some conditions a 44 cm barrier is placed in the high reinforcement density arm to create an effort-related obstacle. Under baseline or control conditions, rats will climb the barrier to receive the high-density reinforcement, while making relatively few selections of the low reward/low cost arm. However, accumbens DA depletions, or administration of DA D1 or D2 family antagonists (e.g. ecopipam or haloperidol), produce a shift in this behavior, thereby increasing low-density arm selection and decreasing selection of the high cost/high reward arm. It has been suggested that these drug-induced shifts in effort-based choice that are seen in animals could be analogous to symptoms

such as psychomotor retardation, anergia, and fatigue, which can be observed in individuals with depression, parkinsonism, and other disorders. For that reason, the current studies investigated the effects of the catecholamine depleting agent tetrabenazine on effort-related choice behavior using the T-maze procedure. Tetrabenazine inhibits the vesicular monoamine transporter 2 (VMAT-2) and is used to treat Huntington's disease. Tetrabenazine has been shown to produce depressive side effects in some patients, including motivational symptoms such as psychomotor slowing, anergia and fatigue. In rats tested on the T-maze choice procedure, administration of tetrabenazine in a dose range of 0.25-0.75 mg/kg IP decreased high-density arm selection but increased low-density arm selection. Initial evidence indicates that these effects of tetrabenazine on effort-related choice behavior can be reversed by co-administration of the adenosine A2A antagonist MSX-3. Collectively, these results demonstrate that administration of low doses of tetrabenazine can alter effort-related choice behavior, biasing animals towards low effort alternatives. The present research may contribute to the development of novel treatments for effort-related motivational symptoms in humans.

Disclosures: S.E. Yohn: None. **E.J. Nunes:** None. **P.A. Randall:** None. **Y. Baqi:** None. **C.E. Müller:** None. **M. Correa:** None. **J.D. Salamone:** None.

Poster

923. Dopamine and Behavior

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Program#/Poster#:923.13/FFF5

Topic: F.03. Motivation and Emotion

Support: ESF DoRa Program Activity 6

European Union Regional Develpment Grant

Estonian Government SF 1800027s08

Swedish Science Foundation VR-Med

Title: Galanin GalR1 but not GalR2 receptors influence mesolimbic dopamine-mediated behavioral energizing.

Authors: M. E. ANDERSON[1], J. RUNESSON[2], I. SAAR[3], Ü. LANGEL[2], *J. K. ROBINSON[1]
[1]SUNY Stony Book, Stony Brook, NY; [2]Stockholm Univ., Stockholm, Sweden; [3]Tartu Univ., Tartu, Estonia

Abstract: Galanin is a 29-amino acid long neuropeptide that has been implicated in many physiological and behavioral functions. Previous research has shown that i.c.v. administration of galanin strongly stimulates food intake in sated rats when food is freely available, but fails to stimulate this consumption when an operant response requirement is present. Mesolimbic dopamine (DA) pathways are also thought to play a role in this appetitive motivation to obtain a reward. Notably, it has been shown previously that fixed ratio schedules involving very little work are largely unaffected by DA depletions, while schedules that involve high work requirements are greatly impaired by these same depletions. Based upon this clear dissociation, the present study had several aims. First, using fixed ratio (FR) schedules, we sought to test the theory that galanin interacts with mesolimbic dopamine by administering galanin i.c.v. to rats working fixed ratio schedules requiring either a low work condition (FR1) or a high work condition (FR >1) to obtain a 0.2% saccharine reward. Consistent with actions of galanin only when dopamine would be released by the high work requirement, the rate of reinforcer receipt decreased for only the FR>1 group as compared to saline controls. Furthermore, injections of GalR1 receptor agonist M617 led to a similar selective decrease in the number of reinforcers received in the galanin-injected rats of the FR>1 group, but a similar decrease was not seen after injections of GalR2 receptor agonist M1153. Taken together, these results show a clear galanin-dopamine interaction at times of appetitive behavior, mediated by the GalR1 receptor.

Disclosures: M.E. Anderson: None. **J. Runesson:** None. **I. Saar:** None. **Ü. Langel:** None. **J.K. Robinson:** None.

Poster

923. Dopamine and Behavior

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Program#/Poster#:923.14/FFF6

Topic: F.03. Motivation and Emotion

Support: NIMH Grant MH078023

Fundació Bancaixa-UJI (P1.1B2010-43)

Title: Winners, losers, workers and slackers: Individual differences in exertion of effort on a progressive ratio/ chow feeding choice task are related to differences in DARPP-32 (Thr34) expression in nucleus accumbens core

Authors: *M. PARDO[1,2], P. A. RANDALL[2], E. J. NUNES[2], L. LÓPEZ-CRUZ[1], M. CORREA[1,2], J. D. SALAMONE[2]

[1]Univ. Jaume I, Castelló, Spain; [2]Dept. of Psychology, Univ. of Connecticut, Storrs Mansfield, CT

Abstract: Several behavioral tests have been developed to assess effort-based choice behavior in rats, including a T-maze barrier choice task and various operant procedures. Recently, a progressive ratio (PROG)/chow feeding concurrent choice procedure was developed, in which rats can press on a PROG schedule reinforced by presentation of preferred high carbohydrate operant pellets, or alternatively, can approach and consume a less preferred chow that is available in the chamber. The rat passes through each ratio level by completing 15 ratios, after which the ratio requirement is incremented by one additional ratio (e.g. FR1 15 times, FR2 15 times, etc.). The PROG schedule stays active as long as the rats receive a reinforcer every 2 min. Typically, rats lever press until the PROG schedule times out, and the animals then consume considerable chow. Thus, the rats eventually reach a point in the session at which the ratio requirement becomes too high, so they switch to chow. Administration of the dopamine (DA) D2 antagonist haloperidol (0.025-0.1 mg/kg IP) significantly reduced number of lever presses, highest ratio achieved, and active lever time. Haloperidol did not reduce chow intake, and if anything there was a tendency to increase chow intake in some rats. Performance on the PROG/chow feeding choice task was highly variable; some rats lever pressed fewer than 100 times and had high levels of chow intake, while others lever pressed more than 1000 times and consumed only small amounts of chow. Moreover, the effects of haloperidol were more marked in rats with higher control levels of lever pressing; analysis of effect sizes showed that the suppressive effect of haloperidol on number of lever presses was greater in high responders (eta2 = 0.236) than low responders (eta2 = 0.157). For these reasons, an additional experiment investigated potential neurochemical differences between high and low responders, using pDARPP-32(Thr34) immunocytochemistry as a marker of signal transduction activity that has been linked to DA receptor activation. To analyze the DARPP-32 expression data, a median split based upon behavioral performance during the final test day was performed. After the behavioral session, rats with high levels of PROG lever pressing showed significantly greater pDARPP-32(Thr34) expression in nucleus accumbens core relative to low responders. There were no differences in accumbens shell and cingulate cortex expression. These results highlight the importance of DA transmission, as well as signal transduction activity in accumbens core neurons, as markers of individual differences in effort-related performance.

Disclosures: M. Pardo: None. **P.A. Randall:** None. **E.J. Nunes:** None. **L. López-Cruz:** None. **M. Correa:** None. **J.D. Salamone:** None.

Poster

923. Dopamine and Behavior

Location: Hall F-J

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Program#/Poster#:923.15/FFF7

Topic: F.03. Motivation and Emotion

Support: CIHR

OGS

Title: Responding for conditioned reinforcement in mice: Effects of strain, operant response type, and methylphenidate

Authors: *C. BROWNE[1], P. J. FLETCHER[2,3]
[1]Univ. of Toronto/Camh, Toronto, ON, Canada; [2]Biopsychology, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [3]Psychology, Univ. of Toronto, Toronto, ON, Canada

Abstract: Environmental stimuli associated with primary rewards such as food or drugs of abuse acquire incentive motivational significance through associative learning and can function as conditioned reinforcers (CR). In drug addiction, these types of stimuli may induce cravings and lead to relapse. Attribution of salience to reward-associated stimuli may be mediated partly through a dopamine (DA) dependent mechanism. Although much has been learned about incentive motivation from studies of conditioned reinforcement in rats, few studies have examined this phenomenon in mice. This is surprising due to the potential for using transgenic mice to explore brain mechanisms in incentive motivation. These experiments assessed 1) whether mice would learn a stimulus-reward association, 2) whether that stimulus would function as a CR, 3) the temporal stability of responding for this CR, and 4) whether responding would be altered by enhancing DA function. We also explored factors related to strain differences and nature of the operant response.
Water-restricted mice (C57Bl/6 or CD1) received 30 pairings of a conditioned stimulus (CS; light+noise) with 0.01 ml 0.2% saccharin in each of 14 daily 40 min sessions. Subsequently, mice were allowed access to 2 response levers or 2 nose-poke ports. Responding on one lever or port delivered the CS (now a CR); responses on the other lever or port had no effect. Mice were tested four times following an i.p. injection of saline or the DA reuptake blocker methylphenidate (MPH; 2.5, 3.5, 5 mg/kg).
All mice learned to approach the saccharin magazine when the CS was presented. Subsequently, mice learned to selectively respond on the lever or port delivering the CR. Within each strain, responding for the CR on the levers versus the ports was roughly equivalent, while CD1 mice showed more responding than C57 mice overall. MPH increased lever responding for the CR but only in the CD1 strain.
The results show that mice can learn a simple stimulus-reward relationship and respond for that stimulus when it functions as a CR. Responding for CR may be more pronounced in the CD1 strain and is enhanced by increasing DA function in this strain. These findings potentially set the stage for using transgenic mice to explore brain mechanisms involved in incentive learning.

Disclosures: C. Browne: None. **P.J. Fletcher:** None.

Poster

923. Dopamine and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:923.16/FFF8

Topic: F.03. Motivation and Emotion

Support: Mercer Univ. internal support

Title: The effects of Salvinorin A on conditioned place preference formation, locomotor behavior, and immediate-early gene expression in midbrain dopaminergic neurons

Authors: W. JENKINS[1], J. SALVATIERRA[2], *K. NORTHCUTT[2]
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Abstract: Salvinorin A (SA), the primary psychoactive agent in the increasingly popular recreational drug salvia, has been classified as a selective kappa opioid agonist. However, recent research suggests that SA may directly interact with the dopamine (DA) system because some of its effects are blocked by a DA antagonist, but not by a kappa opioid antagonist. Given DA's role in mediating the reinforcing properties of various drugs of abuse, SA may have abuse potential. Currently, there is very little published research investigating this possibility, and the research that does exist is somewhat equivocal with some doses resulting in conditioned place preference (CPP) for SA -paired chambers and others showing conditioned place aversions. In addition, the ability of SA to induce locomotor sensitization, another marker of abuse potential, is unknown. The current study investigated whether several different doses (0.1, 10, or 50 μg/kg) of SA would support the development of CPP in adult male rats, and whether repeated SA administration at these same doses would affect psychomotor activation. Finally, because little is known about the dopaminergic networks that SA acts on in the brain, we examined immediate-early gene expression in midbrain DAergic neurons one hour after SA administration using double-label immunohistochemical procedures for tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, and the immediate-early gene product Fos. We observed a CPP only in those animals receiving 10 μg/kg SA, and these same animals had greater locomotor activity after repeated administration of this dose of SA. In addition, there were significantly more TH/Fos double-labeled cells in the substantia nigra of animals receiving an injection of 10 μg/kg SA compared to all other doses of SA and the control animals, but we did not find differences among treatment groups in the ventral tegmental area. The other doses we tested did not support CPP, and they did not significantly affect locomotor activity or the number of

double-labeled cells in the brain areas examined. We conclude that SA, at least at some doses, may indeed represent a potential drug of abuse that markedly affects the DA system and the motor responses that it generates.

Disclosures: W. Jenkins: None. **J. Salvatierra:** None. **K. Northcutt:** None.

Poster

923. Dopamine and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:923.17/FFF9

Topic: F.03. Motivation and Emotion

Support: NADIA Project U01-AA019972

NADIA Project U24-AA020024

UNC Bowles Center for Alcohol Studies

Title: Does adolescent ethanol exposure change the response to acute ethanol in adulthood? Preliminary results on social behavior, dopamine release and gene expression

Authors: *D. L. ROBINSON[1], W. F. MATHES[2], D. L. ZITZMAN[1], W. H. CHAPMAN[1], L. P. SPEAR[3]
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Abstract: Adolescence marks a dynamic period within the mesolimbic dopamine system. During this developmental stage, dopamine release and receptor expression peak, followed by a gradual decline into adulthood. In addition, prefrontal cortical projections to subcortical targets are undergoing myelination and pruning. The brain changes occurring during adolescence are thought to underlie the enhanced sensitivity to reinforcing stimuli, such as social reward and addictive drugs that is observed in adolescent humans and animal models. Given this marked remodeling of mesocorticolimbic dopamine systems and the sensitivity of dopamine to social stimuli, we investigated the functional impact of adolescent intermittent ethanol exposure (AIE) on aspects of dopamine transmission in adulthood. We exposed male rats to i.g. injections of vehicle (“super-sac”: 0.125% saccharin and 3% sucrose) or ethanol using an AIE schedule of 2.5g/kg i.g. (25% ethanol v/v in super-sac) every other day from P25 - P45. This dosing produced ~80mg% in tail blood samples collected 30 min after administration. Rats were tested in early adulthood at P80±1 day for dopamine release after saline or ethanol challenge (0.5 or 1

g/kg ethanol, i.p.). Dopamine release in the ventral striatum was measured with fast scan cyclic voltammetry at baseline, after challenge injection, and during three brief periods of interaction with another rat. We confirmed that 1 g/kg ethanol administration and brief social interactions both increase the frequency of dopamine transients, consistent with our previous reports. Moreover, analysis of behavior during social interaction shows that AIE treatment reduces social contacts in the first interaction, while ethanol yielded a biphasic effect, with 0.5 g/kg facilitating and 1 g/kg inhibiting social contacts in the third interaction. A concurrent experiment evaluated gene expression in animals having undergone the same treatments. Gene expression in the prefrontal cortex and striatum was assessed with Affymetrix microarrays. Preliminary analysis found that 68 genes in the PFC and 191 genes in the striatum were significantly differentially expressed in animals exposed to AIE and/or acute ethanol challenge, and several of these genes have a direct effect on DA signaling, receptor binding, and DA neuronal development.

Disclosures: D.L. Robinson: None. **W.F. Mathes:** None. **D.L. Zitzman:** None. **W.H. Chapman:** None. **L.P. Spear:** None.

Poster

923. Dopamine and Behavior

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Program#/Poster#:923.18/FFF10

Topic: F.03. Motivation and Emotion

Support: NIH Grant MH079292 (PEMP)

NSF Graduate Research Fellowship (NGH)

NIH T32 DA007278

Title: Nucleus accumbens dopamine transmission during decisions involving trade-offs between reward value and effort requirement

Authors: *N. G. HOLLON[1,2], M. M. ARNOLD[1], J. O. GAN[4], M. E. WALTON[5], P. E. M. PHILLIPS[1,2,3]
[1]Psychiatry & Behavioral Sci., [2]Grad. Program in Neurobio. & Behavior, [3]Pharmacol., Univ. of Washington, Seattle, WA; [4]Neurol. & Neurosci., Weill Cornell Med. Col., New York, NY; [5]Exptl. Psychology, Oxford Univ., Oxford, United Kingdom

Abstract: As a corollary of signaling errors in reward prediction, the magnitude of phasic dopamine transmission to the unexpected presentation of a reward-predictive cue reflects the

expected value associated with that cue. We recently demonstrated that dopamine release in the nucleus accumbens to the presentation of single cues encodes their expected reward value but not response cost involving different effort requirements. Here we use this insight to investigate the unresolved question of what dopamine transmission signals during choices between options of different value. In particular, there remains a discrepancy in the literature regarding whether dopamine encodes the value of the subsequently chosen option or the greatest value available regardless of what the animal ultimately chooses. Unlike these previous studies, however, our current design does not depend on analysis of anomalous choices for an option with low overall utility.
We used fast-scan cyclic voltammetry to record rapid changes in dopamine concentration in the nucleus accumbens of rats performing a decision-making task in which two levers were associated with different reward sizes and effort requirements. Each session included single-option "Forced" trials intermixed with two-option "Choice" trials. In one session type, one lever earned a small reward for a low response cost, and the other lever earned a large reward for a moderate cost. Rats significantly preferred the large reward / moderate cost option, and cue-evoked dopamine release showed the expected coding of reward value on Forced trials, with greater release for the large than the small reward option. In other sessions, rats instead preferred the small reward / low cost option over a large reward / high cost option. We observed greater sustained dopamine concentration following the large reward / high cost option but no difference in peak dopamine release to the two options. Cue-evoked dopamine release during Choice trials was similar to that in the corresponding Forced trials in both session types. These results support the notion that accumbens dopamine transmission signals the value of the subsequently chosen option. The magnitude of cue-evoked phasic dopamine, however, reflects an interaction between expected reward value and net utility (as revealed by behavioral preference) but not a simple encoding of either in isolation.

Disclosures: N.G. Hollon: None. **M.M. Arnold:** None. **J.O. Gan:** None. **M.E. Walton:** None. **P.E.M. Phillips:** None.

Poster

923. Dopamine and Behavior

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:923.19/FFF11

Topic: F.03. Motivation and Emotion

Title: Hyperactivity is reduced by environmental enrichment during growing period in an ADHD model rat accompanied with enhanced CART expression in medial prefrontal cortex

Authors: *Y. SHIMIZU, R. NISHIGAKI, Y. WATANABE, Y. UEDA, S. MISUMI, T. MASUDA, H. HIDA
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Abstract: Attention deficit hyperactivity disorder (ADHD) is characterized by hyperactivity, impulsivity and inattention. Spontaneously hypertensive rat (SHR) is often used as a behavioral animal model of ADHD. Dysfunction of mesocorticolimbic dopamine (DA) system such in nucleus accumbence (NAc), amygdala (Amy) and medial prefrontal cortex (mPFC) is related to ADHD: deficits of DA transporter and DA type 4 receptor (D4R) are known as genetic factor while environmental condition are important in brain DA system as well. To investigate the effect of environmental enrichment (EE) during the growing period on ADHD-like behavior in SHR, SHR were allowed to grow in EE, standard environment (SE) or isolated environment (IE) from weaning (P25) to adulthood for 5 weeks, followed by behavioral assessment in open-field test and cylinder test. In open-field test, the decrease in hyperactive locomotion was shown in rats reared in EE, and the velocity was slow in EE compared to SE or IE. In cylinder test, the rats grown in EE showed the significant decrease in anxiety behavior (rearing and locomotion) as compared to those in SE or IE. Therefore, we investigated gene expression of D4R and cocaine and amphetamine regulated transcript (CART) in mPFC, NAc and amygdala. There were no differences of D4R expression in those areas. Significant enhancement of CART mRNA was shown in mPFC of EE group. Immunostaining revealed that CART did not colocalized with parvalbumin-positive GABAergic neurons in mPFC. Data suggest that EE during the growing period improves hyperactivity with change of CART expression in mPFC.

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Poster

923. Dopamine and Behavior

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Program#/Poster#:923.20/FFF12

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA027679

NIH Grant UL1RR024153

NIH Grant UL1TR000005

Grant from OSVC, Health Sciences, Univ Pittsburgh

Title: CS-evoked ERK activation in the nucleus accumbens during Pavlovian-instrumental transfer is mediated by D1 dopamine receptors

Authors: *M. L. REMUS[1,2], C.-A. DOMJAN-YUHAS[3], E. THIELS[4,2,3]
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Abstract: When a neutral stimulus is repeatedly paired with reward, it becomes a cue predictive of the reward. Studies have shown that exposure to such a conditioned stimulus (CS) potentiates behavior aimed at obtaining the reward, as occurs during Pavlovian-instrumental transfer (PIT). We showed that an appetitive CS causes activation of extracellular signal-regulated kinase (ERK) in both the core and shell of the nucleus accumbens (NAc), and that NAc ERK activation is crucial for PIT (Shiflett et al., 2008; Remus & Thiels, submitted). The receptor pharmacology of the CS-evoked ERK activation during PIT is not known. D1 dopamine receptor (D1R) activation is coupled positively to ERK activation in striatal cells (Bertran-Gonzalez et al., 2008). We therefore wanted to determine whether D1R activation plays a role in the CS-evoked increase in active ERK (pERK) in both the core and the shell during PIT. Rats were fitted bilaterally with cannulae aimed between the core and shell of the NAc, and trained with a Pavlovian and an instrumental conditioning paradigm, as described previously. Immediately before the PIT test, the D1R-type antagonist SCH23390 was infused into the NAc on one side and vehicle solution on the other. Unilateral infusion of SCH23390 had no effect on PIT. Comparison of pERK immunoreactive cell counts between the drug- and vehicle-treated NAc revealed that SCH23390 completely blocked the CS-evoked increase in ERK activation in both the core and the shell. Consistent with a requirement of NAc ERK activation for PIT, bilateral infusion of SCH23390, but not of vehicle solution, disrupted PIT, without effect on basal instrumental responding for food reward. Our findings indicate that D1Rs play a critical role in mediating PIT-relevant ERK activation in the NAc, and they suggest D1Rs as a pharmacological target by which NAc ERK and, by extension, PIT can be modulated. We now are determining the NAc cell phenotype in which the D1R-mediated CS-evoked ERK activation occurs using a combination of pharmacological D1R blockade and dual-immunoflurorescence staining for pERK and DARPP32, a protein expressed only by NAc projection neurons, or preprodynorphin (PPD), a protein expressed only by D1R-containing NAc projection neurons. We expect overlap of the pERK signal with DARP32 and PPD staining, and that intra-NAc infusion of SCH23390 will abolish the pERK signal but have no effect on the DARP32 or PPD signal. These data would indicate that CS-evoked ERK activation in the NAc occurs selectively in D1R-containing medium spiny neurons. The results thereby suggest that inputs to and targets of D1R-expressing medium spiny neurons are critical elements of the circuit underlying PIT.

Disclosures: M.L. Remus: None. **C. Domjan-Yuhas:** None. **E. Thiels:** None.

Poster

923. Dopamine and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:923.21/FFF13

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA027679

NIH-Grant UL1RR024153

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Grant from OSVC, Health Sciences, Univ Pittsburgh

Title: Repeated pre-treatment with amphetamine enhances cue-potentiation of reward seeking and associated ERK activation in multiple structures of the reward system

Authors: *J. C. MAUNA[1,2], E. THIELS[2,3,4]
[1]Neurosci., Univ. Pittsburgh, PITTSBURGH, PA; [2]Ctr. for Neurosci., [3]Neurobio., [4]Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Stimuli that repeatedly were paired with reward can potentiate behaviors aimed at obtaining the reward. This effect of conditioned stimuli (CS) on reward seeking [Pavlovian-Instrumental Transfer (PIT)] depends on the reward system, most notably the nucleus accumbens (NAc). Exposure to an appetitive CS increases ERK2 activation in the NAc of rats, and blockade of NAc ERK interferes with PIT (Shiflett et al. 2008). Amphetamine (AMPH) treatment after daily Pavlovian conditioning enhances CS-evoked ERK2 activation as well as CS-potentiation of reward seeking in drug-free rats (Mauna et al. SfN 2011). Here, we examined whether the AMPH-induced enhancement of PIT is associated with increased ERK activation in both subregions of the NAc, and whether CS-evoked ERK activation is modulated by AMPH pretreatment in other structures of the reward system. Rats received daily sessions of tone-food (CS-US) pairings and were treated with either AMPH (1 mg/kg, i.p.) or saline (SAL) immediately after each session. Rats then were trained to press a lever for food. Seven days after the last drug treatment, rats underwent PIT testing, and then brains were removed for analysis of ERK activation. Basal NAc ERK activation was determined in control rats that received tone presentations only (no food) during Pavlovian training but otherwise were treated identically to rats that received tone+food (T+F) pairings. The enhancing effect of AMPH pre-treatment on CS-evoked ERK activation, as determined by Western blot, and on CS-potentiated lever pressing, was maintained for at least 7 days after the last drug treatment. The AMPH effect was observed throughout the NAc: pERK(+) cells were more abundant in both the core and the shell of AMPH- compared to SAL-treated T+F rats, while the pERK(+) cell count was equally low in

both control groups. A similar pattern of results was observed in the prefrontal cortex (PFC), in particular in the deeper layers, as well as in the basolateral amygdala (BLA). Interestingly, pERK(+) cells in the dorsolateral striatum (DLS) were more numerous, relative to control rats, only in AMPH- but not SAL-treated T+F rats. Neither training nor drug treatment affected pERK staining in the dorsomedial striatum. Consistent with previous work, our findings implicate the BLA in the mediation of PIT. Additionally, our findings suggest that the PFC also is part of the PIT circuit, and, importantly, that the AMPH effect on PIT entails recruitment of all three key elements of the reward system studied here. Our results suggest that the dorsal striatum does not play a role in PIT, although the DLS may contribute to enhancement of PIT caused by prior exposure to a psychostimulant.

Disclosures: J.C. Mauna: None. **E. Thiels:** None.

Poster

923. Dopamine and Behavior

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:923.22/FFF14

Topic: F.03. Motivation and Emotion

Title: The effects of pramipexole on conditioned responding in “sign-trackers” and “goal-trackers”

Authors: *J. HAIGHT[1], R. KELLEY[1], S. B. FLAGEL[1,2]
[1]Psychiatry, [2]Mol. Behavioral Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: Pramipexole, a dopamine D2/D3 receptor agonist, is a therapeutic drug used to treat the symptoms of various maladies including Parkinson’s Disease, restless-leg syndrome and fibromyalgia. A subset of patients treated with pramipexole have been found to develop impulse control disorders (ICDs), centered around compulsions such as gambling, eating, illicit drug-taking or sexual behavior. Why only certain individuals are predisposed to develop these ICDs is largely unknown. In recent years, a rodent model exploring individual differences in response to a reward-paired stimulus has shed light on the psychological and neurobiological processes that likely contribute to such impulsive behaviors. When trained in a Pavlovian conditioning task, outbred Sprague-Dawley rats will develop a conditioned response that consists primarily of either “sign-tracking” or “goal-tracking” behavior. Sign-trackers (STs) direct their behavior towards the “sign” or conditioned stimulus (CS) associated with reward delivery, while goal-trackers (GTs) approach the location of reward delivery upon CS presentation. Importantly, it has been shown that the CS comes to have motivational value, or incentive salience, for STs, and

that learning of the sign-tracking (but not the goal-tracking) response is dependent on dopamine. Using this model of individual differences in the attribution of incentive salience, we sought to identify distinct traits that might render one susceptible to developing ICDs when administered pramipexole. Outbred Sprague-Dawley rats were trained in a Pavlovian-conditioning task, where presentation of a lever-CS predicted delivery of a food pellet. After seven days of training, animals were classified as STs or GTs based on their conditioned response (CR) to lever presentation. Animals from each group were then treated with either saline or three different doses in descending order (0.32, 0.1 and 0.032 mg/kg, s.c.) of pramipexole immediately prior to the next three conditioning sessions, and changes in the expression of the conditioned responses were assessed. Since higher doses of pramipexole can increase nonspecific responding, this was controlled for and was not a factor within this dose range. Preliminary results indicate that 0.032 and 0.1 mg/kg of pramipexole attenuates CR expression in both STs and GTs, while the higher dose of 0.32 mg/kg decreases expression only in STs. Additional studies and analyses are currently underway to further our understanding of the effects of pramipexole on CR expression in STs and GTs.

Disclosures: J. Haight: None. **R. Kelley:** None. **S.B. Flagel:** None.

Poster

924. Reward: Neuropharmacology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:924.01/FFF15

Topic: F.03. Motivation and Emotion

Support: NHMRC Project Grant

Title: The mGlur5 antagonist MTEP impairs the acquisition of the extinction of alcohol-seeking

Authors: K. E. HUTTON-BEDBROOK, *G. P. MCNALLY
Univ. New South Wales, Sydney, Australia

Abstract: Extinction learning in several conditioning preparations depends on glutamatergic neurotransmission, but the role of glutamate in extinction of drug seeking, and the specific receptors involved, remain poorly understood. Recent evidence has identified a role for metabotropic glutamate receptors in drug seeking behaviours. We investigated the role of mGluR5 and NMDA receptors in the acquisition and expression of the extinction of alcohol-seeking as well the neuroanatomical locus for this effect. Male Long-Evans were trained in 1 hr sessions across 7 days to respond for alcoholic beer. Following this, animals underwent extinction training during which responding was no longer reinforced. In Experiment 1, injection

of the mGluR5 antagonist MTEP (1 mg/kg) impaired the acquisition but not expression of extinction. In contrast, in Experiment 2 injection of the NMDA receptor antagonist ifenprodil (3 mg/kg) did not impair extinction learning. In Experiment 3 the impairment of extinction learning by MTEP was shown to be dose-dependent. Finally, Experiment 4 studied the role of basolateral amygdala (BLA) mGluR5 receptors in extinction learning by microinjecting MTEP into the BLA prior to extinction training. Taken together, these experiments identify a role for mGluR5 receptors in extinction learning during drug self-administration.

Disclosures: K.E. Hutton-Bedbrook: None. **G.P. McNally:** Research Grant; NHMRC project grant.

Poster

924. Reward: Neuropharmacology

Location: Hall F-J

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Program#/Poster#:924.02/FFF16

Topic: F.03. Motivation and Emotion

Support: Marie Curie FP7-Reintegration-247841 – "Alco_CaMK"Grant-

Title: alpha-CaMKII autophosphorylation regulates morphology of post-synaptic densities in the hippocampus and mice behaviour after alcohol treatment

Authors: *K. RADWANSKA, M. LIPINSKI, M. SZYMANSKI, K. ŁUKASIEWICZ
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Abstract: Ca2+/calmodulin-dependent kinase II (alpha-CaMKII) is a major protein of glutamatergic synapses in the forebrain. Its role in learning and memory processes is very well documented. Its function in addiction-related processes, is however largely unknown. Here we show that alpha-CaMKII autophosphorylation deficient mutants (CaMKII-T286A) have altered behavioural response to alcohol. The T286A mutants do not show increased locomotor activity or decreased anxiety in open field and elevated zero-maze, as observed in wild-type mice. These behaviours are also not sensitised in the mutants after chronic alcohol treatment, as it is observed in the wild-type animals. Furthermore, we show that alcohol treatment (2.5 g/kg) decreases an average size of immunopositive puncta for a marker of post-synaptic densities, PSD95, in the CA1 field of the hippocampus of the wild-type mice. This phenomenon does not occur in alcohol-treated T286A mutants. Thus our data show that alpha-CaMKII autophosphorylation is involved both in the regulation of psychostimulant effects of alcohol, and alcohol-induced changes in the morphology of post-synaptic densities in the hippocampus. These observations

suggest that psychostimulant effects of alcohol involve remodelling of post-synaptic densities in the hippocampus and this process is regulated by CaMKII autophosphorylation.

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Poster

924. Reward: Neuropharmacology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:924.03/FFF17

Topic: F.03. Motivation and Emotion

Support: NIDA IRP

Title: Effect of the Toll-like receptor 4 antagonist (+)- naltrexone on incubation of heroin craving

Authors: S. KAMBHAMPATI[1], H. EICHENBAUM[1], C. L. PICKENS[1], J. M. BOSSERT[1], K. RICE[2], L. R. WATKINS[3], Y. SHAHAM[1], *F. R. THEBERGE[1]
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Abstract: Recent evidence implicates the glial toll-like receptor 4 (TLR4) in morphine analgesia, tolerance, and reward, as assessed in the conditioned place preference procedure. Here we used the selective TLR4 antagonist (+)-naltrexone (a μ-opioid receptor inactive isomer) to determine the role of this receptor in the time-dependent increases in cue-induced heroin seeking during forced abstinence (incubation of heroin craving). We trained rats for 9 h/day to self-administer heroin (0.1 mg/kg/infusion) for 9 days; lever presses were paired with a 5-sec tone-light cue. We then assessed cue-induced heroin seeking in 30-min extinction sessions on abstinence day 1; immediately after testing, we implanted the rats with Alzet minipumps delivering (+)-naltrexone (0, 15.0, or 30.0 mg/kg/day, s.c) for 14 days. We then tested the rats for incubated cue-induced heroin seeking in 3-h extinction tests on abstinence day 13. We found that both doses of (+)-naltrexone decreased cue-induced heroin seeking on abstinence day 13. Results suggest a critical role of the glial TLR4 in the development of incubation of heroin craving. We currently study the effect of acute injections of (+)-naltrexone on incubation of heroin craving.

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Poster

924. Reward: Neuropharmacology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:924.04/FFF18

Topic: F.03. Motivation and Emotion

Support: NIAAA Intramural funding

Title: Dietary tyrosine/phenylalanine depletion blunts human mesolimbic recruitment by prospective rewards

Authors: *J. M. BJORK[1], S. J. GRANT[2], G. CHEN[3], D. W. HOMMER[4]
[1]DCNBR-Clin Neurosci Br., NIH/NIDA, BETHESDA, MD; [2]DCNBR, NIDA, Bethesda, MD; [3]Statistical and Scientific Computing Core, NIMH, Bethesda, MD; [4]Lab. of Clin. and Translational Studies, NIAAA, Bethesda, MD

Abstract: In humans, functional magnetic resonance imaging (fMRI) experiments have demonstrated that anticipation of instrumental responses for various rewards recruits the ventral striatum (VS). This has been attributed to activation of mesolimbic dopaminergic (DA) circuitry by cues for incentives, once cue-contingencies have been learned or trained. To confirm a causal role of dopaminergic functioning in human instrumental behavior, subjects responded to cues to win or avoid losing money in a monetary incentive delay (MID) task during temporary dietary restriction of DA precursor availability to the brain. In a repeated-measures, double-blind design, 16 healthy adults performed the MID task five hours after ingestion of one of two amino acid beverages in a counterbalanced order. The “depletion” (DEP) beverage consisted of large neutral amino acids (LNAA) that lacked catecholamine precursors Tyrosine (Tyr) and Phenylalanine (Phe). The “balanced” (BAL) amino-acid beverage included a proportionally-balanced amount of Tyr and Phe. Relative to pre-drink baseline, the DEP beverage caused a 94% reduction in the plasma (Tyr+Phe)/LNAA ratio at the time of scanning, while the BAL drink caused a modest 15% decrease. However, there were no dose differences in self-reported mood or in MID task reaction times. However, relative to the BAL beverage, the DEP beverage halved the peak modeled BOLD signal recruitment by large MID task rewards in a volume-of-interest anatomically localized in the VS, and also resulted in blunted patterns of brain-wide activation that did not reach a significant dose difference. In addition, notification of losses activated anterior insula under DEP conditions, but not BAL conditions. These data suggest that

mesolimbic incentive-related circuitry in humans is physiologically sensitive to changes in dopamine precursor availability, but the system maintains significant reserve or buffering capacity to resist a concomitant effect on behavior.

Disclosures: J.M. Bjork: None. **S.J. Grant:** None. **G. Chen:** None. **D.W. Hommer:** None.

Poster

924. Reward: Neuropharmacology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:924.05/FFF19

Topic: F.03. Motivation and Emotion

Support: NSERC

Title: Effects of perinatal delta-9-tetrahydrocannabinol (THC) exposure on brain c-Fos immunoreactivity following the reinstatement of morphine conditioned place preference by THC

Authors: *G. WILLEMS, P. E. MALLET
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Abstract: Early life experiences, which occur during critical periods of development, are thought to influence vulnerability to adulthood drug addiction. While perinatal THC exposure has been shown to exacerbate the rewarding qualities of opioids in adulthood, exposure to an enriched environment (EE) during adolescence is implicated in the inhibition of adulthood opioid reward. Here, we intend to demonstrate that animals perinatally exposed to THC show differential degrees of morphine reward in adulthood dependent on housing in an enriched or standard environment (SE).Perinatal THC injections (5 mg/kg, subcutaneous) were administered from post-natal day 4 to 14, a time period which is reflective of the third trimester of human gestation. Animals housed in SE are expected to establish a more salient conditioned place preference (CPP) and take longer to extinguish morphine CPP (5 mg/kg, intraperitoneal) compared to animals housed in EE indicating that EE may have a protective effect against perinatal THC exposure. Furthermore, it is expected that THC will reinstate an extinguished morphine-induced CPP following a priming dose of THC (0.75 mg/kg, intraperitoneal) in animals perinatally exposed to THC, but not in untreated controls suggesting perinatal THC exposure increases cross-sensitization of animals to opioids. Neural activity in several brain regions associated with the mesocorticolimbic dopamine pathway (shell and core of nucleus accumbens, dorsomedial caudate putamen, dorsolateral periaqueductal gray, ventral tegmental area, and the bed nucleus of the stria terminalis) will be examined using c-Fos immunohistochemistry following the reinstatement of the extinguished morphine CPP. Based on

previous research conducted in our lab, it is expected that some regions will exhibit an increase in neural activity due to perinatal THC exposure, while other regions will have decreased c-Fos expression. These expected changes in c-Fos immunoreactivity may elucidate the underlying neural correlates of the observed changes in the rewarding properties of opiates due to perinatal THC exposure and environmental complexity.

Disclosures: G. Willems: Other Research Support; Canadian Consortium for the Investigation of Cannabinoids. **P.E. Mallet:** None.

Poster

924. Reward: Neuropharmacology

Location: Hall F-J

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Program#/Poster#:924.06/FFF20

Topic: F.03. Motivation and Emotion

Support: Supported by NIH grants DA017178 and DA020725 to T.LWhite.

Title: Amphetamine modulates fMRI BOLD response during active risk-taking in healthy young adults.

Authors: K. MILMAN[1], L. H. SWEET[2], M. WORDEN[1], R. A. COHEN[2], *T. L. WHITE[3]
[1]Neurosci., [2]Dept of Psychiatry and Human Behavior, [3]Ctr. for Alcohol & Addiction Studies, Brown Univ., PROVIDENCE, RI

Abstract: Amphetamine is a psychostimulant used for treatment of ADHD, and for military air operations because it increases alertness and attention. Clinical evidence suggests that impulsivity increases after drug consumption in addicted individuals. This increase in impulsive behavior could facilitate the transition to drug abuse through increased drug exposure in vulnerable young adults. To date, however, brain mechanisms involved in the effects of drugs on impulsive behavior are not well understood in healthy individuals. Objective: The present study was designed to determine the brain regions involved in acute drug effects on impulsive behavior, as measured using fMRI and a computerized measure of risk-taking behavior (Balloon Analogue Risk Task, BART) in healthy young adult volunteers. Methods: Healthy participants (N=52; 18-35 years) performed 4 runs of the BART during fMRI using standard echo planar conditions, 90 minutes after consumption of 20 mg d-amphetamine or placebo on two separate test days in a double-blind, within-subjects design. Results: Preliminary analyses (*n*=46) indicate significant drug effects on regions involved in attention, default network, and reward approach. During high incentive trials, BOLD response was elevated in midline thalamus, right parahippocampal gyrus, and right cerebellum ($p \leq 9.5 \times 10^{-4}$, FDR corrected p-value $\leq .05$,

cluster sizes > 150 voxels) after consumption of amphetamine compared to placebo, suggesting stimulant augmentation of activity in regions involved in emotional gating and volitional approach. BOLD responses to cash-out choices during the task were elevated in right lentiform nucleus/ right putamen, right posterior cingulate and left precentral gyrus after consumption of amphetamine compared to placebo, suggesting a facilitation of volitional motor approach of reward under drug ($p \leq 3.3 \times 10^{-5}$, FDR corrected p-value $\leq .05$). In contrast, BOLD responses in regions involved in spatial attention, sensory attention, and default mode network appeared to be reduced after amphetamine compared to placebo during the high incentive trials (right postcentral gyrus/ BA 40, left cuneus, bilateral lingual gyrus, left superior parietal lobule, left superior temporal gyrus, $p \leq 9.5 \times 10^{-4}$, FDR corrected p-value $\leq .05$). Conclusions: These data suggest that amphetamine selectively heightens the processing of relatively salient, impulsivity-relevant reward targets in ways that facilitate an internally-generated pattern of reward approach, within the larger context of reduced processing of unrelated external cues. Supported by National Institute on Drug Abuse (NIDA) grants DA017178 and DA020725 to T.L.White.

Disclosures: K. Milman: None. **L.H. Sweet:** None. **M. Worden:** None. **R.A. Cohen:** None. **T.L. White:** None.

Poster

924. Reward: Neuropharmacology

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Program#/Poster#:924.07/FFF21

Topic: F.03. Motivation and Emotion

Support: R01 DA019942-05

K05 DA015267

Title: Effects of orexin receptor antagonism on methamphetamine seeking in adolescent and adult rats

Authors: *N. E. ZLEBNIK, N. A. HOLTZ, M. E. CARROLL
Univ. of Minnesota, Minneapolis, MN

Abstract: Previous studies have shown enhanced vulnerability to addiction in adolescent (vs. adult) as well as female (vs. male) rats. The orexin system recently has been implicated in addiction and reward processing, with orexin receptor blockade more effective in reducing drug seeking in male vs. female rats. However, to date, it is not known whether there are age differences in orexin antagonism of drug seeking. In the present study, we investigated treatment

of methamphetamine (METH) seeking in both adolescent (PND 23-60) and adult (> PND 90) male and female rats using selective orexin-1 receptor (Orx1A, SB-334867, 20 mg/kg) and orexin-2 receptor (Orx2A, TCS OX2 29, 20 mg/kg) antagonists. Rats were trained to self-administer METH (0.05 mg/kg/inf, iv) during two 2-h sessions/day for 5 days. Subsequently, auditory and visual stimuli that signaled drug delivery were unplugged, and rats were allowed to extinguish lever pressing for 20 additional sessions over 10 days. Next, a within-subjects design was used to test for METH seeking precipitated by METH (1 mg/kg, ip) or METH-paired stimuli following systemic (ip) pretreatment with Orx1A or Orx2A. Preliminary results indicate no sex differences in METH self-administration, extinction responding, or reinstatement responding under these conditions. In agreement with prior work, both Orx1A and Orx2A failed to significantly reduce METH-elicited reinstatement. However, both Orx1A and Orx2A worked equally well to reduce METH-paired cue-elicited reinstatement. These results extend previous work to show a role for orexin signaling in cue-induced, but not METH-induced, reinstatement of METH seeking behavior.

Disclosures: N.E. Zlebnik: None. **N.A. Holtz:** None. **M.E. Carroll:** None.

Poster

924. Reward: Neuropharmacology

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Program#/Poster#:924.08/FFF22

Topic: F.03. Motivation and Emotion

Support: NHMRC Grant 508976

NHMRC Grant 509246

Title: The effect of relaxin-3 related antagonists on alcohol seeking

Authors: *P. J. RYAN[1], H. KASTMAN[1], E. V. KRSTEW[1], K. J. ROSENGREN[2], M. A. HOSSAIN[1], J. D. WADE[1], A. L. GUNDLACH[1], A. J. LAWRENCE[1]
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Abstract: Alcohol use and abuse is a major cause of morbidity and mortality, accounting for an estimated 4% of all global deaths and 5% of the global burden of disease and injury. Despite the huge impact of alcohol use disorders on society, current first-line therapeutic agents, such as naltrexone are inadequate, with high relapse rates and compliance problems. New therapeutic agents are required, particularly for the reduction of hazardous drinking and prevention of

relapse, which represent the most difficult clinical problems. Relaxin-3 is a highly conserved brain neuropeptide and its cognate G-protein coupled receptor is RXFP3. Relaxin-3/GABAergic neurons in hindbrain project widely to forebrain areas that express high levels of RXFP3, including amygdala, hippocampus and hypothalamus. This anatomical data and findings that relaxin-3 can modulate food intake, stress responses, and interact with CRF systems, supports the hypothesis that relaxin-3 may modulate drug seeking behavior (1). In studies using a model of alcohol use and alcohol-seeking in alcohol-preferring (iP) rats, we demonstrated that central administration of RXFP3 antagonists (3-30 µg, icv) decreased self-administration of alcohol in a dose-related manner (1-way ANOVA, $F(4,70) = 10.28$, $p < 0.0001$) and attenuated cue-induced reinstatement following extinction (1-way RM ANOVA, $F(2,26) = 30.07$, $p < 0.0001$). By comparison, RXFP3 blockade (10 µg, icv) produced no significant change in self-administration of sucrose, suggesting a selective effect for alcohol. Interestingly, RXFP3 antagonists decreased cue-induced reinstatement following extinction for sucrose and alcohol (1-way RM ANOVA, $F(2,30) = 13.96$, $p < 0.0001$), suggesting RXFP3 blockade may reduce reward-seeking behavior more generally. RXFP3 antagonist treatment had no effect on general ingestive behavior, activity, or cognition in the paradigms used. These data suggest relaxin-3/RXFP3 signaling regulates alcohol intake and relapse-like behavior, adding to current knowledge of the brain chemistry of reward-seeking. Further experimentation is required to determine the neural circuits and signaling mechanisms involved and to investigate effects on other drugs of abuse.
1. Smith CM *et al*. (2011) J Chem Neuroanat **42**, 262-275

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Poster

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Program#/Poster#:924.09/FFF23

Topic: F.03. Motivation and Emotion

Support: Psi Chi Undergraduate Reseach Award

Title: Examining the role of fenfluramine and sibutramine treatment on cue-induced reinstatement of food-seeking behaviors in the rat

Authors: *W. E. PRATT, R. T. FORD
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Abstract: Individuals struggling with obesity often have difficulty maintaining dietary regimens. One source of dietary relapse is the reinstatement of previous feeding behaviors following the presentation of cues indicating the availability of palatable and highly caloric food reward. The drugs sibutramine and fenfluramine have been previously prescribed because they enhance satiety mechanisms and decrease meal size. However, it is unclear whether these anorectic agents are also effective in blocking the cue-induced reinstatement of food-seeking behaviors. In these experiments, three separate groups of rats (N = 12/group) were food-deprived and trained to lever press for sugar pellets in the presence of a light/tone cue according to procedures developed by Floresco, McLaughlin, and Haluk (2008). Once trained, rats underwent daily 20-min extinction sessions in which lever press resulted in no programmed consequences. Upon extinguishing to 10% of their pre-extinction performance, rats received two separate reinstatement sessions following drug or saline injections (counterbalanced across rats and separated by 48 hr) in which lever pressing resulted in delivery of the light/tone cue. 30 min prior to these reinstatement tests, rats received IP injection of saline or the selective D_1 receptor antagonist SCH 23390 (6 microgram/kg, Experiment 1), fenfluramine (3 milligrams/kg, Experiment 2), or sibutramine (3 milligrams/kg, Experiment 3). Consistent with previous reports (Ball, Combs, & Beyer, 2011), rats did not reinstate lever pressing relative to their final day of extinction following systemic injections of the D_1 antagonist. Similarly, fenfluramine treatment blocked the cue's ability to reinstate lever pressing as compared to the saline injection day. However, there was no difference in lever pressing across extinction, drug, and saline days within the sibutramine group; all animals reinstated their lever pressing during the first reinstatement test, and this was unaffected by sibutramine treatment. These experiments suggest that the effect of fenfluramine is not limited to the promotion of satiety once a meal has been initiated, and demonstrate that some anorectic treatments may inhibit the effectiveness of conditioned cues to elicit relapse into food-seeking behavior. As continued effort is placed into developing pharmacotherapy for weight loss, it will be of interest to determine the effectiveness of anorectic drugs upon cue-induced relapse, across both animal models and within human diet regimens.

Disclosures: W.E. Pratt: None. **R.T. Ford:** None.

Poster

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Title: Effects of triazolam on caudate and nucleus accumbens dopamine levels at doses that are self-administered by rhesus monkeys.

Authors: *E. K. SAWYER[1], K. S. MURNANE[2], L. L. HOWELL[2], J. K. ROWLETT[1]
[1]Div. of Neurosci., New England Primate Res. Center, Harvard Med. Sch., Southborough, MA; [2]Div. of Neuropharm. and Neurologic Dis., Yerkes Natl. Primate Res. Center, Emory Univ., Atlanta, GA

Abstract: Benzodiazepines are abused widely, yet the neurobiological basis for this effect is poorly understood. It has been proposed that, as with other drugs of abuse, an increase in dopamine (DA) release in the nucleus accumbens underlies benzodiazepine self-administration. However, there is to date no direct evidence documenting such an increase and, in fact, benzodiazepines may decrease extracellular DA levels in rodents. Therefore, we examined the reinforcing and neurochemical effects of triazolam in rhesus monkeys. We first determined doses of the representative benzodiazepine triazolam that were reliably self-administered above vehicle levels. In this study, triazolam (0.0003-0.03 mg/kg/injection) was available to adult monkeys (n=4) for self-administration under a progressive ratio schedule of i.v. drug delivery. Triazolam maintained self-administration levels significantly greater than vehicle at unit doses of 0.003 and 0.01 mg/kg/injection (average numbers of injections/session= 11.7 and 10, respectively). The total cumulative dose was calculated as the unit dose (mg/kg/injection) x average number of injections/session, resulting in total cumulative dose values of approximately 0.035 and 0.1 mg/kg. In a separate group of adult monkeys (n=4), doses equivalent to the total cumulative dose values for triazolam were evaluated for changes in extracellular DA levels in the caudate and nucleus accumbens using in vivo microdialysis. Cocaine (1 mg/kg, i.v.) served as a positive control. DA levels in the caudate were not significantly affected following 0.03 or 0.1 mg/kg, i.v., of triazolam. However, DA levels in the nucleus accumbens were significantly decreased following 0.1 mg/kg triazolam. Cocaine significantly increased extracellular DA in the nucleus accumbens in all animals. Together, these data suggest that the mechanism(s) underlying the reinforcing effects of benzodiazepines may differ from that of other abused drugs and form the basis for further studies investigating the neurochemical correlates of benzodiazepine abuse.

Disclosures: E.K. Sawyer: None. **K.S. Murnane:** None. **L.L. Howell:** None. **J.K. Rowlett:** None.

Poster

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Program#/Poster#:924.11/FFF25

Topic: F.03. Motivation and Emotion

Title: Attenuation of nicotine-induced alterations in dopamine signaling during adolescence via 5-HT1A receptor antagonism

Authors: *M. YUAN, C. A. FENG, A. J. CABRERA, J. J. ZENG, J. D. BELLUZZI, F. M. LESLIE
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Abstract: Adolescence is a developmental transition period when the initiation of tobacco use and the onset of neuropsychiatric disorders most often emerge. Nicotine exposure during this vulnerable period may elicit long-lasting biochemical alterations in brain signaling and development, potentiating the likelihood for substance abuse. Previous studies in our lab have implicated serotonin (5-HT), acting via 5-HT1A receptors, as a mediator of nicotine-induced alterations of dopamine (DA) signaling during adolescence (Dao et al., 2011). Brief, low dose nicotine exposure in adolescent rats enhances acquisition of cocaine self-administration, an effect blocked by WAY-100,635 (5-HT1A antagonist). Furthermore, this adolescent nicotine treatment enhances sensitivity to direct DA agonists, increasing locomotor activity induced by quinpirole (D2-like agonist), an effect mimicked by 8-OH-DPAT (5-HT1A agonist) and blocked by co-administration of WAY-100,635 with nicotine. The present study was designed to determine whether 5-HT1A antagonism with WAY-100,635 can rescue alterations in the functional sensitivity of D2 receptors in adolescents after nicotine exposure. Nicotine (60µg/kg) was administered intravenously (i.v.) for four consecutive days in adolescent and adult male Sprague-Dawley rats. The following day, rats were treated with WAY-100,635 (100µg/kg i.v.) 20min prior to administration of quinpirole (0.4mg/kg, i.p.), and locomotor activity and erectile response were recorded. Acute administration of WAY-100,635 prior to D2 activation attenuated nicotine's age-specific enhancement of quinpirole-induced locomotion but not erectile response. These findings further implicate 5-HT1A receptor regulation of D2 signaling in adolescent brain and offer an exciting potential for the rescue of functional alterations in dopamine signaling following adolescent nicotine exposure.

Disclosures: M. Yuan: None. **C.A. Feng:** None. **A.J. Cabrera:** None. **J.J. Zeng:** None. **J.D. Belluzzi:** None. **F.M. Leslie:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: NIH Grant DA021870

Title: Methamphetamine-induced conditioned place preference in C3H/HeN mice is observed during the day (ZT 6-8) but not at night (ZT 19-21).

Authors: *S. J. CLOUGH[1], A. J. HUTCHINSON[1], I. STEPIEN[1], R. L. HUDSON[2], M. L. DUBOCOVICH[1]
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Abstract: Methamphetamine (METH) has been extensively studied for its ability to produce reward through its action on the mesolimbic dopamine system. The pineal hormone melatonin is synthesized following a circadian rhythm, with low levels during the day and high levels at night (Reiter, *Mol Cel Endocrinol* 1991;79:C153-C158). Melatonin exerts its effects through action on two G-coupled protein receptors, the MT_1 and MT_2, which are located in several brain regions including areas of the reward pathway (UZ *et al*., Mol Brain Res 2005, 136:45-53). During the day (Zeitgeber Time [ZT] 6-8; 12h:12h light-dark cycle, ZT 0 = lights on) METH (1.2 mg/kg, ip) induces a strong conditioned place preference in C3H/HeN wild-type mice. However deletion of either the MT_1 or MT_2 melatonin receptor blocks this effect, suggesting a role for melatonin in the modulation of METH-induced reward. The goal of this study was to examine the contributions of endogenous melatonin in the modulation of METH-induced place preference by comparing preference scores at ZT 6-8 and ZT 19-21 when melatonin is low and high respectively. Male C3H/HeN wild-type mice were subjected to a conditioned place preference test during ZT 6-8 and ZT 19-21. Compartment duration was measured during the drug-free habituation and pre-test sessions using LocoScan System (Clever Inc, Reston, VA). Animals were conditioned for 6 days with alternating treatments of METH (1.2 mg/kg, ip) and vehicle (VEH) and tested for place preference 1 day after the last conditioning session. A preference score was derived by subtracting the duration spent in the VEH-paired compartment from the duration spent in the METH-paired compartment during the post-test. At ZT 19-21 mice exhibited a similar preference score regardless of receiving METH (70.1$\pm$37.8 s, n=17) or VEH (23.3$\pm$21.5 s, n=17). This is in stark contrast to mice tested at ZT 6-8, which displayed a strong place preference for METH (226.0$\pm$35.5 s, n =11, $p<0.001$) compared to VEH controls (-

36.3±28.1 s, n=12). This time dependent difference suggests the involvement of a regulatory mechanism subject to a circadian rhythm, such as melatonin, which may act through inhibition of long-term potentiation (Wang *et al*., Eur J. Neuroscience 22:22-31, 2005). *Supported by DA 021870 to MLD*

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Poster

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Topic: F.03. Motivation and Emotion

Support: Fondazione Compagnia di San Paolo (Turin, Italy) – Neuroscience Call 2008

Title: Comparative effects of lithium and antidepressants in a rat model of anhedonia

Authors: *C. GAMBARANA, S. SCHEGGI, A. FERRARI, M. DE MONTIS
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Abstract: Anhedonia, a key symptom of major depression, defines the inability to experience pleasure from normally pleasurable life events. The reduced hedonic capacity of depressed patients may not be solely sustained by a reduced capacity to experience pleasure, but also by an inability to emit positive behavioral responses to pleasurable stimuli. Repeated exposure to unavoidable stress induces in rats an escape deficit to avoidable stressors, disrupts the dopaminergic response in the nucleus accumbens shell (NAcS) to palatable food consumption and competence to acquire an instrumental behavior sustained by palatable food. The phasic increase in NAcS dopamine output in response to a relevant stimulus confers incentive salience to the stimulus representation. Non food-deprived rats exposed to repeated unavoidable stress don't learn to choose the baited arm in a T-maze and to self-administer sucrose pellets under FR1 or FR5 schedule, and under a progressive response (PR) schedule of reinforcement, that is a measure of reward strength. Thus, repeated stress exposure conforms to the criteria for an experimental anhedonia model. We studied the effects of repeated lithium administration on sucrose self-administration (SA) in anhedonic rats using the following experimental protocol: after a 7-day stress exposure, rats were trained for SA with the FR1 schedule, then underwent 3 training sessions with the FR5 schedule, while continuing stress exposure. Control rats were trained in parallel. At day 21 stressed rats were divided in 2 groups: saline- and lithium-treated, while continuing stress exposure. After 7 days of treatment, rats were exposed to a single SA

session with the FR1 schedule, 5 sessions with the FR5 schedule and then to 3 sessions with the PR schedule while continuing treatments and stress exposure. Lithium treatment reverted the stress-induced learning deficit and reinstated the PR score, i. e. the reward and incentive values of sucrose, to control values in chronically stressed rats. Using the same experimental protocol, different results were obtained in rats treated with fluoxetine (FLX) as this treatment failed to modify the disruptive effects of unavoidable stress on the competence to acquire sucrose-sustained SA and escape response. We previously demonstrated that administration of imipramine, FLX, or lithium for 1-2 weeks before stress exposure and during behavioral training under stress prevent the development of the neurochemical (lack of dopaminergic response to a palatable meal) and behavioral deficits. Thus, FLX prevents the development of stress-induced disruption of SA, while lithium also reverted an established anhedonia condition.

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Poster

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Topic: F.03. Motivation and Emotion

Support: R01 DA023947

Title: Potential of partial negative allosteric modulators of the metabotropic glutamate receptor subtype 5 for reversal of cocaine self-administration in rats.

Authors: *R. J. AMATO, IV[1,2], M. E. JOFFE[1,2], R. D. MORRISON[1,2], A. L. RODRIGUEZ[1,2], A. S. FELTS[1,2], K. A. EMMITTE[1,2], J. S. DANIELS[1,2], P. J. CONN[1,2], C. W. LINDSLEY[1,2,3], C. K. JONES[1,2]
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Abstract: Recent evidence indicates that the metabotropic glutamate receptor subtype 5 ($mGlu_5$) plays an important role in the modulation of the rewarding effects of various drugs of abuse, including cocaine. For example, $mGlu_5$ knockout mice do not self-administer cocaine and are insensitive to other cocaine-induced behavioral changes. Moreover, selective negative allosteric modulators (NAMs) of $mGlu_5$ can attenuate cocaine self-administration in animals. Unfortunately, first generation full $mGlu_5$ NAMs, including MPEP, are associated with dose-limiting adverse effects, including psychotomimetic-like activity and cognitive impairments,

which limit their therapeutic potential. We have recently discovered a chemical series with several partial mGlu5 NAMs that act by partially blocking the maximal activation of $mGlu_5$ by glutamate in a calcium mobilization assay and exhibit a range of activities from 10% maximal inhibition to 90% maximal inhibition of the glutamate response. Compounds within this series also bind to the allosteric MPEP site on $mGlu_5$ and exhibit favorable pharmacokinetic properties for systemic administration in animals. For the behavioral studies, male Sprague Dawley rats were trained to respond under a schedule of cocaine self-administration and were administered vehicle or a dose of either a full or partial $mGlu_5$ NAM (1-30 mg/kg) prior to each test session. During each session, responding (lever press) under a fixed-ratio (FR)-10 resulted in the delivery of 0.5 mg/kg/infusion cocaine. Dependent measures of responding under this schedule were the total number of infusions and response rate (response/minute). Preliminary findings indicate that varying degrees of $mGlu_5$ inhibition of the full and partial $mGlu_5$ NAMs produce dose-related decreases both in the rate of responding for cocaine and the total number of infusions earned per session. The dose-related efficacy in the cocaine self-administration paradigm was correlated with the degree of central $mGlu_5$ occupancy and the potential to disrupt performance in a cognitive task of memory function. These novel partial $mGlu_5$ NAMs provide important tool compounds to establish whether partial inhibition of $mGlu_5$ is sufficient to produce efficacy in preclinical models of cocaine self-administration and seeking behaviors without the associated side-effects observed with first generation, full $mGlu_5$ NAMs.

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Poster

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Program#/Poster#:924.15/FFF29

Topic: F.03. Motivation and Emotion

Title: Evaluation of the effects of withdrawal from acute pcp on progressive ratio responding-relevance to negative symptoms of schizophrenia?

Authors: *K. M. DLUGOLENSKI, R. GRAF, Z. A. HUGHES
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Abstract: Negative symptoms of schizophrenia such as lack of motivation or drive are largely untreated by typical and atypical antipsychotics. As this symptom domain involves reward

circuitry, the challenge is to specifically address deficits in motivation without causing increased responding in healthy subjects which could raise concerns regarding abuse liability. Therefore, identification of preclinical models which recapitulate deficits in motivation observed in schizophrenia would be of great value. Given the NMDA-hypofunction model of schizophrenia, one manipulation with potential relevance is withdrawal from an acute NMDA antagonist. Spielewoy and Markou (Neuropsychopharmacology, 2003, 1106-1116) used intracranial self stimulation (ICSS) to demonstrate increased reward threshold 8, 12 and 24 h after a single dose of phencyclidine (PCP). Here we have investigated whether this protocol also affects progressive ratio responding.

All studies were performed in accordance to the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the NIH(Pub. 85-23, revised 1996) and approved by the Institutional Animal Care and Use Committee (IACUC). Adult male Sprague Dawley rats were trained to lever press for a food reward on a progressive ratio schedule. Progressive ratio responding was assessed 21 h after administration of PCP (5 mg/kg s.c.) or vehicle. Rats dosed with PCP exhibited decreases in all response measures (breakpoint, rewards, active lever presses, inactive lever presses) as well as moderate decreases in general locomotor activity. Using a cross-over design, the ability of D-cycloserine to reverse these effects was tested. D-cycloserine (32 or 100 mg/kg s.c.) was administered to vehicle or PCP treated rats 30 minutes prior to assessing progressive ratio responding. Neither dose of D-cycloserine affected responding in vehicle treated rats or attenuated the deficit caused by PCP treatment.

D-cycloserine was tested due to the clinical reports suggesting efficacy against negative symptoms in schizophrenia (Goff et al, Schizophrenia Bulletin, 2008, 106(2-3): 320-327). This treatment has not been studied extensively in patients so it is far from a ‘gold standard’ treatment for negative symptoms. A number of novel mechanisms (e.g. D-amphetamine prodrugs, GlyT inhibitors, glycine site agonists) are being evaluated in clinical trials, some of which are showing early signals of efficacy. Additional preclinical testing of such mechanisms is required to determine whether reversal of PCP-induced deficits in progressive ratio responding has value in predicting efficacy in negative symptoms of schizophrenia.

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Poster

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Topic: F.03. Motivation and Emotion

Support: R15 DA030618

Title: An examination of the effects of serotonin 2A, 2B, and 3 receptor activation of the nucleus accumbens on feeding behavior in the rat

Authors: *P. LIN, K. A. CLISSOLD, C. P. LEWIS, X. ZHU, W. E. PRATT
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Abstract: Central serotonin (5-HT) signaling has been widely implicated in the regulation of feeding behaviors in both humans and animal models. We have previously reported that individual serotonin receptors of the medial nucleus accumbens regulate feeding and food-directed motivation. For example, nucleus accumbens stimulation of the 5-HT_{1A} receptor inhibits feeding behavior, whereas activation of the 5-HT_6 receptor increases food intake and the effort that rats will exert to earn sugar reinforcement. The current experiments sought to further examine the role of individual serotonin receptors by testing the effects of pharmacological manipulation of the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_3 receptors within the anterior medial nucleus accumbens on feeding in hungry rats. Male Sprague-Dawley rats were acclimated to laboratory conditions and surgically implanted with bilateral guide cannulas above the nucleus accumbens shell. After recovery, the rats were food deprived to 90% of their ad libidum body weight and habituated to 2-hour feeding sessions during which they had free access to rat chow and water. Food consumption, water intake, ambulation, and rearing behaviors were monitored throughout each session. Across multiple experimental days, individual groups of rats (N= 7-8/group) received counterbalanced bilateral injections of one of the following selective serotonergic agents into the medial nucleus accumbens prior to being placed into the food intake chambers: the 5-HT_{1A} agonist TCB-2 (at 0.0, 1.0, 5.0, and 10.0 microg/0.5 microl/side); the 5-HT_{2B} agonist BW 723C86 (at 0.0, 2.0 and 5.0 microg/0.5 microl); or the 5-HT_3 agonist m-chlorophenylbiguanide (at 0.0, 10.0, 20.0 and 40.0 microg/0.5 microl). Stimulation of the 5-HT_{2A} receptor modestly increased ambulation, but had no effect on food intake across the 2 hr session. In contrast, stimulation of either the 5-HT_{2B} or the 5-HT_3 receptor subtype increased food intake across time without significantly affecting measures of locomotion or water intake. In separate experiments, blockade of these receptor subtypes had no effect on feeding behavior. Together, these results contribute to a growing understanding of the function of individual serotonin receptors within the nucleus accumbens on food-motivated behavior, and suggest that individual serotonin receptor subtypes serve unique behavioral roles within ventral striatal circuitry.

Disclosures: P. Lin: None. **K.A. Clissold:** None. **C.P. Lewis:** None. **X. Zhu:** None. **W.E. Pratt:** None.

Poster

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Title: Neuropeptide Y Y1 receptors in the bed nucleus of the stria terminalis modulate binge-like ethanol consumption by C57BL/6J mice.

Authors: *E. G. LOWERY[1], T. E. THIELE[1,2], T. L. KASH[1,3]
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Abstract: Recent evidence suggests that neuropeptide Y (NPY) modulates binge-like ethanol consumption via the Y1 and Y2 receptors (R). The brain regions that are involved in this behavior have not been elucidated, though regions of the extended amygdala, such as the bed nucleus of the stria terminalis (BNST), are known to be critical for behavioral and neurophysiological phenotypes associated with ethanol dependence. Therefore, we investigated the role of NPY Y1R in the BNST in binge-like ethanol consumption by bilaterally infusing the Y1R agonist [Leu31,Pro34]-Neuropeptide Y (LeuPro; 0.42 µg/0.5 µl) into the BNST of male C57BL/6J mice one hour prior to binge-like ethanol consumption. Mice treated with LeuPro displayed significantly lower levels of ethanol consumption than vehicle-treated mice (3.32 ± 0.76 vs. 5.17 ± 0.31 g/kg/4h, respectively) and achieved blood ethanol concentrations (BECs; 65.43 ± 25.45 mg/dl) below both the NIAAA-defined level characteristic of binge drinking (≥ 80 mg/dl) and the levels achieved by the vehicle-treated mice (108.53 ± 18.89 mg/dl). Importantly, bilateral infusions of LeuPro did not impair locomotor activity. These results demonstrate that Y1Rs of the BNST are involved in regulating binge-like ethanol consumption and provide the first direct evidence that NPY signaling in the BNST has a role in the modulation of ethanol intake. Future studies will assess the role of Y2Rs in the BNST in binge-like ethanol consumption.

Disclosures: E.G. Lowery: None. **T.E. Thiele:** None. **T.L. Kash:** None.

Poster

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Title: The effects of rearing condition and chronic methylphenidate treatment on anxiety-like behavior and ethanol consumption

Authors: *K. E. GILL, A. CHAPPELL, J. WEINER, L. J. PORRINO
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Abstract: Methylphenidate (MPH) is the most commonly prescribed medication in childhood, with an estimated 5-6% of children using it as treatment for Attention Deficit Hyperactivity Disorder. However, the drug's long term effects on the dopamine (DA) system and future vulnerability to substance abuse remain unclear. Previously we found that chronic administration of 8 mg/kg/day MPH for three weeks during pre- and peri-adolescence significantly reduced the density of DA D2/D3 receptors in the striatum of young rats. Low D2/D3 receptor density is associated with increased vulnerability to alcohol abuse. Additionally, environmental rearing conditions are known to affect future vulnerability to alcohol abuse, with isolation during early development increasing future ethanol intake. Here we examined the effects of MPH treatment and early rearing condition on anxiety-like behavior and two-bottle choice ethanol drinking in adulthood. Forty-eight young (PND 21) male Sprague Dawley rats (n = 16/housing group) were assigned to isolated (I), pair-housed (PH), or environmentally enriched (EE) conditions for 4 weeks. During 3 of those weeks, 8 rats per group received 8 mg/kg/day MPH via ALZET© osmotic mini-pump. At PND 50, rats were tested for anxiety-like behavior on the elevated plus maze. Subsequently, rats were individually housed and began a two-bottle choice ethanol drinking procedure that lasted 5 weeks. For one hour each day, rats had access to two bottles: one containing a 1% sucrose/8% ethanol solution and the other containing water. Total intake (g/kg ethanol) was calculated. There was a significant effect of housing on anxiety-like behavior ($F_{2,42} = 8.961$, $p < 0.001$). Isolated rats had significantly greater expression of anxiety-like behavior than PH or EE rats. A three-way analysis of variance (day*drug*housing condition) showed a significant interaction of drug*housing condition ($F_{2,42} = 4.626$, $p < 0.02$) on total intake of ethanol over the course of the procedure. Post-hoc tests confirmed that MPH increased ethanol intake in I rats only. Pair-housed and EE rats drank similar amounts of ethanol regardless of drug treatment. Previous studies have shown that isolation housing increases both the expression of anxiety-like behavior and ethanol intake. Here, while isolation did increase

anxiety-like behavior, only I rats that were treated with methylphenidate had greater ethanol intake than other groups. Thus, while 4 weeks of isolation alone during adolescence was not sufficient to alter drinking, the addition of a drug known to lower DA D2/D3 receptor density in reward related brain regions did increase vulnerability to alcohol drinking in adulthood.

Disclosures: K.E. Gill: None. **A. Chappell:** None. **J. Weiner:** None. **L.J. Porrino:** None.

Poster

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Title: Effects of dopamine beta-hydroxylase (DBH) inhibition on cocaine seeking behavior in squirrel monkeys

Authors: *D. A. COOPER[1,2], H. L. KIMMEL[2], L. L. HOWELL[2]
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Abstract: Disulfiram (Antabuse) has shown promise in clinical trials as a pharmacotherapy for cocaine dependence. One of many biochemical actions of disulfiram is inhibition of dopamine beta-hydroxylase (DBH), the enzyme that converts dopamine to norepinephrine (NE); consequently, reducing brain NE levels**.** It is believed that this mechanism is responsible for the therapeutic effects of disulfiram. Noradrenergic signaling is required for reinstatement of drug seeking in rats, a paradigm which is thought to model drug relapse. In rats, disulfiram blocks cocaine-primed reinstatement, corroborating with clinical results. Furthermore, nepicastat, a selective DBH inhibitor also blocks cocaine-primed reinstatement in rats, supporting the therapeutic use of DBH inhibitors. In this study, we attempted to replicate these results in non-

human primates. Squirrel monkeys trained to self-administer cocaine under a second-order schedule were pretreated with disulfiram or nepicastat prior to cocaine-primed reinstatement sessions. Neither pretreatment altered cocaine-primed reinstatement. Unexpectedly, nepicastat alone was sufficient to induce a modest reinstatement effect. We are currently assessing the extracellular neurochemical effects of nepicastat in squirrel monkeys using in vivo microdialysis to corroborate with the behavioral results we have found. Additionally, the discrepancies found between squirrel monkeys and rats may be explained by differences in pharmacokinetic or metabolic profiles in the two species. Ongoing studies are investigating whether metabolic differences are present, which will ultimately be instrumental in influencing the translation of these therapies to a human population.

Disclosures: D.A. Cooper: None. **H.L. Kimmel:** None. **L.L. Howell:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: DA018114

Title: Iptakalim attenuates the stimulus and reinforcing effects of nicotine in rats.

Authors: *S. CHARNTIKOV[1], S. PITTENGER[1], N. SWALVE[1], K. FINK[1], S. SCHEPERS[1], M. LI[1], G. HU[2], R. BEVINS[1]
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Abstract: Smoking and its associated nicotine dependence remains a significant health problem worldwide. Although advancements in treatment have been made in recent years, the high relapse rate indicates a need for continued research into potential therapeutics. Iptakalim, approved in China to treat hypertension, is an ATP sensitive potassium channel opener and an antagonist for alpha4-beta2 containing nAChRs. Further, pretreatment with iptakalim diminishes dopamine and glutamate release induced by nicotine. This neuropharmacological profile suggests that iptakalim may be used to treat smoking. To this end, in one study, we assessed whether iptakalim would block the stimulus effects of nicotine. Rats first received discrimination training with intermixed nicotine (0.4 mg/kg, SC) and saline sessions. Sucrose was intermittently presented in a response-independent manner only on nicotine session. Upon stable discrimination performance, greater anticipatory dipper entries (goal-tracking) on nicotine than saline sessions,

rats were tested for whether pretreatment with iptakalim (10, 30, 60 mg/kg, IP) blocked responding evoked by nicotine. Iptakalim blocked responding in a dose-dependent manner. Further, simultaneous collection of chamber activity indicated that iptakalim also blocked the locomotor stimulant effects of nicotine. When iptakalim was given alone, baseline chamber activity and goal-tracking was not affected, decreasing the feasibility of an account based on a non-specific effect of iptakalim. In a separate study, rats were prepared with IV catheters and then trained to self-administer nicotine (0.03 mg/kg/infusion) on a VR3 schedule of reinforcement. Pretreatment with iptakalim (1, 3, 6 mg/kg, IV) decreased the intake of nicotine (i.e., less active lever responding). Inactive lever presses were not affected by iptakalim. However, general chamber activity was attenuated by pretreatment with 3 and 6 mg/kg iptakalim. An analysis of covariance revealed that this reduction of activity did not significantly contribute to the decreased active lever presses. This suggests that the observed antagonism was not due to a non-specific effect. In sum, iptakalim blocked the stimulus, the reinforcing, and the stimulant effects of nicotine. Together, these finding support the notion that iptakalim may be an effective pharmacotherapy for increasing smoking cessation.

Disclosures: S. Charntikov: None. **S. Pittenger:** None. **N. Swalve:** None. **K. Fink:** None. **S. Schepers:** None. **M. Li:** None. **G. Hu:** None. **R. Bevins:** None.

Poster

924. Reward: Neuropharmacology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:924.21/FFF35

Topic: F.03. Motivation and Emotion

Support: ARC DP110100297

Title: Oxycodone-induced activation of Toll-Like Receptor 4 contributes to drug reward

Authors: *M. R. HUTCHINSON[1], K. VAN STEEG[2,3], J. THOMAS[2]
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Abstract: Traditional opioid action to create reward was thought to result solely via the initial agonism of neuronal opioid receptors. Previous studies have implicated proinflammatory immune signaling within the brain in potentiating the rewarding properties of opioids. However, the mechanism for the initiation of this proinflammatory central immune signal was unclear. Here we present evidence for an additional novel contributor to opioid reward: the innate immune pattern-recognition receptor, Toll-like receptor 4 (TLR4), and its MyD88-dependent

signaling as the trigger of the proinflammatory central immune signaling. Conditioned place preference for oxycodone was examined in wild-type Balb/c, *TLR4* $^{-/-}$ and *MyD88* $^{-/-}$ mice. Oxycodone (20 mg/kg) induced a 127 ± 31 sec change in preference for the oxycodone-paired environment, compared to 32 ± 32 sec for *TLR4* $^{-/-}$ and -25 ± 32 for *MyD88* $^{-/-}$ mice. Two-way ANOVA revealed significant effects of oxycodone ($p = 0.049$), strain ($p = 0.046$) and interaction ($p = 0.01$). Post-hoc Bonferroni multiple comparisons revealed a significant difference in the change in preference between oxycodone and saline conditioned wild-type mice ($p < 0.001$), but not in *TLR4* $^{-/-}$ or *MyD88* $^{-/-}$ mice ($p > 0.05$). Importantly, oxycodone-TLR4 actions were not a unidirectional suppressive influence on opioid pharmacodynamics. Hotplate latency dose responses were generated in wild-type and TLR4 $^{-/-}$ mice. There was a 5-fold leftward shift in the *TLR4* $^{-/-}$ oxycodone dose response curve (ED_{50} wild-type 1.36 mg/kg versus *TLR4* $^{-/-}$ 0.26 mg/kg; $P < 0.0001$) compared to wild-type mice. Moreover, immediately following behavioral testing in these mice their spinal cords were collected and quantification of the phosphorylation of Mitogen Activated Protein Kinases was performed. Oxycodone induced significant dose dependent p38 and JNK phosphorylation in wild type, but not *TLR4* $^{-/-}$ mice ($P < 0.05$). Collectively, these data indicate that the actions of opioids at classical opioid receptors, together with the newly identified actions at TLR4/MD2, possibly affects the mesolimbic dopamine system and may explain altered opioid reward behaviors. Thus, the discovery of TLR4/MD2 recognition of opioids as foreign xenobiotic substances (xenobiotic associated molecular patterns; XAMPs) adds to the existing hypothesized opioid reward mechanisms, identifies a new drug target in TLR4/MD2 for the treatment of addiction, and provides further evidence supporting a role for central inflammation in drug reward. Moreover, questioning whether XAMP-TLR4 activity contributes to the rewarding properties of diverse drugs of abuse now appears warranted.

Disclosures: M.R. Hutchinson: None. **K. van Steeg:** None. **J. Thomas:** None.

Poster

924. Reward: Neuropharmacology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:924.22/FFF36

Topic: F.03. Motivation and Emotion

Support: Royal Scoeity of New Zealand Marsden Fund

Title: 5,7 dht lesions facilitate mdma self-administration

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Abstract: Our previous studies have shown that the acquisition of MDMA self-administration is relatively slow and a larger number of rats fail to meet acquisition criteria compared to other drugs of abuse. During the initial sessions, only a small number (1-2) infusions are self-administered. With repeated testing, however, there is an escalation of intake over days and eventually some rats self-administer 20-30 mg/kg/day during 2 or 6 hr test sessions. We have suggested that the preferential effects of MDMA on serotonin neurotransmission initially limits its self-administration. Following repeated exposure, however, tissue levels of 5HT decrease and the MDMA-produced increase in synaptic dopamine increases. We hypothesise that the increased DA response underlies the development of high rates of MDMA self-administration. This idea was tested in the present study by examining the effects of neurotoxic 5,7 DHT lesions on the acquisition of MDMA self-administration. Intracerebroventricular administration of 5,7 DHT produced an 80% decrease in tissue levels of 5HT in a number of brain sites. Acquisition of MDMA self-administration in control rats proceeded with a protracted time course, as we have reported. In this control group about half the rats failed to acquire self-administration within a 25 day cut-off period, as we have also previously reported. The lesioned rats, however, acquired self-administration more rapidly and all of the rats met the criterion for self-administration. These data support the idea that MDMA self-administration is, at least initially, limited by 5HT effects.

Disclosures: S. Schenk: None. **S. Bradbury:** None.

Poster

924. Reward: Neuropharmacology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:924.23/FFF37

Topic: F.03. Motivation and Emotion

Support: CIHR to JY

Title: Anatomical and functional organization of the mouse rostromedial tegmentum (RMTg)

Authors: *D. I. WASSERMAN, H. WANG, J. S. YEOMANS
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Abstract: Previous studies in rats found that activation of RMTg neurons can inhibit ventral tegmental area (VTA) dopamine output, and that opiates can inhibit these RMTg GABA neurons. Last year, we reported opposing effects of VTA versus RMTg activation on morphine-induced locomotion in mice. Transfecting the excitatory M5 muscarinic receptor into the VTA doubled the locomotor response to morphine. Conversely, transfecting the M5 receptor into the RMTg blocked the stimulant effect of morphine. We investigated the organization of the mouse RMTg and VTA and their connections. TH and GAD immunoreactivity was observed in VTA sites, but only GAD in the RMTg. The mouse RMTg was also rich in mu-opiate receptors specifically on GAD-positive neurons, as previously found in rat (Jhou et al, 2009).A strong projection from RMTg GABA neurons to VTA DA neurons has previously been reported in rats. To map the connections between these regions in mice, we injected cholera toxin B retrograde tracer into the VTA. While many areas of the brain displayed retrograde labeling, the strongest projections were from RMTg neurons. Specifically, the region that showed the strongest VTA projections, immediately dorsolateral to the caudal IPN, was the same location where morphine-induced locomotion was blocked. These data support the hypothesis that RMTg GABA neurons that inhibit VTA DA neurons are important for morphine-induced locomotion in mice.

Disclosures: D.I. Wasserman: None. **H. Wang:** None. **J.S. Yeomans:** Research Grant; CIHR.

Poster

924. Reward: Neuropharmacology

Location: Hall F-J

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Program#/Poster#:924.24/FFF38

Topic: F.03. Motivation and Emotion

Support: NSERC

Wilfrid Laurier University

Title: Effects of perinatal delta-9-tetrahydrocannabinol (THC) exposure and environmental complexity during adolescence on motivation, working memory and anxiety in rats

Authors: *A. CELEJEWSKI, G. WILLEMS, S. DANIELS, P. E. MALLET
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Abstract: Early life cannabinoid exposure has been shown to produce a broad range of behavioural and cognitive impairments. Among these are changes in reward system function, memory, and emotional reactivity. Furthermore, environmental enrichment during rearing has been shown to alter cognitive function in adulthood. The present study sought to determine if

environmental complexity during adolescence offered protection against the detrimental cognitive effects of early life cannabinoid exposure. Changes in motivation, working memory, and anxiety were examined in rats perinatally exposed to THC, then reared in either an enriched environment (EE) or standard environment (SE) during adolescence. Male Wistar rat pups received either daily injections of THC (5 mg/kg, s.c.) or its vehicle on postnatal days (PND) 4-14. Rats were weaned at PND21 and then reared in either SE or EE conditions until PND52. Beginning at PND81, animals were food restricted, and trained to lever press for food reward on a progressive ratio (PR) schedule to compare motivation for food reward across groups. Perinatally THC exposed rats displayed higher breakpoints, but no effect of rearing environment was observed. To assess working memory, performance on a delayed-matching-to-position (DMTP) task was assessed following administration of THC (0.1, 0.5, 1 or 2 mg/kg, i.p.) or its vehicle. We intend to determine if rearing environment exacerbates (SE) or ameliorates (EE) the detrimental impact of perinatal THC exposure. We will also examine baseline anxiety in the elevated plus maze and light dark emergence tests to determine if early life THC exposure and rearing conditions also affect emotional reactivity in adulthood.

Disclosures: A. Celejewski: None. **G. Willems:** None. **S. Daniels:** None. **P.E. Mallet:** None.

Poster

924. Reward: Neuropharmacology

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:924.25/FFF39

Topic: F.03. Motivation and Emotion

Title: Impact of sub-chronic ketamine exposure on the reinforcing effects of nicotine in rats: Implications for self-medication in schizophrenia

Authors: *M. SHOAIB, N. HARUN
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Abstract: Cognitive deficits represent the core deficits within schizophrenia, accountable for the majority of disruption to everyday life. Over 80% of patients with schizophrenia smoke compared to just 30% of the general population. This is proposed as a form of self-medication to help combat cognitive problems as nicotine. Our laboratory has shown that a sub-chronic, sub-anaesthetic regimen of NMDA antagonist ketamine can induce cognitive deficits on a variety of cognitive domains such as memory capacity of working memory and executive decision-making in the attentional set-shifting task. The objective was to examine the effects of treating rats with ketamine (daily injection of 30mg/kg IP for 5 days) on acquisition to self-administer intravenous

nicotine (0.03mg/kg/inj) using two-lever operant conditioning chambers. Following 3 days of a 'washout' period from the sub-chronic treatment, groups of rats (n=12) were given access to nicotine in 1hr sessions under a fixed-ratio schedule of reinforcement. Both ketamine- and vehicle-treated subjects showed a steady rate of acquisition with no significant difference between the groups. There was a tendency for ketamine-treated rats to self-administer fewer nicotine injections which was reflected in the dose-response curves generated under a fixed-ratio of 3. Similarly, under a progressive ratio, no significant difference between the groups was apparent. Saline substitution extinguished responding in both groups but with no overall difference between the groups. The results from this experiment suggest that the sub-chronic regimen of ketamine treatment used to model cognitive deficits associated with schizophrenia in rats does not extend to the increased intake of nicotine in the self-administration model. Moreover, the findings suggest that the reinforcing effects of nicotine are not enhanced by ketamine and thus highlight a therapeutic role for nicotine in tobacco that serves to enhance cognition in patients with schizophrenia.

Disclosures: M. Shoaib: None. **N. Harun:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:925.01/FFF40

Topic: F.03. Motivation and Emotion

Support: NIDA Intramural Research Program

Title: Extinction of cocaine-seeking with cocaine methiodide attenuates priming-induced but not footshock-induced reinstatement of cocaine-seeking

Authors: S. MYAL, B. WANG, *R. A. WISE
NIDA IRP, BALTIMORE, MD

Abstract: Cocaine hydrochloride (cocaine HCl) is habit-forming because of its ability to elevate extracellular dopamine levels in the brain. Cocaine methiodide (MI), a charged analog of cocaine that does not cross the blood-brain barrier, is not rewarding in its own right, but acquires conditioned rewarding properties when animals learn_through repeated experience with intravenous injections of cocaine HCl_that the rewarding central effects effects are predicted by the more immediate peripheral actions of the drug. The peripheral actions come to trigger the release of glutamate in the ventral tegmental area in cocaine HCl-trained but not cocaine HCl-naïve animals. The peripheral cocaine effects_triggered experimentally by cocaine MI_lose their

reinforcing efficacy as they are repeatedly experienced without the subsequent central effects that are experienced with cocaine HCl. Here we examined extinction of cocaine seeking when cocaine MI or saline were substituted for cocaine HCl, and we determined the abilities of footshock or experimenter-administered cocaine to reinstate cocaine-seeking following extinction with cocaine MI or saline. On the first day of extinction testing, responding was attenuated more with saline than with cocaine MI substitution; however, after the first day, extinction proceeded about equally with the two substitutions. Although footshock reinstated cocaine seeking equally in cocaine MI-extinguished and saline-extinguished animals, drug-primed reinstatements with cocaine HCl-induced and cocaine MI-induced reinstatements were much weaker in cocaine MI-extinguished animals. Thus breaking down the learned association between the peripheral and central effects of cocaine may be useful to reduce cocaine craving in users who lapse.

Disclosures: S. Myal: None. **B. Wang:** None. **R.A. Wise:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

Location: Hall F-J

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Program#/Poster#:925.02/FFF41

Topic: F.03. Motivation and Emotion

Title: Nucleus accumbens core and shell complementary inhibit redundant actions with different temporal pattern

Authors: *C. SATO[1], H. FURUDATE[1], T. KOBAYASHI[1], T. KIMURA[2,3]
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Abstract: We previously reported that, in the novel paradigm, 8-arm food foraging task (8-arm FFT) that limited the quantity of rewards and had a longer period of the reward-unavailable situation after acquisition of the last reward, animals expected no available reward resulting from their own reward acquisition behavior and inhibited redundant actions. Moreover we suggested that the nucleus accumbens core (AcbC) was necessary for inhibition of redundant activity based on the expectation of 'no-reward' during learned reward-unavailable situation. In the present study we examined how accumbens core and shell (AcbS) were involved in the inhibition of behavioral activity. The AcbC- or AcbS-lesioned rats trained by the 8-arm FFT showed higher activity during 10 min after last-reward-acquisition than intact rats, suggesting that both AcbC and AcbS inhibit activity after last-reward-acquisition. We compared the temporal change of

activity after last-reward-acquisition in three groups. AcbC-lesioned rats showed higher activity during 2 min after last-reward-acquisition, and same level of activity during 6-10 min compared to intact rats. Although AcbS-lesioned rats showed no significant effects on behavioral activity during 2 min after last-reward-acquisition, they showed higher activity during 2-10 min after last-reward-acquisition compared to intact rats. These results suggest that, AcbC and AcbS are involved in the inhibition of redundant activity during learned reward-unavailable situation with different temporal patterns.

Disclosures: C. Sato: None. **H. Furudate:** None. **T. Kobayashi:** None. **T. Kimura:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

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Program#/Poster#:925.03/FFF42

Topic: F.03. Motivation and Emotion

Support: GM-08224 CAJR

MBRS-RISE, R25-GM061838

Title: Cytochrome oxidase metabolic mapping of cocaine sensitized rats

Authors: *M. E. VELEZ[1], E. PADILLA[2], F. GONZALEZ-LIMA[2], C. A. JIMENEZ-RIVERA[1]
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Abstract: Addiction has been considered an aberrant form of learning that incorporates synaptic plasticity. When changes in neuronal activity occur throughout the brain, the energy demands of a cell also fluctuate. Cytochrome oxidase (CO) is a mitochondrial membrane protein that helps supply energy demand by generating ATP via oxidative phosphorilation. Here we wish to characterize which neural systems modify their CO activity as a result of cocaine sensitization. Rats were injected with 15mg/kg cocaine i.p. or isovolumic saline for five days. One hour after last injection brains were removed and slices were stained for CO activity. Subjects injected with cocaine had developed a strong sensitized response measured by an increased locomotor activity (5828 ± 433 pcc) when compared to day one (3062 ± 599 pcc). CO activity was significantly decreased in cocaine-treated animals in the superficial layers of the dorsal and lateral frontal cortex regions (DFS = 217 ± 8 and LFS = 242 ± 9) when compared to saline-treated animals (DFS = 244 ± 8 and LFS = 265 ± 6). Inter-regional correlations of CO activity showed that noradrenergic paths between the locus coeruleus and deep layers of the infralimbic cortex were positively correlated in animals treated with cocaine (r = 0.802) but not in control rats. In

dopaminergic pathways, significant positive correlations were observed between the substantia nigra compacta and the superficial layer of the medial frontal cortex (r =0.809), the deep layer of the lateral frontal cortex (r=0.770) and the superficial layer of the lateral orbital cortex (r=0.717) only in cocaine treated animals. In cholinergic pathways, CO activity in the interpeduncular nucleus was negatively correlated with the activities of the deep layer of the dorsal frontal cortex (r = -0.851), the superficial and deep layers of the anterior insular cortex (AIS, r = -0.672 and AID, r = -0.954), the superficial and deep layers of the lateral orbital cortex (LOS, r = -0.697 and LOD, r = 0.847) in saline treated animals but not in rats that received cocaine. Taken together our data suggest that prefrontal networks change from inhibitory cholinergic influences to networks driven by noradrenergic and dopaminergic pathways after cocaine exposure. Cocaine sensitization to cocaine may release behaviors that normally are inhibited by the prefrontal cortex. When prefrontal networks for inhibitory control are impaired, impulsive behaviors may arise.

Disclosures: M.E. Velez: None. **E. Padilla:** None. **F. Gonzalez-Lima:** None. **C.A. Jimenez-Rivera:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:925.04/FFF43

Topic: F.03. Motivation and Emotion

Support: NSERC

Brescia University College

Title: Consumption of highly palatable foods and nociceptive sensitivity in free-feeding adult male mice

Authors: *C. M. TENK[1], R. N. KIMANI-DUPUIS[1], M. KAVALIERS[2]
[1]Psychology, Brescia Univ. Col., London, ON, Canada; [2]Psychology & Grad. Program in Neurosci., Univ. of Western Ontario, London, ON, Canada

Abstract: Highly palatable foods, such as those high in sugars, fats or both, are highly preferred both by humans and rodents. These foods act as natural rewards and activate endogenous dopamine and opioid systems. These systems play an integral role in pain modulation with results of prior research having shown that palatable foods affect nociceptive and pain sensitivity. Consumption of fats has been reported to decrease pain/nociceptive sensitivity while

consumption of sugars either increases or decreases pain/nociceptive sensitivity depending on both the duration of ingestion as well as the time period between ingestion and presentation of the noxious stimulus. The current study with mice examined the consumption of highly palatable foods (sugar-fat or sugar) during repeated, limited access feeding and the subsequent effect on nociceptive sensitivity. For four consecutive days, nociceptive responses (latency of a foot lift/lick response to a 50 C surface) in free-feeding CD-1 male mice (n=8 per group) were assessed before, and after, 1hr access to one of four foods (sugar, sugar-fat, lab-chow, no food). Locomotor activity was also assessed during the feeding period using an automated open-field. Animals fed sugar-fat consumed significantly more than all other groups during the feeding period. In addition, the patterns of consumption significantly differed among groups with the sugar-fat animals, despite consuming significantly more food, making significantly fewer entries into, and spending significantly less time in, the food dish area than other groups. Furthermore, consumption of both palatable foods, sugar and sugar-fat, significantly decreased paw lick latency relative to chow and no-food controls, indicating an increase in nociceptive sensitivity (hyperalgesia) in these groups. These results indicate that a highly palatable sugar-fat food is readily consumed by adult male mice but the patterns of consumption are significantly different than those for sugar. However, despite these differences in consumption, these data suggest that sugar-fat ingestion influences nociceptive sensitivity in the same manner as sugar, increasing nociceptive sensitivity and inducing hyperalgesia in this experimental model.

Disclosures: C.M. Tenk: None. **R.N. Kimani-Dupuis:** None. **M. Kavaliers:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

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Program#/Poster#:925.05/FFF44

Topic: F.03. Motivation and Emotion

Support: State of Claifornia

Title: A selective role for lmo4 in cue-reward learning.

Authors: *R. MAIYA[1], U. HEBERLEIN[2,1]
[1]Ernest Gallo Clin. and Res. Ctr., Emeryville, CA; [2]Dept. of Anat., Univ. of California, San Francisco, San Francisco, CA

Abstract: Cues associated with drug seeking play an important role in initiating drug craving and seeking in abstinent addicts. Hence, understanding the molecular mechanisms that underlie the formation of cue-reward associations may have important implications for the treatment of

addictive disorders. We have previously demonstrated a role for the transcriptional regulator *Lmo4*, which is highly expressed in pyramidal projection neurons of the basolateral complex of the amygdala (BLC) in fear learning. The BLC has also been shown to modulate selective aspects of cue-reward learning. Hence, in this study we sought to examine the role of *Lmo4* in the formation of cue-reward associations and the subsequent ability of such reward-paired cues to control goal-directed behavior. We first examined cue-reward learning in mice heterozygous for a genetrap insertion at the *Lmo4* locus, which results in 50% reduction in *Lmo4* levels. Both wild type (WT) and *Lmo4gt/+* mice readily learned to associate the presentation of stimuli with the delivery of an appetitive reinforcer. Furthermore, reward-paired cues were able to elicit approach behavior in the absence of the primary reinforcer (goal tracking) equivalently in both genotypes. We next examined conditioned reinforcement, which is a measure of the motivational properties of the reward-paired cue, in both genotypes. Interestingly, our results suggest that reward-paired cues served as conditioned reinforcers in WT but not *Lmo4gt/+* mice, suggesting a deficit in assigning incentive value to the cue in *Lmo4gt/+* mice. Since the BLC along with several forebrain regions plays a critical role in attributing incentive value to the cue, we examined the effects of knockdown of *Lmo4* specifically in the BLC on conditioned reinforcement. Preliminary results suggest that shRNA-mediated knockdown of *Lmo4* expression in the BLC, also results in a selective deficit in conditioned reinforcement. In summary, these results suggest a novel and selective role for the transcriptional regulator *Lmo4* in the BLC in attributing incentive salience to conditioned cues.

Disclosures: R. Maiya: None. **U. Heberlein:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

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Program#/Poster#:925.06/FFF45

Topic: F.03. Motivation and Emotion

Support: University of Missouri Research Board Grant

Title: Basolateral amygdala mediation of palatable diet consumption driven by intra-accumbens DAMGO administration and 24-hour food deprivation: A behavioral and neural activation examination

Authors: *K. PARKER, M. P. MCCABE, F. ODU, R. SHARMA, M. M. THAKKAR, M. J. WILL
Univ. Missouri, Columbia, MO

Abstract: The amygdala has been of considerable interest in learning, motivation, reward, and addiction research, but a recent surge of reward and feeding research has raised awareness of the potential role of the amygdala in the overconsumption of palatable foods. Administration of μ-opioid receptor agonists into the nucleus accumbens or 24-hour food deprivation increases consumption of a palatable, high-fat diet. Studies from our laboratory have suggested an important role of the basolateral amygdala (BLA) in mediating the feeding behavior following intra-accumbens DAMGO administration. Blocking neural activity within the basolateral amygdala via GABAA agonist muscimol has been shown to block the feeding following intra-accumbens administration of DAMGO (Will et al., 2004). Although muscimol inactivation of the BLA reduced DAMGO-induced food intake to control levels, this treatment led to exaggerated number and duration of food hopper entries after food intake had ended (Will et al., 2009). A subsequent experiment under conditions of limited access to the diet (8 grams) found the identical pattern of exaggerated hopper entries and duration following intra-Acb administration of DAMGO, regardless of whether the BLA was inactivated. Additionally, BLA muscimol inactivation had no influence on feeding driven by 24-hour food deprivation, suggesting the BLA selectively influences DAMGO-induced feeding. The present experiments were conducted to replicate these behavioral findings in rats that were immediately sacrificed following the feeding session to assess patterns of neural activity in key feeding related brain regions. Therefore, c-Fos double-labeling fluorescent immunohistochemistry was conducted on tissue from multiple brain areas shown to contribute to feeding behavior, including the orbital frontal cortex, nucleus accumbens, ventral pallidum, hypothalamus, amygdala, and ventral tegmental area.

Disclosures: K. Parker: None. **M.P. McCabe:** None. **F. Odu:** None. **R. Sharma:** None. **M.M. Thakkar:** None. **M.J. Will:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

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Program#/Poster#:925.07/FFF46

Topic: F.03. Motivation and Emotion

Support: NSERC

Title: Long-term consequences of sweet solution consumption in rats.

Authors: G. SENTHINATHAN, A. CELEJEWSKI, *R. EIKELBOOM
Wilfrid Laurier Univ., Waterloo, ON, Canada

Abstract: Access conditions may have profound effects on consumption, beyond those driven by need. Restricted access models can be used to control and manipulate consumption of natural rewards and drugs. Non-food deprived rats consume excessive amounts of 4% sucrose solution when daily access is restricted to 24h out of every 72 or 96h, but consume much smaller amounts when given 24h ad libitum (AL) access (Hewitt & Eikelboom, 2008). When all rats are subsequently given alternate day access, rats from the restricted group continue to show significantly higher consumption. It is unclear if a) the consumption difference will be maintained with an extended alternate day access period and b) if the consumption difference is due to elevated intake induced by restricted access or if a period of AL access prevents rats from increasing intake. The present study investigates these questions, examining the longer-term consequences of sweet solutions consumption.
Sprague-Dawley rats (~ 65 days old) were randomly assigned to AL or restricted (once every third or fourth day) access to 0.25% saccharin (Exp. 1) or 4% sucrose (Exp. 2) until consumption stabilized and in Exp. 3 access to AL sucrose or no sucrose (NS). Subsequently all rats in Exp. 1 received alternate day access to saccharin for an extended period. In Experiments 2 and 3 following stabilization of sucrose consumption, groups were split and rats received either AL or restricted (once every 3 days) access to sucrose.
With an extended alternate day access period (Exp. 1) the saccharin consumption difference was maintained for over 45 days. AL rats switched to restricted access (Exp. 3) immediately increased intake, matching control NS restricted access rats. In Exp. 2 restricted rats showed double the consumption of AL rats, restricted access rats continued to consume high levels of sucrose solution when switched to AL access. After about 30 days of AL access the consumption was similar to chronic AL rats. Ad lib access to sucrose does not prevent rats from developing high consumption when access conditions are suddenly restricted. The elevated intake caused by a period of restricted access is robust and resistant to change but eventually returns to lower levels of consumption with ad lib access.

Disclosures: G. Senthinathan: None. **A. Celejewski:** None. **R. Eikelboom:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

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Program#/Poster#:925.08/FFF47

Topic: F.03. Motivation and Emotion

Support: NIH-NIDA 1R01DA029147-01A1 (YZ)

NIH-NIDA DA05130 (MJK)

Title: Adolescent oxycodone self-administration enhances subsequent oxycodone-induced conditioned place preference and locomotor activity in C57BL/6J mice

Authors: *Y. ZHANG[1], A. HO[2], M. KREEK[2]
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Abstract: Illicit use of opioid analgesics (e.g., oxycodone) in adolescence is a rising problem in the United States. *Objective:* Our goal was to determine whether adolescent oxycodone self-administration affects subsequent oxycodone-induced conditioned place preference (CPP) and locomotor activity in mice. *Methods:* Groups of adolescent C57BL/6J mice (4 weeks old) underwent surgery during which a catheter was implanted into their jugular veins. Mice were given five days to recover from the surgery and were placed in the self-administration chambers for acquisition of oxycodone self administration. Mice self administered oxycodone (0.25mg/kg/infusion) 2 hrs daily for 14 days while controls received yoked saline administration. Fifteen days after the last oxycodone self-administration session, mice underwent a 10-day oxycodone CPP training procedure (1, 3, 10, mg/kg, i.p., separate groups). *Results:* Mice that had self-administered oxycodone during adolescence spent more time in the oxycodone-paired chamber ($p < 0.05$ and $p < 0.01$ for 1 and 3 mg/kg CPP groups, respectively) than those that were oxycodone-naïve (yoked saline controls). Further, oxycodone increased locomotor activity significantly more in mice that had self-administered oxycodone than the yoked saline control ($p < 0.05$). *Conclusion:* Adolescent oxycodone self-administration increased the rewarding effect of oxycodone upon re-exposure to oxycodone.

Disclosures: Y. Zhang: None. **A. Ho:** None. **M. Kreek:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:925.09/FFF48

Topic: F.03. Motivation and Emotion

Support: NICH/HD to MFC

Title: Is reward system involved in pair bonding in ring doves?

Authors: A. DIOS[1], K. ALEXANDER[2], S. J. HANSON[2], *M.-F. CHENG[2]
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Abstract: Although the term “pair bonding” is used freely in academic literature, much is not known about how it is characterized. Previous work done in our lab suggests that pair bonded ring doves, operationally defined as doves that have completed a breeding cycle and successfully reared a squab (Erickson 1971), prefer to spend time with their mates during behavioral preference tests. These tests, however, are subject to environmental factors, such as housing conditions and testing parameters, that can diminish their sensitivity as a predictor of pair bonding. Because of these testing constraints, our lab sought to find a neural marker that would be a better predictor for pair bonding. Our present study examines whether ZENK expression in the nucleus taeniae (avian amygdala) serves as a predictor for pair bonding in female ring doves. We found that we were able to predict pair bonding using a quadratic discriminant analysis on principal component scores conducted on ZENK cell counts in the nucleus taeniae. Additionally, we found there are no gender differences in the prediction of pair bonding. This method appears to be more sensitive in predicting pair bonding than behavioral preference tests. Previous tract tracing studies in our laboratory suggest that regions involved in the reward system circuitry, including the nucleus accumbens and hypothalamatic regions, project to the nucleus taeniae. Lesion studies will be done to determine if the nucleus taeniae and these regions contribute to pair bonding behavior.
Key Words: Pair bonding, Taeniae (Avian Amygdala), ZENK expression, Ring Doves, Electrolytic Lesion

Disclosures: A. Dios: None. **K. Alexander:** None. **S.J. Hanson:** None. **M. Cheng:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:925.10/FFF49

Topic: F.03. Motivation and Emotion

Support: Research Board, Univ. of Missouri

Title: Involvement of the basolateral amygdala in homeostatic and hedonic feeding on a progressive-ratio breakpoint task

Authors: H. W. JOHNS, *D. K. MILLER, K. E. PARKER, A. S. SAGE, M. J. WILL
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Abstract: The amygdala has been long recognized as an important structure for motivation, emotion, and learning. Integral to motivational and appetitive systems, the basolateral nucleus of the amygdala (BLA) monitors and updates changes in a stimulus' incentive value, and,

consequently, mediates goal-directed behavior. One important afferent of the BLA is the nucleus accumbens (Acb). Stimulation by the synthetic opioid DAMGO of the nucleus accumbens of the ventral striatum has been shown to robustly increase intake of palatable food (e.g., fat, sugar, and salt); however, this process is dependent upon the BLA. Pharmacological inactivation of the BLA via infusion of GABAA agonist muscimol abolishes palatability-driven feeding, but not homeostatic feeding.
Our lab uses a rat model to study the neural mechanisms of both motivational states (ie, palatability- versus homeostatic-driven feeding). Previous work from our lab in a free-feeding experiment has demonstrated that rats pre-treated with BLA muscimol and Acb DAMGO tend to approach, but not consume, a high-fat chow at a significantly greater rate than those receiving either drug in isolation. These data suggest that the approach and consummatory phases of feeding are dissociable. To more closely study these phases of feeding in the aforementioned motivational states, we have established a dose of DAMGO that produces operant lever-pressing behavior for sucrose pellets equivalent to that of 24-hour food deprivation. We will next investigate the role of the BLA in producing lever-pressing behavior in two separate experiments. The first will measure lever-pressing breakpoint and possible place conditioning behavior as mediated by several doses of Acb DAMGO and BLA muscimol (DAMGO dose: saline, .025, .25 micrograms/.5 microliter; muscimol dose: saline, 20 nanograms); the second will measure the same variables using BLA muscimol and food deprivation (0, 24, 48 hours deprived) instead of DAMGO infusion.

Disclosures: H.W. Johns: None. **D.K. Miller:** None. **K.E. Parker:** None. **A.S. Sage:** None. **M.J. Will:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:925.11/FFF50

Topic: F.03. Motivation and Emotion

Title: Nucleus accumbens deep brain stimulation in a rat model of binge eating

Authors: *W. DOUCETTE, J. MACLEOD, J. LEITER, A. GREEN
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Abstract: Binge eating disorder affects 0.7-4% of the general population, and bingeing behavior is present in 30% of dieting obese individuals. Binge eating is defined as repeated, discrete, and intermittent bouts of consuming unusually large amounts of food. Those with binge eating

disorder have binges that are calorically dominated by sugar and fats and often consist of foods such as ice cream and cake - foods that have been shown to be potent activators of the brain reward circuit. Work of others has demonstrated that a restricted sweet-fat diet in rats can produce binge eating behavior. We have used this rat model to test the hypothesis that treatment with deep brain stimulation in the nucleus accumbens (NAc), a crucial component in the brain reward circuit, will decrease the percentage of total calories consumed during restricted access to the sweet-fat diet. This hypothesis is based on the following: 1) brain areas thought to be dysfunctional in both addiction and eating disorders include the NAc; and 2) deep brain stimulation in the NAc has been shown to decrease alcohol use in animal models of addiction. Sprague-Dawley rats were given restricted access (2 hours/day, from 6 to 8 hr after the onset of the dark cycle) to a highly palatable sweet-fat chow (*Teklan diets*), with standard chow provided *ad libitum,* and daily measurements of consumption of each type of food, water and body weight. Following insertion of electrodes into the NAc animals were exposed to this feeding paradigm until binging was stable from day to day. A *Grass* stimulator with an optical isolation unit delivered high frequency (150 Hz), constant current (0.2 mA), bilateral monophasic stimulation with a pulse duration of 60 µsec during the restricted access phase in the treatment group and was compared to an implanted sham stimulation group. Pilot data collected to date demonstrate a statistically significant ($p < 0.0005$) 30% reduction in caloric intake during the binge period in the active stimulation group compared to the sham control group. Statistical analysis of the two groups was performed using an ANOVA with a post-hoc Tukey test. Preliminary data suggest that deep brain stimulation within the NAc can decrease caloric intake during the binge eating period in an animal model of binge eating disorder. While this study only addresses stimulation to reduce bingeing, future work will be needed to determine if chronic stimulation can not only reduce bingeing but also produce sustained weight loss.

Disclosures: W. Doucette: None. **J. MacLeod:** None. **J. Leiter:** None. **A. Green:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

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Topic: F.03. Motivation and Emotion

Support: NIH Grant R01DK071738

NSF Grant 0641943

NIH Grant RO3DA020802

NIH Grant T32MH67631-07

Title: Microstructural studies of the ingestive behavior evoked from the nucleus accumbens shell and ventral palllidum

Authors: *I. RIVERO-COVELO, I. SALIJA, D. WIRTSHAFTER, T. R. STRATFORD
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Abstract: Dramatic increases in the intakes of solid and liquid diets can be produced by injections of the GABA-A agonist muscimol into the nucleus accumbens shell (AcbSh). Similar effects are seen after injections of the GABA-A antagonist bicuculline into the medial ventral pallidum (VPm), a structure which receives GABAergic projections from the AcbSh.
Alterations in food intake can result from a number of different types of behavioral changes. In order to determine how behavior is altered by manipulations of the AcbSh and the VPm, it is necessary to actually examine the animals' behavior, rather than relying on measurements of intake. In these experiments, we used microstructural techniques to determine exactly how licking behavior is altered by drug injections in the AcbSh or VPm.
Injections of muscimol into the AcbSh of rats consuming a 10% sucrose solution led to large increases in total consumption and in the number of licks emitted, without altering mean lick volume. Rats normally emit licks in "clusters", which we define as strings of licks separated from each other by interlick intervals (ILIs) of more than 0.5 sec. Increases in intake could thus result either from increases in the mean size of clusters (SC) or in the total number of clusters emitted (NC). We found that effects of intra-AcbSh muscimol were entirely due to increases in NC, with no change in SC. The "within burst lick rate," i.e. the mean value of ILIs less than 0.25 sec, was unaffected. When examined across time (t), licking behavior can be well described by the exponential decay equation "cumulative licks = ab(1-exp(-t/b))", where the "a" parameter reflects the initial rate of licking, and "b" is the decay constant. Nonlinear curve fits indicated that the effects of intra-AcbSh muscimol were due entirely to increases in the time constant, with no alterations in initial rate.
In animals receiving bicuculline in the VPm, increases in licking again resulted entirely from increases in the number of clusters and in the time constant "b" of the exponential curve fits. In this case both cluster size and the rate constant "a" were significantly reduced, although these changes were not large enough to offset the effects on NC and "b".
These results indicate that there are both similarities and differences between the hyperphagia syndromes induced from the AcbSh and VPm. Many studies have suggested that changes in dietary palatability are associated with alterations in SC and "a". The current findings provide no support for the view that effects of muscimol in the AcbSh are mediated through changes in palatability, and raise the possibility that the effects of bicuculline in the VPm may occur in spite of a reduction in palatability.

Disclosures: I. Rivero-Covelo: None. **I. Salija:** None. **D. Wirtshafter:** None. **T.R. Stratford:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

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Program#/Poster#:925.13/FFF52

Topic: F.03. Motivation and Emotion

Support: NSERC Grant

Title: Using genetic tools to examine ventral hippocampal mediated exploration

Authors: *R. NGUYEN[1], V. MAHADEVAN[2], B. RICHARDS[3], M. WOODIN[2], J. S. YEOMANS[1], J. C. KIM[1]
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Abstract: Chemical and electrical stimulation of the ventral hippocampal formation (vHPC) activates dopamine release in the nucleus accumbens and increases locomotion in rats. We extended these findings in mice using optogenetic and pharmacogenetic tools delivered by adeno-associated virus. Open field locomotion was examined upon light-stimulation of channelrhodopsin-2, or clozapine-N-oxide (CNO) stimulation of the hM3D receptor in the vHPC. Both methods of activation significantly increased spontaneous locomotion. Furthermore, activation of the hM3D receptor with CNO increased the firing rate of CA1 pyramidal neurons recorded in vitro. Our findings suggest that genetically induced vHPC activity enhances locomotion in mice. Additionally, retrograde tracing from the nucleus accumbens shell and medial prefrontal cortex (mPFC) resulted in cell body labeling in the ventral CA1 and subiculum. Therefore, we investigated the locomotor effect of silencing the mPFC while stimulating the vHPC. Genetic methods will help define the specific neural populations in vHPC and mPFC modulating dopamine system function.

Disclosures: R. Nguyen: None. **V. Mahadevan:** None. **B. Richards:** None. **M. Woodin:** None. **J.S. Yeomans:** None. **J.C. Kim:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

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Program#/Poster#:925.14/FFF53

Topic: F.03. Motivation and Emotion

Support: R01 DA 016423

R01 DA 022520

R01 AT 001415

Title: Prepronociceptin polymorphisms predict inter-individual variability in placebo-induced opioid release

Authors: *M. SIKORA, M. PECINA, E. AVERY, V. DIAZ, J.-K. ZUBIETA
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Abstract: The neuropeptide nociceptin, also called orphanin FQ (N/OFQ), is the endogenous agonist of the N/OFQ peptide receptor (NOP receptor). Both N/OFQ and the NOP receptor share a high degree of homology with classical opioid peptides and opioid receptors, respectively, and use similar signal transduction pathways. Among many biological roles suggested for the N/OFQ/NOP receptor system, modulation of opioid analgesia has been most widely investigated and this system has been reported to counteract several opioid-mediated effects. The N/OFQ/NOP receptor system is present in vlPAG neurons, where there is also a high density of opioid peptides and receptors. N/OFQ microinjection in the vlPAG antagonizes the acute antinociceptive effects of DAMGO and morphine. Here we hypothesized that genetic variation within the precursor prepronociception will influence placebo response and the Δ u-opioid BPND associated to it.

Disclosures: M. Sikora: None. **M. Pecina:** None. **E. Avery:** None. **V. Diaz:** None. **J. Zubieta:** None.

Poster

925. Neural Substrates of Reward: Animal Studies II

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Program#/Poster#:925.15/FFF54

Topic: F.03. Motivation and Emotion

Support: DARPA W911NF-10-0059

NIH DP2-OD2441

NIH R01-DA28299

Title: Molecular level functional MRI of dopamine release in the ventral striatum

Authors: *T. LEE, L. CAI, A. JASANOFF
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Abstract: Dopamine signaling is critical to brain systems controlling voluntary movement, learning, and the experience of reward. Although extensive studies of dopaminergic neural activity have been performed using electrophysiology and pharmacological approaches, very little information has been obtained about spatial characteristics of dopamine signaling in the brain. To address this issue, we recently developed a family of protein-based contrast agents that can detect dopamine in magnetic resonance imaging (MRI). Here we use these probes to perform molecular-level functional imaging of dopamine release during delivery of a widely-studied artificial reward, electrical stimulation of the medial forebrain bundle (MFB) in rats.
Rats were implanted with electrodes targeted to the MFB, and operant responses to MFB stimulation were verified behaviorally. Animals were then anesthetized, tracheostomized, artificially ventilated, and injected with 80 mg/kg L-DOPA in preparation for dopamine imaging. They were placed in a cross coil system and inserted into a 9.4 T Bruker Avance II MRI scanner. During imaging with gradient echo T1-weighted contrast (TR/TE = 250/10 ms), the dopamine sensor BM3h-9D7 was infused into brain (0.1 μL/min.) in order to fill large regions of the ventral striatum. Following 50 minutes of infusion, MFB stimuli (2 ms pulses, 60 Hz, 250 μA) were delivered in blocks of 16 s, alternating with 300 s epochs of rest. Statistical analysis using AFNI revealed significant stimulation-associated MRI signal changes across the ventral striatum, with the strongest responses observed in the medial part of nucleus accumbens shell. Average time series data indicated signal changes of up to 2%, consistent with peak dopamine concentrations of approximately 10 μM binding to the sensor. These responses varied in a manner dependent on the stimulus parameters, and were not observed using an inactive form of the MRI sensor, WT BM3h. Parallel measurements using electrochemical detection verified that dopamine concentrations determined by MRI were realistic, and confirmed that BM3h-9D7 binds dopamine in vivo.
These experiments offer a novel and qualitatively unique spatiotemporal view of phasic dopamine signaling, and provide direct evidence for the spatial heterogeneity of dopamine release in the ventral striatum.
Ann Graybiel, Mark Howe, and Charles Blaha are thanked for assistance with voltammetry methods.

Disclosures: T. Lee: None. **L. Cai:** None. **A. Jasanoff:** None.

Poster

926. Motivation and Emotion: Risk and Uncertainty

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Topic: F.03. Motivation and Emotion

Support: Leverhulme Trust grant F/00 407/BF

Cardiff125 Grant

Title: Is risk-taking behaviour mediated by imprinted genes?

Authors: C. L. DENT[1], A. WARD[2], A. PLAGGE[3], J. WILKINS[4], *T. HUMBY[1], A. R. ISLES[1]
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Abstract: Impulsivity and risk taking are natural parts of human behaviour, however it seems that in certain individuals, these behaviours become pathological and maladaptive. Increased impulsivity and risk-taking are recognised as clinical traits of many psychiatric disorders, including schizophrenia and attention deficit/hyperactivity disorder (ADHD), as well as drug addiction and gambling. A prominent feature of impulsive choice is an intolerance of a delay of a reward or gratification. One method used to target the measurement of this behaviour is the 'delayed-reinforcement task' (DRT). By employing the use of mouse models with genetic knock-outs, we have discovered that impulsive and risk-taking behaviours are mediated by epigenetic factors, and specifically by brain-expressed imprinted genes.
Imprinted genes, defined by their parent-of-origin specific monoallelic expression, have been shown to have an increasingly important role in mediating adult behaviour1. Using separate strains of mice with genetic deletions of the genes Nesp55 and Grb10, oppositely imprinted in the adult brain, DRT performance was assessed. Maternally expressed Nesp55 promoted the riskier choice of larger delayed rewards, whereas paternally expressed Grb10 reduced the choice of larger delayed rewards. This indicates that maternal genomes can contribute differentially, and perhaps, antagonistically, to impulsive and risk-taking behaviour.
To explore this effect further, we are developing a novel test of risk-taking behaviour. The 'predator odour risk-taking task' uses a predator odour as a fear stimulus, to induce a conflict with obtaining a reward. Mice were trained to cross the central chamber of a three-chambered apparatus to collect a liquid reward. It was observed that when a predator odour (rat, cat, fox) was present in the middle chamber, mice took significantly longer to cross the apparatus to collect the reward; suggesting that the presence of a predator odour successfully induced fear, and therefore provided an index of risk-taking behaviour. This behaviour is being further explored using pharmacological manipulation, specifically taxing the dopaminergic, serotoninergic and noradrenergic systems, in order to examine the role that these neurotransmitter systems may have upon risk-taking behaviour in this task. We will then

examine the behaviour of Nesp55 and Grb10 KO mice in this task, which will not only provide a novel measure of their risk-taking behaviour, but will also help define the specific mechanism that Grb10 and Nesp55 have on risk-taking behaviour.
Wilkinson, L.S., Davies, W., Isles, A.R. (2007) Nature Reviews Neuroscience 8(11):832-843

Disclosures: C.L. Dent: None. **A. Ward:** None. **A. Plagge:** None. **J. Wilkins:** None. **T. Humby:** None. **A.R. Isles:** None.

Poster

926. Motivation and Emotion: Risk and Uncertainty

Location: Hall F-J

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Program#/Poster#:926.02/FFF56

Topic: F.03. Motivation and Emotion

Title: Single neuronal encoding of reward uncertainty in the frontal midline structures of the monkey

Authors: *I. E. MONOSOV, O. HIKOSAKA
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Abstract: Uncertainty about the future modulates decision-making, motivation, and emotion. This suggests that the brain may convert outcome-distributions used for valuation to degrees of uncertainty. The ventral portion of the anterior midline of the primate brain, including ventromedial prefrontal cortex (vmPFC) and septum, is thought to play a role in processing information about reward and modulating emotional states. But if and how single neurons there are modulated by uncertainty about rewards and punishments is unknown. While four monkeys were presented visual cues associated with different probabilities of rewards and punishments, we recorded from single neurons over a wide area of the anterior ventral midline, including the vmPFC (primarily in areas 25 and 14), septum, and the anterior hippocampal continuation (AHCC) (i.e., the area dorsal to the vmPFC and septum). We found that neurons in the AHCC responded strongly to cues predicting upcoming rewards probabilistically (i.e., 50%, 25%, 75%), but showed little response to cues predicting upcoming rewards certainly (0%, 100%). In contrast, they were not influenced by uncertainty about punishments. When a different set of cues was used to predict different amounts of reward, AHCC neurons showed little response to any of the cues. Based on our findings, we hypothesize that AHCC promotes learning and enhances vigilance in an uncertain environment. We also found that neurons in ventral vmPFC were influenced by reward-uncertainty in a manner analogous to hope: they responded similarly to cues predicting reward delivery certainly (100%) and to cues predicting rewards

probabilistically (50%). In summary, AHCC signals the degrees of reward-uncertainty, whereas ventral vmPFC signals the possibility of obtaining reward. Both schemes may play important roles in motivatated behavior in uncertain environments.

Disclosures: I.E. Monosov: None. **O. Hikosaka:** None.

Poster

926. Motivation and Emotion: Risk and Uncertainty

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Topic: F.03. Motivation and Emotion

Support: Michael J. Fox Fdn.

Daniel F. and Ada L Rice Fdn

USPHSG #NS074014

Title: Intracranial self-stimulation as the positive reinforcer to study impulsivity in a delayed discounting paradigm in rats

Authors: *S. TEDFORD[1], A. PERSONS[1], L. CHEN[1], S. L. ROKOSIK[1,2], T. C. NAPIER[1]
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Abstract: Alterations in impulsivity are associated with drug and behavioral addictions. Delayed discounting is an established method used to assess impulsive choice in both humans and rodents. Impulsive choice is reflected in the selection of a small, immediate reward over a larger, delayed reward. To test impulsive choice in rats, food is often used as the positive reinforcer which poses potential confounding factors including the need to food deprive animals and subsequent satiation. To model mechanisms that underlie the many facets of impulsivity, a common universal reinforcing property is needed. A realistic alternative to food reward is intracranial self-stimulation (ICSS). ICSS directly targets a common reward system that is activated regardless of modality. We recently implemented a rodent model of ICSS-mediated probability discounting (Rokosik and Napier, JNeurosci Methods, 198:260, 2011; Rokosik and Napier, NPP, 37:1397,2012). Here, we tested the hypothesis that ICSS can provide a positive reinforcer for delayed discounting tasks. Rats were implanted with bipolar stimulating electrodes targeted at the lateral hypothalamus (LH), and after recovery, they were trained to associate the positively reinforcing electrical stimulation of the LH with a lever press with a forepaw.

Stimulation of 50Hz and 160Hz were used for the small and large reinforcer, respectively. Our prior work demonstrated that these values elicited near EC50 and Emax levels of responding with no aversive effects. Rats were trained in a Choice Test that determined their ability to discriminate between the small and large reinforcers. The task was successfully acquired within one week, and then rats entered the delayed discounting task. Several delays were tested. Data collected thus far have shown that with a three sec delay, rats prefer the large reinforcer (by ~80%) over the small, immediate reinforcer. By 15 sec, the rats consistently switch to the small, immediate reinforcer. These data indicate that an ICSS-mediated delayed discounting paradigm is feasible and a streamlined alternative to food reward studies.
Acknowledgements: Michael J. Fox Fdn., Daniel F, Ada L Rice Fdn. and USPHSG #NS074014

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Poster

926. Motivation and Emotion: Risk and Uncertainty

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Program#/Poster#:926.04/FFF58

Topic: F.03. Motivation and Emotion

Title: Adolescent risk taking and resting state functional connectivity.

Authors: S. J. DEWITT[1], S. ASLAN[2], N. CHUKWUEKE[3], V. GERMER[3], *F. FILBEY[4]
[1]Brain and Behavioral Sci., Univ. of Texas at Dallas, Dallas, TX; [2]AdvanceMRI, LLC., Frisco, TX; [3]Ctr. for BrainHealth, Dallas, TX; [4]Univ. of Texas At Dallas, Dallas, TX

Abstract: Funding Support: Center for BrainHealth Seed Grant
Resting state functional connectivity (rsFC) allows the assessment of intrinsic network connectivity of brain areas in the absence of specified goal-directed behaviors. In the present study, we examined how circuits that include the amygdala, a region of emotion processing, may distinguish risk-taking from non-risk-taking youth. Resting fMRI data were collected in 25 risk-taking (risky) and 18 non-risk-taking (control) youth as measured by the Youth Risk Behavior Scale(YRBS). We hypothesized that there would be disruptions in rsFC between the amygdala and prefrontal control areas, such as the anterior cingulate in the risk group compared to the control group. Furthermore, we hypothesized that the rsFC (i.e. z-scores) will correlate with temperament and impulsivity as measured by the Junior Temperament Childhood Inventory (JTCI) score and the Impulsive and Sensation Seeking Scale (IMPSS), respectively. Voxel-based-analysis of z-score functional maps (seed region: amygdala) revealed greater connectivity

with the left cingulate gyrus, bilateral precuneus, right middle frontal gyrus and supramarginal gyrus at cluster threshold of K≥ 17 and $p \leq 0.001$(using AFNI). Analysis of behavioral data revealed significantly higher scores on the IMPSS for the risky group vs. controls, t(41) = 2.23 p =0.03, but IMPSS did not correlate with amgydalar connectivity in either group . A significant negative correlation was found between amygdala-right middle frontal gyrus coupling and the reward dependence subscale of the JTCI for controls (r = -.58 p = 0.01), such that the greater the reward-driven emotionality, the weaker the connectivity between amygdala-PFC. There were no significant correlations found in the risky group. Enhanced rsFC in the risky group between amygdala and posterior cingulate gyrus, and right precuneus has been postulated to underlie intense automatic emotional reactivity (Pavuluri et al. 2009). Similar enhanced connectivity between amygdala and PFC regions in high-risk youth (i.e., adolescents with family history of substance abuse) (Silveri et al., 2011) suggests that this pattern could be a biomarker for addiction. To conclude, these findings suggest systems-level disruptions in risk-taking youth that are not present in non-risk taking youth. These findings could inform treatment development for at-risk youth.

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Poster

926. Motivation and Emotion: Risk and Uncertainty

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Program#/Poster#:926.05/FFF59

Topic: F.03. Motivation and Emotion

Support: KOSEF Grant M10644000028-06N4400-02810

Title: Impaired risky decision making in depressed patients with and without suicide attempt history

Authors: *K. BAEK[1], J.-H. KWON[1], J.-H. CHAE[2], J.-A. MIN[2], K. CHOI[2], K. JANG[2], N.-B. LEE[2], J. JEONG[1]
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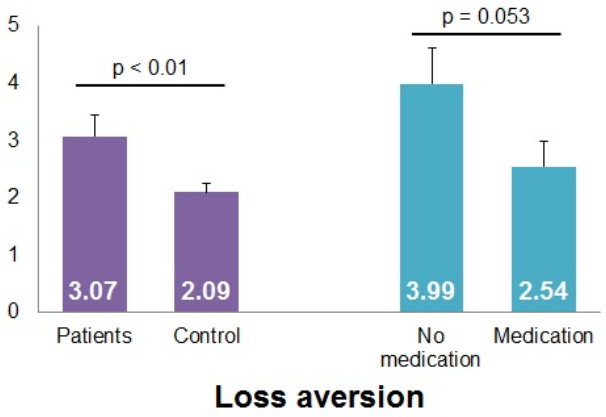
Abstract: Depression is depressive mood state characterized by negative bias in cognition, and often results in impairment in decision making. Suicide attempts might be viewed as a non-optimal decision making process. Decision making tasks such as Iowa gambling task has been used in experimental studies with depressed patients and suicide attempters, but the deficit in

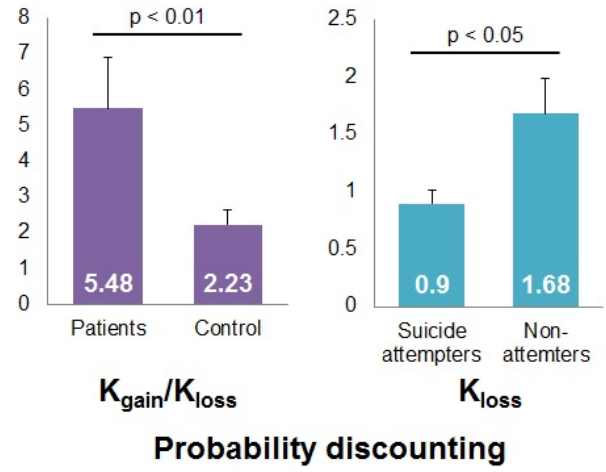
decision making was still not well clarified. We hypothesized that the depressed patients, particularly ones with suicide attempt history, would differ in sensitivity to loss during decision making, since depressed patients are thought to be susceptible to negative outcomes than positive ones.

We examined the sensitivity to loss in 42 patients with major depressive disorder (patients with suicide attempt history, n=22) and 58 healthy controls using loss aversion and probability discounting task (in gain and loss frame).

Depressed patients, particularly non-medicated patients, exhibited significantly higher loss aversion than healthy controls as shown in Fig. 1 (Mean ± S.D: 3.07±2.46 vs. 2.09±1.18, $p < 0.01$). Antidepressant treatment affected loss aversion in depressed patients marginally ($p = 0.053$), suggesting that neuromodulator system such as 5-HT might be underlying decision making process regarding negative outcomes. In probability discounting, depressed patients showed higher ratio of (discount rate of gain)/(discount rate of loss) than healthy controls, which indicates higher risk aversion (5.48±8.77 vs. 2.23±3.01, $p < 0.01$). Discount rate of loss was significantly differed between patients with and without suicide attempt history (0.90±0.50 vs. 1.68±1.23,$p < 0.05$). In our preliminary fMRI experiment, the insula, the medial prefrontal cortex and the dorsolateral prefrontal cortex were implicated in probability discounting task.

In conclusion, high loss aversion and risk aversion were distinguished as key features of patients with major depression. These loss sensitivity tasks might be useful in quantifying affective/cognitive bias in depressed patients and predicting risk of suicidal attempt.





Disclosures: K. Baek: None. **J. Kwon:** None. **J. Chae:** None. **J. Min:** None. **K. Choi:** None. **K. Jang:** None. **N. Lee:** None. **J. Jeong:** None.

Poster

926. Motivation and Emotion: Risk and Uncertainty

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:926.06/FFF60

Topic: F.01. Human Cognition and Behavior

Support: NINDS NS035115

Title: Changes in perception of risk and valuation of monetary rewards with transition from acute to chronic back pain

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Abstract: Recent observations show the involvement of nucleus accumbens in chronic pain. This region is part of the mesolimbic neural mechanisms mediating reward valuation, decision-making, and motivation. However, it remains unclear the extent to which the circuitries of pain and reward overlap with and influence one another. Since nociceptive input provides motivational information and value of its own via its function as a primary punisher and as a negative reinforcement, we are interested in exploring the link between reward and pain, specifically for the transition from an acute to a chronic state. Here we assess performance, and compare brain activity, for a monetary gambling task –behavioral loss aversion – and compare the performances of healthy controls (HC, n=21) to those of people with subacute back pain (SBP, n=56) and chronic back pain (CBP, n=14). All SBPs and CBPs were diagnosed by a clinician following IASP criteria; CBPs had back pain >1 year and HCs had no history of back pain. Additionally, SBPs were segregated into two groups: recovering SBP patients (SBPr) were defined as people whose pain was decreased by >20% at the time of the task; all other SBPs were defined as persisting (SBPp).
The task was performed during an fMRI scan. For each trial, subjects were shown a display depicting an amount of potential gain and an amount of potential loss, and after a decision period, had to accept or reject the gamble with a computer mouse. We computed the regression coefficients for each person's loss and gain sensitivity, and their ratio equaled their individual level of loss aversion (lambda). We found no group effect in average lambda, gain, or loss sensitivity values ($p=0.19$); however, there was a significant gender affect, with male participants having higher gain sensitivity than females overall ($p<0.01$). There was also a significant interaction between group and gender, such that males' gain sensitivities increased as they moved farther away from a healthy baseline, with SBP males performing somewhere in between HC and CBP males. Preliminary results of brain activity indicate significant differences in activity within the anterior cingulate during the task. Additional analyses are underway, and more results will be presented at the meeting.
So far, our findings suggest that individuals with back pain exhibit riskier decision-making and may be less loss-averse, implying that the experience of pain changes the experience of monetary reward, although this interaction seems relatively weak and dependent upon gender differences.

Disclosures: S.E. Berger: None. **M.N. Baliki:** None. **A.T. Baria:** None. **E.L. Parks:** None. **S. Torbey:** None. **K.M. Herrmann:** None. **T.J. Schnizter:** None. **A.V. Apkarian:** None.

Poster

926. Motivation and Emotion: Risk and Uncertainty

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:926.07/FFF61

Topic: F.01. Human Cognition and Behavior

Support: NIA Grant R01 AG021632

NIA Grant R37 AG12713

WRIISC VA Palo Alto

MIRECC VA Palo Alto

Title: Neural correlates of risk during decision-making in older pilots

Authors: *M. M. ADAMSON, D. HERALDEZ, K. MAIN, K. CASTILE, J. TAYLOR, J. YESAVAGE
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Abstract: INTRODUCTION: The most lethal accidents in General Aviation are usually caused by improperly executed instrument landing approaches involving descents below the minimum altitude of 200 ft without the use of visual references prescribed by the Federal Aviation Administration (FAA). Older pilots are more likely than younger pilots to take the risk of landing in worse weather conditions than acceptable by FAA rules. Although the individual differences in flight simulator performance have been explained in part by age, expertise and general cognitive ability, the contributions of a specific brain network associated with risky decision making remains unexplored. METHOD: This study evaluates the role of age-related neural changes during a pilot's crucial decision to land or not under difficult weather conditions. Ten young (19-45) and 10 middle-age to old (46-79) instrument certified pilots "fly" a series of short simulated "cockpit view" landing scenarios in the fMRI scanner with conditions in which low visibility (high risk) explicitly favors the no-land choice and high visibility (low risk) favors the land choice. Measures include: landing decision scores (Land(low risk) vs. No Land (high risk)), task-related and resting-state networks (default mode, salience and executive), and structural brain volumes.RESULTS: Initial analyses of age-related differences in task related functional brain activity shows lower activation during the No Land condition in some but not all regions of the default mode network (which consists of posterior cingulate/precuneus, lateral parietal and middle temporal gyrus, and anterior cingulate, superior, and middle frontal gyri) in older pilots compared to younger pilots. CONCLUSION: Decreased activity in default mode network during a risky situation (No Land condition) is modulated by age-related brain changes during real-world everyday task performance.

Disclosures: M.M. Adamson: None. **D. Heraldez:** None. **K. Main:** None. **K. Castile:** None. **J. Taylor:** None. **J. Yesavage:** None.

Poster

926. Motivation and Emotion: Risk and Uncertainty

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:926.08/FFF62

Topic: F.01. Human Cognition and Behavior

Title: Dopaminergic contributions to risk taking and temporal discounting: Evidence from Parkinson's disease

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Abstract: When making decisions, subjective factors -- such as one's willingness to take risks or wait for better outcomes -- often influence choices. Previous research has shown that both risk taking and temporal discounting are modulated by striatal dopamine, but questions remain about the causal role of dopamine in these behaviors. Parkinson's disease is characterized by a loss of dopaminergic inputs to the striatum, providing a model for better understanding the role of striatal dopamine in specific aspects of decision making. Here, we tested patients in the mild to moderate stage of Parkinson's disease and healthy controls on two decision making tasks: one that assesses risk taking (the Columbia Card task) and one that assesses temporal discounting (Intertemporal Choice task). Additionally, to characterize how modulating dopamine levels in patients alters risk taking and temporal discounting behavior, we tested Parkinson's patients both on and off dopaminergic medication. We found that Parkinson's patients made riskier choices than matched controls. Specifically, Parkinson's patients were less sensitive to an increased probability of loss, and this effect was enhanced when patients were tested on dopaminergic medication. On the Intertemporal Choice task, we found that Parkinson's patients were more patient than controls, that is they discounted less for time than controls. Preliminary results suggest that this effect was especially strong among Parkinson's patients tested while on medication. Together, these results support the idea that striatal dopamine modulates risk-taking and temporal discounting. These results also raise important questions regarding the mechanisms by which dopamine modulates different aspects of decision making in both health and disease.

Disclosures: E.K. Braun: None. **K. Foerde:** None. **B. Figner:** None. **E. Weber:** None. **D. Shohamy:** None.

Poster

926. Motivation and Emotion: Risk and Uncertainty

Location: Hall F-J

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Program#/Poster#:926.09/FFF63

Topic: F.01. Human Cognition and Behavior

Support: P20 DA022539

R01 DA020726

T32 DA024635

PM 20063287

Title: Neurobiological determinants of risk-taking: The influence of recent experience, frontolimbic activation, and dopamine D2/D3 receptors

Authors: *M. KOHNO[1], D. G. GHAHREMANI[2], A. M. MORALES[1], C. L. ROBERTSON[3], K. ISHIBASHI[2], A. T. MORGAN[2], R. A. POLDRACK[6], M. A. MANDELKERN[4], E. D. LONDON[3,5]
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Abstract: Despite progress in our understanding of risky decision-making, the factors that influence decisions involving risk are not well understood. Neuroimaging studies have provided evidence of a distributed neural network involved in decision-making; however, decision-making has often been evaluated independent of recent experience. The experience of losses and rewards has been shown to affect fronto-limbic and dopaminergic-brain regions respectively, but their affects on the circuitry underlying risky decision-making are poorly understood. In addition, animal studies have shown a reduction in the number of risky choices with administration of D2-receptor agonists suggesting a major role for dopamine in risky decision-making. The links between dopamine and the effects of choice-outcome on the circuitry underlying risky decision-making, however, are unknown. Using the Balloon Analog Risk Task (BART), a computerized task used to evaluate the propensity for risk-taking, we sought to evaluate the role of striatal D2-like dopamine receptors in the circuitry underlying risky decision-making and the effects of recent outcomes on risk-taking behavior and associated neural activity. In the BART, participants are presented with a series of choices in which they risk losing accumulated earnings for greater potential gains by pumping a virtual balloon. The alternate choice is to cash out and retain accrued earnings. Neural activation was assessed using fMRI paired with the BART in

healthy participants (n=60,18-51yrs.; 27 F/33 M), and striatal-dopamine D2-like-receptor availability was measured by [18F]fallypride positron emission tomography in a subset of the sample (n=13).
After explosion but not after cashing out, participants made fewer pumps, and activation in amygdala and hippocampus showed greater sensitivity to risk ($p<0.05$, whole-brain corrected). Ventral striatal D2-like receptor availability and sensitivity of activation to level of risk and reward while cashing out was negatively related to (i.e., predicted reduction in) risk-taking on the next trial ($r= -0.798$, $p= 0.002$; $r= -0.778$, $p= 0.002$). D2-like receptor availability was also negatively correlated with sensitivity of activation to level of risk in the DLPFC while pumping ($r= -0.751$, $p= 0.004$). Both measures were related to total earnings (BP(ND): $r= -0.596$, $p= 0.027$, BOLD: $r= 0.740$, $p= 0.005$), suggesting a mechanism by which an interactive system of frontal and striatal regions supports adaptive risk-taking. These findings are the first direct evidence for a potential mechanism by which dopaminergic pathways within frontostriatal networks determine risk-taking behavior.

Disclosures: M. Kohno: None. **D.G. Ghahremani:** None. **A.M. Morales:** None. **C.L. Robertson:** None. **K. Ishibashi:** None. **A.T. Morgan:** None. **R.A. Poldrack:** None. **M.A. Mandelkern:** None. **E.D. London:** Other Research Support; Support was provided in part by Philip Morris USA. None of the sponsors were involved in data collection, analysis or interpretation of results..

Poster

926. Motivation and Emotion: Risk and Uncertainty

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:926.10/FFF64

Topic: F.01. Human Cognition and Behavior

Support: NSERC Discovery Grant

Alberta Gambling Research Institute

Title: Gambling for the big win: Enhanced memory for extreme outcomes drives risky decisions

Authors: C. R. MADAN[1], E. A. LUDVIG[2], *M. SPETCH[1]
[1]Psychology, Univ. of Alberta, Edmonton, AB, Canada; [2]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: Would you rather a guaranteed $20 win or a 50/50 chance at winning $40? What about sustaining a guaranteed $40 loss vs. a 50/50 chance of losing $40 dollars? A classic result

from behavioral economics is that people usually choose the guaranteed win (i.e., are risk averse) in the gain context, but choose the gamble (i.e., are risk seeking) in the loss context.
This change in risk sensitivity for gains and losses, known as the reflection effect, holds for a broad range of experiments when people are explicitly told the outcomes and probabilities. Recent results, however, have shown that when people learn about the outcomes from their own experience, this reflection effect can reverse with people becoming risk seeking for gains and risk averse for losses.
In a series of experiments, we show that this reversal in risk preference is driven by an overweighting of the extreme or peak outcomes in decisions made from experience. That is, the biggest win and the biggest loss in a given context are overweighted when evaluating risky options that can lead to these outcomes. This overweighting creates a bias toward risk seeking for gains and risk aversion for losses.
We hypothesize that extreme outcomes are overweighted in decision processes due to memory biases. In the example above, this hypothesis supposes that a win of $40 is more memorable than an outcome of $0. As a result, the risky option has a subjectively higher value than the guaranteed win, leading to risk seeking. In contrast, when an extreme loss of $40 is similarly overweighted, the risky option has subjectively more negative value than the guaranteed loss, leading to risk aversion.
In the experimental task, participants were presented with pairs of doors, with each door leading to a fixed or risky gain or loss. Experiment 1 confirmed that participants showed a reversed reflection effect with experience in our procedure. Experiment 2 manipulated reward magnitude and showed that the reversed reflection effect occurred only for doors leading to the relatively most extreme outcomes. Experiment 3 split gains and losses between subjects and found risk seeking for the highest value outcome in both cases.
These results suggest that memory biases for extreme outcomes may drive behavior for experience-based risky decisions. More broadly, gambling behavior may also be driven by this memory bias, where people continue to gamble for the memorable big win despite sustaining multiple smaller, and thus forgettable, losses.

Disclosures: C.R. Madan: None. **E.A. Ludvig:** None. **M. Spetch:** None.

Poster

926. Motivation and Emotion: Risk and Uncertainty

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:926.11/FFF65

Topic: F.01. Human Cognition and Behavior

Support: Institute for Research on Gambling Disorders

Title: An MEG study of risk and overconfidence in pathological gamblers

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Abstract: Studying the neural correlates of confidence and risky decision making may be useful for understanding mechanisms associated with pathological gambling. The present study compared MEG responses of 34 pathological (PG) and 34 non-pathological (NPG) gamblers during the Georgia Gambling Task (Goodie, 2003). Participants completed the Diagnostic Interview for Gambling Severity and South Oaks Gambling Screen, measures of gambling pathology, and a standard confidence assessment task using 180 pairwise comparisons of populations of states. In a subsequent MEG task, participants viewed the same comparisons (Judgment; 2000 ms), or a probability-matched random event, followed by the number of points that could be lost during that trial (Decision; 1500 ms), and indicated with a button press whether they chose to bet on their decision. After bet acceptance or rejection, participants received feedback on whether they won or lost.
Recordings were obtained using a 143 channel whole head MEG system. Using spherical head models and L2 minimum norm, we localized ERFs. Baseline differences and task related changes during the initial 750ms of the Judgment task phase were computed as a function of group and confidence level (2-way ANOVA).
There were a number of interactions between gambling pathology and high (HC) versus low confidence (LC). NPLC (Non-pathological gambler, low confidence) initially show decreased activation in right occipital temporal cortex. During later processing, however, NPLC show increased activity in this region, while NPHC show greater activity than NPLC in left occipital temporal cortex. As these regions support identification of behaviorally relevant stimuli, it is interesting that occipital temporal cortex in PG did not distinguish between confidence levels at any time. This complements other insensitivities in this region in PG (Camchong et al, 2007) and suggests that manifestations of top-down bias signals in early sensory processing are disregulated in PG. During early and late processing, NPLC show decreased activity in parietal cortex, consistent with electrophysiological studies in rats (Kiani et al, 2009). PGLC show early increased activation throughout dorsal and ventral visual processing streams, which persists in the left lateral prefrontal cortex. While right anterior PFC is associated with higher levels of confidence (Camchong et al, 2007) and metacognitive awareness (Flemming et al, 2012)., NPHC show decreased activity in right posterior PFC. These findings suggest that regions of right PFC differentially code confidence information, and suggest a deficit in PGs' higher cognitive processing related to decision making.

Disclosures: M.E. Hudgens-Haney: None. **J.P. Hamm:** None. **A.S. Goodie:** Research Grant; Institute for Research on Gambling Disorders. **E.A. Krusemark:** None. **J.E. McDowell:** None. **B.A. Clementz:** None.

Poster

926. Motivation and Emotion: Risk and Uncertainty

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:926.12/FFF66

Topic: F.01. Human Cognition and Behavior

Title: Are Ambiguity aversion and ambiguity intolerance identical? : A neuroeconomics investigation.

Authors: Y. TANAKA[1], T. IDENO[3], S. OKUBO[3], K. TAKEMURA[3], J. MIYATA[1], R. KAWADA[1], S. FUJIMOTO[1], *M. KUBOTA[1], A. SASAMOTO[1], N. SAWAMOTO[2], H. FUKUYAMA[2], T. MURAI[1], H. TAKAHASHI[1]
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Abstract: 1. Background:
In the field of decision making research, ambiguity aversion describes an attitude of preference for a gamble in which the probability of an outcome is known over a gamble in which the probability of an outcome is unknown. On the other hand, ambiguity intolerance has been defined as the tendency to resort to black-white solutions to arrive at premature closure. It is not clear whether these two notions derived from different disciplines are identical or not. To clarify this issue, we first compared the degree of ambiguity aversion with the degree of ambiguity intolerance in behavior level. Then, we investigated neural correlates of these two measures using voxel-based morphometry (VBM).
2. Methods:
Twenty-four healthy volunteers participated in this study. We investigated the relationship between individual ambiguity aversion tendency and individual ambiguity intolerance tendency by behavioral experiments (Choice under risk and ambiguity Task) and questionnaire (The Need for Closure Scale (NFCS)). We also administered the self-report version of the Revised NEO Personality Inventory (NEO-PI-R). Furthermore, the neuroanatomical correlates of ambiguity aversion tendency and ambiguity intolerance tendency were investigated by measuring the gray matter (GM) concentration using VBM.
3. Results:
Most subjects exhibited ambiguity aversion. The ambiguity aversion parameter measured by a

decision-making task was not correlated with the score of ambiguity intolerance, subscale of NFCS. However, ambiguity aversion parameter was correlated with agreeableness score of NEO-PI-R. While ambiguity aversion parameter were correlated with GM density in right inferior parietal lobule and left inferior frontal gyrus, score of ambiguity intolerance was not correlated with GM density in any region.

4. Conclusions:

Ambiguity aversion described in decision theory may not necessarily identical to ambiguity intolerance referred in clinical psychology. Cautious applications of decision theory to clinical neuropsychiatry are recommended.

Disclosures: Y. Tanaka: None. **T. Ideno:** None. **S. Okubo:** None. **K. Takemura:** None. **J. Miyata:** None. **R. Kawada:** None. **S. Fujimoto:** None. **M. Kubota:** None. **A. Sasamoto:** None. **N. Sawamoto:** None. **H. Fukuyama:** None. **T. Murai:** None. **H. Takahashi:** None.

Poster

926. Motivation and Emotion: Risk and Uncertainty

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:926.13/FFF67

Topic: F.03. Motivation and Emotion

Title: Dissociative relationships between impulsivity and dopamine D2/D3 receptor availability in the striatal subregions

Authors: H. PARK, B. MOON, B. LEE, *S. KIM
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Abstract: Impulsivity is significantly associated with the risk-taking behaviors including addiction and is linked to the dopamine D2/D3 receptor availability in the striatum which is separable into functional subregions (limbic, associative and sensorimotor). We investigated the relationships between the impulsiveness and dopamine D2/D3 receptor availability in the striatal subregions using [18F]fallypride and positron emission tomography (PET).
Six healthy men underwent dynamic [18F]fallypride PET studies to measure striatal dopamine D2/D3 receptor availability in terms of binding potential (BPND). Non-planning (NPimp), motor (Mimp) and attentional (Aimp) impulsiveness of subjects were assessed using the Barratt Impulsiveness Scale version 11 (BIS-11), which is consisted of total 23 likert items of 4-point scale. Striatal subregions were delineated on the individual magnetic resonance images and five subregions were defined as the precommissural dorsal caudate (preDCA), -putamen (preDPU), postcommissural-caudate (postCA), -putamen (postPU), and the ventral striatum (VST). BPND

and BIS-11 score were incorporated into the linear and non-linear regression analysis. Subjects showed varied levels of impulsiveness in BIS-11 score; NPimp (ranged 8-23 points), Mimp (ranged 4-20 points) and Aimp (ranged 7-18 points). In the preDCA, a part of the associative striatum implicated in the cognitive function, significant linear correlation was found between BPND and NPimp, Pearson R = .87, P = .026. The relationship between BPND and the sum of NPimp, Mimp and Aimp was better described by non-linear regression analysis, Pearson R = .98, P = .008, in the preDCA. BPND of the postCA, a part of the associative striatum, was linearly correlated with NPimp, Pearson R = .79, P = .062, Mimp, Pearson R = .79, P = .059, and Aimp, Pearson R = .81, P = .049. In the VST, a part of limbic striatum, the inverted-U-shaped relationships were shown between BPND and NPimp, Mimp and Aimp scores.
These data demonstrates that striatal dopamine D2/D3 receptor availability is associated with impulsivity. They also suggest that striatal subregions may play differential roles in the modulation of impulsivity.

Disclosures: H. Park: None. **B. Moon:** None. **B. Lee:** None. **S. Kim:** None.

Poster

926. Motivation and Emotion: Risk and Uncertainty

Location: Hall F-J

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Program#/Poster#:926.14/FFF68

Topic: F.03. Motivation and Emotion

Support: Swiss National Fondation

Title: Differences in dopaminergic response to unpredictable rewards in the right ventral Striatum in Fibromyalgia patients: A 11C-raclopride bolus plus constant infusion PET-study

Authors: K. LEDERMANN[1], J. JENEWEIN[1], H. SPROTT[4], G. HASLER[5], U. SCHNYDER[1], C. BURGER[2], A. JOHAYEM[2], T. CSERVENYAK[2], S. KOLLIAS[3], A. BUCK[2], *C. MARTIN-SOELCH[6]
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Abstract: Emerging evidence demonstrates the important role of the mesolimbic dopamine system (DA) in the processing of pain and reward. A DA hypofunction has been reported in chronic neuropathic pain. Fibromyalgia syndrome (FMS) is a non-neuropathic chronic pain disease, in which first indication of DA dysfunction was also evidenced. FMS is frequently

associated with depression: Reduced DA function and reduced responses to reward were also evidenced in depression.
Therefore reduced DA reactions to rewarding stimuli could be involved in the depressive or pain symptoms observed in FMS. Here, we tested differences between FMS patients and healthy subjects in striatal DA release in response to unpredictable monetary rewards using the bolus plus infusion (B/I) PET-method. We expected FMS patients to show a reduced endogenous DA release, expressed by increased [11C] raclopride binding and expected these changes to correlate with depression and pain scores. Eighteen female FMS patients and sixteen healthy subjects were measured at rest and while performing a slot machine task consisting of a sensorimotor control condition and a monetary reward condition where subjects randomly received monetary rewards in an unpredictable fashion. Regional PET measures were acquired in MRI-based striatal regions of interest. Percent of change in [11C] raclopride binding was computed as the difference in ratio region/cerebellum. We found significant higher [11C] raclopride binding ratios in FMS patients than in healthy subjects across the different measurement conditions in the right ventral striatum ($p=0.04$). The percent of change between sensorimotor and reward condition was significantly larger in FMS than in healthy participants. No significant correlations with pain and depression scores emerged. These results point to an involvement of the DA function in the pathophysiology of FMS consisting in a reduced DA function at rest and unexpectedly in a hypersensitive reaction to rewards.

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Poster

926. Motivation and Emotion: Risk and Uncertainty

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Topic: F.03. Motivation and Emotion

Support: NSF Dissertation Improvement Grant

NICHD

UCLA Center for Culture, Brain, and Development

SRCD Dissertation Grant

UC MEXUS Dissertation Grant

Title: Neural activation during risk-taking relates to risky sexual behavior in adolescents

Authors: *D. GOLDENBERG[1], E. H. TELZER[1], A. FULIGNI[2], A. GALVAN[1,3]
[1]Psychology, [2]Psychiatry and Biobehavioral Sci., [3]Brain Res. Inst., UCLA, Los Angeles, CA

Abstract: Relative to children and adults, adolescents engage in increased sexual risk-taking behaviors. Although young people represent only one-quarter of the sexually active population, they account for a disproportionate amount of sexually transmitted infections (STIs) and unintended pregnancies each year. In this study, our goal was to examine the relationship between risky sexual behavior and neural correlates of risk-taking.
Twenty-one sexually active adolescents (ages 14 -16 years) were recruited from local schools; 13 adolescents reported engaging in risky sexual behavior (e.g. lack of condom use). Risk-taking was assessed during functional magnetic resonance imaging (fMRI) using the Balloon Analogue Risk Task (BART). On each trial of the BART, participants
choose whether to inflate a balloon, which increases earnings, or to cash-out before the balloon explodes. Here, inflations and cash-outs are indices of risky and cautious decisions, respectively. fMRI analyses contrasted
the blood oxygen level dependent (BOLD) response during risky and cautious choices. Behaviorally, there was a significant negative correlation between frequency of risky sexual behavior and mean reaction time for inflations ($r=-.30$, $p<.05$); subjects reporting greater risky sexual activity were significantly faster at inflating the balloons, suggesting a greater orientation toward impulsivity. The fMRI results revealed that risky sexual behavior was negatively associated with activation during cautious decisions
(i.e., cash-out trials) in the parietal cortex, a region implicated in decision-making and risk processing, and insular cortex, a region implicated in emotion regulation and risk prediction. These results suggest that risky sexual behavior in adolescents is associated with neural activation during risky decision making. To our knowledge, no research has yet examined the link between risky sexual behavior in adolescents and the brain. The implications of this study may help elucidate neurocognitive indicators for the development of risky
sexual behaviors. More knowledge of these factors could have high utility in informing interventions aimed at preventing and reducing teenage pregnancy and sexually transmitted diseases.

Disclosures: D. Goldenberg: None. **E.H. Telzer:** None. **A. Fuligni:** None. **A. Galvan:** None.

Poster

927. Novel Optical Methods

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Topic: G.04. Physiological Methods

Support: US Army Medical Research Acquisition W81XWH-08-2-0573

Title: Investigating a resting baseline for functional near infrared (fNIR) imaging

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Abstract: Neuroimaging studies often rely on a 'resting baseline condition'. However the definition of such a condition and its interpretation are still elusive. In this preliminary study, we utilize functional near infrared (fNIR) spectroscopy for the investigation of resting conditions. fNIR is a field deployable neuroimaging modality that enables the monitoring of changes in oxyhemoglobin and deoxyhemoglobin concentration in the prefrontal cortex. We investigated these biomarkers during resting baseline in fNIR spectroscopy measures.
While changes in oxyhemoglobin are affected by local blood flow, changes in deoxyhemoglobin levels mostly reflect local oxygen consumption, which should be minimal in absence of meaningful cognitive activation. Hence, we hypothesize that deoxyhemoglobin would overall provide a more stable resting state index than oxyhemoglobin. To test this hypothesis, fNIR signals were collected from three healthy subjects across 11 sessions each. Participants were asked to sit quietly with their eyes closed for 1 minute and with their eyes open for 1 additional minute, while staring at a computer screen. fNIR signals were collected from the subjects prefrontal area at two different wavelengths from 16 channels. Changes in oxy- and deoxyhemoglobin concentrations were computed using the modified Beer-Lambert law. Statistical analyses of the collected fNIR measures indicated that deoxyhemoglobin levels were more stable across the 11 sessions as compared to oxyhemoglobin levels, in agreement with our hypothesis.
Stability of deoxyhemoglobin values can be used as a parameter to validate resting baseline conditions in neuroimaging studies. Furthermore, future studies will be undertaken to investigate if a low cognitive load task, such as the Psychomotor Vigilance Task, could be used as a 'cognitive baseline condition' yielding stable readings not only in terms of deoxyhemoglobin, but also in oxyhemoglobin measures.

Disclosures: M. Cardin: None. **A. Rodriguez Merzagora:** Ownership Interest; fNIR Devices, LLC. **K. Izzetoglu:** Ownership Interest; fNIR Devices, LLC. **P.A. Shewokis:** None. **B. Onaral:** Ownership Interest; fNIR Devices, LLC. Consultant/Advisory Board; Coulter Foundation.

Poster

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Topic: G.04. Physiological Methods

Support: Keck Foundation

NIH Director's Pioneer Award

Stanford Bio-X Program

Title: Laser surgical preparation of awake fruit flies for fluorescence imaging of neural activity.

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Abstract: The fruit fly, Drosophila melanogaster, is a model organism that is used across multiple areas of neuroscience. Recent improvements in genetically encoded calcium indicators (GECIs) and fluorescence microscopy have facilitated in vivo imaging of neural dynamics in fruit flies and are well poised to help address how neural circuit dynamics underlie fly behavior. However, a main experimental limitation in the study of neural circuits is the low rate at which brain-imaging data is acquired, typically limited to a few animals per day. For fruit flies, manual dissection of the cuticle to create an optical window for brain imaging is a time-consuming process that requires considerable surgical skill and practice and introduces undesired variability between experiments. Further, removal of the cuticle with sharp forceps or scissors can cause physical trauma to the brain that compromises the fly's behavior.

To remove the fly cuticle in an automated fashion we developed a microsurgery system based on a nanosecond-pulsed 193-nm laser. We chose the wavelength and pulse duration to enable tissue removal with minimal heating of the fly or bleaching of the fluorescent GECI. Our computer-controlled system can cut optical windows in the cuticle 10-500 μm in width using ~1 s of laser illumination. Following microsurgery, we imaged various brain regions in the fly's olfactory and visual systems. We assessed the effects of the laser surgery on fly behavior by measuring post-surgical phototactic responses. Using a T-maze, we found that phototaxis was not significantly degraded for laser dosages of less than 90 pulses. We thus conducted all imaging experiments below this threshold.

The laser microsurgery protocol we developed provides more precise control of the size and depth of the surgical cut as compared to manual dissection methods. This enables us to minimize the surface area of the brain that is exposed to the environment without compromising the quality

of the measured neuronal calcium transients. To illustrate, we opened a 120 µm x 90 µm hole in the fly cuticle to image the Kenyon cells in the mushroom bodies. Using isoamyl acetate and geranyl acetate as stimulating odors, we recorded odor-evoked neuronal calcium transients. We observed such calcium transients with ΔF/F values exceeding 40% even 18 hours after surgery. This 18-hour imaging duration is apparently at least a two-fold increase over the longest duration for in vivo optical imaging previously reported for live flies. This extension of the experimental duration should help facilitate time-lapse imaging studies of individual flies, such as those intended to examine experience-dependent changes in neural dynamics.

Disclosures: S. Sinha: None. **L. Liang:** None. **L. Luo:** None. **T.M. Baer:** None. **M.J. Schnitzer:** None.

Poster

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The John B. Pierce Laboratory, Inc.

Title: Superior response kinetics of circularly permuted fluorescent proteins as reporters in CiVSP-based voltage sensors

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Abstract: Currently the most successful fluorescent protein-based voltage probes use the voltage sensing domain of the *Ciona intestinalis* voltage sensitive phosphatase (CiVSP). Recent advances in the calcium sensor field have shown that circularly permuted green fluorescent proteins (cpGFP) can produce robust signals. To determine whether such an approach could improve the CiVSP-based voltage sensors, we produced a matrix of different CiVSP/cpGFP fusion constructs (90) and tested these for cellular distribution and/or voltage sensitivity in HEK

cells and cultured hippocampal neurons. We found a direct relationship between a probe's responsiveness to voltage changes and the fluorescent protein's "hole" size and insertion site within the CiVSP. Our best probe found to date, ElectricPk exhibits a modest fluorescence signal change (~-1.2% ΔF/F for +100mV depolarization) but has remarkably fast on and off kinetics (~2ms). The probe exhibits very little of the slow response component which plagues other CiVSP-based probes. This rapid response speed facilitated action potential detection in cultured hippocampal neurons. The optical signal had a shape that was nearly identical to that of the voltage recording of the action potential. In addition, the probe exhibited an unusually linear ΔF/ΔV response profile to both depolarizing and hyperpolarizing voltage changes. These probes are the first CiVSP-based probes capable of accurately capturing the rapid movements of the voltage sensor and as such support the use of circularly permuted versions of fluorescent proteins in the design of voltage sensitive probes.

Disclosures: J. Platisa: None. **L. Barnett:** None. **M. Popovic:** None. **T. Hughes:** None. **V.A. Pieribone:** None.

Poster

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Support: NIH 2R01EB001464-06

Title: ScanImage, software for *in vivo* laser scanning microscopy

Authors: *V. IYER[1], S. PERON[1], G. M. G. SHEPHERD[2,1], K. SVOBODA[1]
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Abstract: *ScanImage* is freely available software (scanimage.org) for laser scanning microscopy, in particular two-photon laser scanning microscopy (TPLSM). Adoption of *ScanImage* is growing rapidly; it is now used by over 150 laboratories. Two versions of *ScanImage* are currently supported: *ScanImage* 3.8 allows galvanometer based scanning and *ScanImage* 4.1 is for resonant scanning hardware (Thorlabs). Galvanometer scanning is widely used and offers flexible scan modes, such as line and point scanning. Resonant scanning supports scan rates up to 16kHz – an order-of-magnitude speed-up compared to galvanometer scanning. *ScanImage* 3.8 and 4.1 have similar user interfaces and capabilities, but distinct codebases. *ScanImage* 3.8 replaces previous 3.x versions, adding 'cycle' mode capability for automation of

imaging sequences, new region-of-interest (ROI) scanning capabilities, and compatibility with a 64-bit operating system. *ScanImage* 4.1 improves several performance characteristics compared to its predecessor (version 4.0). Multiple samples are now averaged for each pixel, optimizing signal-to-noise throughout the field-of-view and for all pixel rates. Data buffering is improved, reducing the probability of losing image frames during continuous acquisition. *ScanImage* 4.1 supports higher frame rates (fewer lines-per-frame) and faster stack acquisitions, and adds frame-count tagging for each individual frame of acquired data logged to a TIF image file. *ScanImage* 4.1 has been optimized for volume imaging, using fast axial scanning based on piezoelectric actuators synchronized to resonant scanning. Both *ScanImage* 3.8 and 4.1 facilitate the binding of customized *user functions* to one or more *ScanImage*-defined events. This approach can be used for a wide range of user requirements, ranging from simple customization to creation of elaborate and powerful 'plugins'. Future development will be aimed at unifying versions 3.x and 4.x into a consolidated release capable of supporting multiple scanheads, e.g. both galvanometer and resonant scanning, either in parallel or in series.

Disclosures: V. Iyer: None. **S. Peron:** None. **G.M.G. Shepherd:** None. **K. Svoboda:** None.

Poster

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Topic: G.04. Physiological Methods

Support: NIH Grant R01-EB001954

NIH Grant P41-RR14075

Title: Simultaneous optical and electrical measurement of neurovascular coupling in awake rats

Authors: J. SUTIN[1,2], W. WU[1], L. RUVINSKAYA[1], *M. A. FRANCESCHINI[1]
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Abstract: Neurovascular coupling, the coordination of changes in metabolic demand from neuronal tissue with vascular regulation of localized blood perfusion, has previously been investigated by diffuse optical imaging and trans-cranial EEG in anesthetized rats. To overcome the profound impact of anesthetics on brain hemodyamics and electrical activity, we have developed an implantable electro-optical probe for longitudinal measurements in fully awake rats.

Our probe allows, in a minimally invasive format, simultaneous electrical measurement of neuronal activity by EEG and optical measurement of the hemodynamic response by diffuse optical spectroscopy. A survival surgery is performed to remove the superficial tissue above the rat skull, install EEG screw electrodes and anchor the implant to the bone. In this manner, cerebral hemodynamic measurements are made without interference from blood flow in the scalp. Furthermore, the skull is kept fully intact, minimizing perturbation to the brain and its vascular networks.
During measurement, a custom designed connector is attached to the implant, making electrical connections to the EEG electrodes and holding optical fibers in rigid contact with the skull. On each side of the skull, a single source fiber illuminates the brain with two wavelengths of near-infrared light. Four detector fibers per side allow for regional measurement of evoked changes of cortical oxy and de-oxy hemoglobin concentrations from changes in light transmission between source and detector. The optical fibers and EEG electrode are positioned for maximum sensitivity over SI. Whisker stimulation is performed electrically via chronic electrodes implanted subcutaneously in the whisker pad.
Rats are conditioned to remain restrained during measurements. Awake subjects enter a tubular body restraint and their heads are immobilized via the skull implant. Animals can be maintained in either fully awake state or various levels of anesthesia can be applied.
We have measured response to different frequency and intensity whisker stimulation on both awake and anesthetized rats. Preliminary results find a larger hemodynamic response in fully awake rats. In addition, higher intensity stimulation results in greater regional activation associated clear changes in the EEG signal. Experiments to systematically measure these effects in awake and anesthetized animals are currently underway.

Disclosures: J. Sutin: None. **W. Wu:** None. **L. Ruvinskaya:** None. **M.A. Franceschini:** None.

Poster

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Vanderbilt Vision Research Center

Vanderbilt University Center for Integrative & Cognitive Neuroscience

DOD/AFOSR F49620-01-1-4029, the Human Frontiers Science Program

Title: Functional tract tracing in non-human primates using pulsed infrared laser light with optical imaging and fMRI

Authors: *G. CHEN[1], J. M. CAYCE[2], R. M. FRIEDMAN[3], F. WANG[4], C. TANG[4], E. D. JANSEN[2], A. MAHADEVAN-JANSEN[2], J. C. GORE[4], A. W. ROE[3]
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Abstract: We have previously shown that pulsed infrared light can be used to excite neurons with high spatial resolution and low invasiveness in the somatosensory cortex of rodents. However, whether this method can be used effectively in the cortex of non-human primates is unknown. In this study, we evaluated pulsed infrared optical stimulation of cortex in squirrel monkeys and its potential for functional tract tracing. Stimulation of the visual cortex generated excitatory neuronal electrical responses as assessed with single unit recordings. Moreover, optical imaging of the cortical region during pulsed infrared stimulation revealed activation of nearby functionally related sites, indicating a novel way to study local intra-areal connectivity. We also investigated the use of optical stimulation in a functional magnetic resonance (fMRI) environment. In a high field (9.4T) scanner we recorded images of primary somatosensory cortex (S1) of squirrel monkeys during optical stimulation of a single digit location in Area 1. Optical stimulation was introduced via a fiberoptic and a newly developed long-term MR-compatible, optically transparent window. We found that optical stimulation evokes significant changes in cerebral blood volume (CBV) weighted MR signals. Such signals are known to correlate with neural activity. We found that the magnitude of the CBV changes and the activated region of cortex increased with radiant exposures. Furthermore, we observed that the light stimulation can not only directly drive the stimulated site, but also peripheral cortical activity. Stimulation of a single digit location in Area 1 resulted in focal, digit-specific activation in Areas 3b, 2, and possibly 3a. Such activations were confined primarily to the middle layers in these nearby areas. These data demonstrate that pulsed infrared laser excitation, used in conjunction with optical imaging or with fMRI, is a useful technique for functional tract tracing technique in vivo, which can reveal both local and global connectivity patterns.

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Poster

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Support: MNDA (UK)

Title: Live imaging and motor unit function during progressive neuromuscular synaptic degeneration

Authors: *R. BROWN, R. R. RIBCHESTER
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Abstract: Diagnosis of Amyotrophic Lateral Sclerosis (ALS) is currently informed by a combination of symptomatic presentation and interpretation of functional deficits, supported by electromyography (EMG). Recent development of new criteria for EMG analysis allows earlier diagnosis, and shows abnormalities in non-symptomatic areas. However, variation in onset times and detection of objective disease signs, for instance in animal models such as the SOD1 mouse model of ALS, still hinders accurate diagnosis and the monitoring of disease progression, or the efficacy of treatment. Therefore it is desirable to develop more direct ways of monitoring disease progression in vivo. EMG recording is also “blind” to the direct and progressive effects of disease on the morphology of motor units and their constituent neuromuscular junctions (NMJ). Direct imaging of motor units should therefore address some of these concerns. Using mice expressing fluorescent protein in their axons we have combined EMG recording via fine monopolar needles with fibre-optic confocal microendoscopy (CME), a method of live imaging of nerves and neuromuscular junctions. We use a Mauna Kea Technologies CellVizio system to image NMJ and an ADI Powerlab system to monitor the EMG. We have successfully monitored slow, progressive degeneration of motor nerve terminals and axons in transgenic thy1.2 YFP16 Wld^{s} mice, in which Wallerian degeneration is about ten times slower than in wild-type mice. We imaged the degeneration of axons and nerve terminals following unilateral sciatic nerve section up to two weeks in vivo, and recorded the EMG from the same vicinity as the motor endplates we imaged. This technique of visualising both structural and functional deficits will not only facilitate the study of animal models but conceivably allow earlier, reliable diagnosis

and monitoring of disease progression in ALS and other neuromuscular diseases, which would allow more rapid entry to and measurement of efficacy in clinical trials. Safe, high-contrast fluorochromes that selectively stain different components of NMJ will be required for full translation of this possibility to human subjects, however.

Disclosures: R. Brown: None. **R.R. Ribchester:** None.

Poster

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Topic: G.04. Physiological Methods

Title: Reliable detection of single action potentials and synaptic calcium signals using improved genetically-encoded calcium indicators

Authors: *T.-W. CHEN, J. YU, R. A. KERR, V. JAYARAMAN, L. L. LOOGER, K. SVOBODA, D. S. KIM
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Abstract: Genetically-encoded calcium indicators (GECIs) allow non-invasive measurement of neuronal activity in vivo. Currently available GECIs, such as the widely used GCaMP3, still suffer from limited sensitivity. Using structure-guided mutagenesis and high-throughput screening, we increased the fluorescence change in response to single action potentials (APs) by >10-fold compared to GCaMP3. We also accelerated the kinetics by ~2-fold. These new GECIs reliably report single APs in single trials in vivo with near 100% accuracy. In the mouse visual cortex, we detected ~5-fold more visually responsive neurons. The sensitivity, dynamic range and speed of the new GECIs exceed those of the synthetic indicator OGB-1. The improved sensitivity further facilitated reliable measurement of synaptic calcium signals in the dendrites of pyramidal cells and parvabumin (PV)-positive interneurons in vivo. Hot spots of orientation-selective domains can be resolved both in single pyramidal cell spines and small segments of PV cell dendrites. These improved GECIs will permit a more complete description of neuronal circuit function and enable long-term functional imaging of single synapses.

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Poster

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Support: Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency

Title: An implantable CMOS-based device with hexagonal shape for wide-area brain functional imaging

Authors: *M. HARUTA, T. KOBAYASHI, C. KITSUMOTO, T. NODA, K. SASAGAWA, T. TOKUDA, J. OHTA
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Abstract: To explore brain functions based on coordinative neural activities in different domains in a brain, there is a large demand for simultaneous multi-domain observation technique. Multi-domain fluorescence imaging will be a very powerful solution to understand inter-region coordinative activities. The authors have been developing techniques to observe neural activity using CMOS-based implantable imaging devices. These devices are advantageous in in vivo fluorescence imaging, especially under freely-moving situations. We implanted the imaging device in the mouse brain and demonstrated continuous imaging of neural activities. It means that our CMOS-based imaging system can be applied to behavior experiments. However, our previous sensors had small imaging area as 1-3 mm2, and thus not suitable to observing multiple domains in a brain in detail. In this study, we have developed a CMOS-based imaging system for a wider-area brain imaging that includes multiple brain functional imaging. We propose a new sensor design with multiple hexagonal CMOS-based imaging devices. The hexagonal shape is advantageous to configure a wider and multi-domain spherical imaging area on the brain. We integrated LEDs for illumination / excitation, and absorptive light filter for fluorescence imaging. We demonstrated fluorescence imaging using the multiple hexagonal CMOS-based imaging devices on the brain of the mouse. In the experiment, we injected fluorescence beads into the brain surface to prove the function of the device, and successfully observed the injected beads that were excited by LEDs mounted on the imaging devices. New imaging system is expected to be a powerful solution for wide-area, multi-domain brain imaging.

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Poster

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Title: Improved blood velocity measurements with a hybrid image filtering and iterative radon transform algorithm

Authors: *P. Y. CHHATBAR, P. O'HERRON, P. KARA
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Abstract: The determination of blood velocity changes in individual vessels is an important aspect of understanding neurovascular coupling. A variety of methods have been proposed to determine blood velocity in space-time image sequences, such as singular value decomposition (Kleinfeld *et al*, 1998, PNAS), radon transform (Drew *et al*, 2009, J Comput Neurosci), signal deconvolution (Kamoun *et al*, 2010, Nat Methods) and global energy minimization (Deneux *et al*, 2011, IEEE Trans Med Imaging). Of these, the radon transform has relatively easy implementation and fast data processing. However, the accuracy of the velocity measurements is dependent on the angle step-size used for the radon transform. This creates a trade-off between the speed and accuracy of velocity measurements at a particular image resolution. Moreover, factors like image contrast, imaging depth, movement artifacts and image acquisition speed can potentially lead to erroneous velocity measurements. Here we develop a robust algorithm with high accuracy and fast implementation that can address these hurdles towards accurate blood velocity measurements.
Two-photon imaging experiments were performed in anesthetized cats and rodents. The vasculature was visualized with circulating fluorescein dextran and arterial walls were selectively labeled with Alexa Fluor 633 Hydrazide (Shen *et al*, 2012, Nat Methods). Sensory-evoked changes in blood velocity were recorded using linescan acquisition routines, where moving red blood cells (RBCs) produced streaks in the recorded space-time images with angles proportional to the their velocity. These space-time images were processed with the hybrid algorithm as follows. An average smoothing filter was applied to suppress noise. Then, RBC streak margins were enhanced using a Sobel filter. Next, a series of iterative radon transforms was performed to find the angle value with maximum variance in the radon transform space. The first iteration used a large angle step-size spanning a full range of angle values. The reliability of the measured angle for the first iteration was assessed using the maximum-to-mean variance

ratio. Subsequent iterations used progressively smaller angle step-sizes with the range of angles centered on the angle that had the maximum variance in the previous iteration. The smallest angle step-size was determined by the image segment resolution and once this angle step-size was achieved, the iterative radon transform was terminated. Compared with previous methods, our hybrid algorithm is more sensitive to detecting changes at high blood velocities, provides superior angle precision and requires fewer radon transform cycles.

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Poster

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Topic: G.04. Physiological Methods

Title: Miniaturized wireless optical recording system for freely moving mice

Authors: *T. SUZUKI[1], A. KAMOSHIDA[1,2], C. HOMMA[1], K. YAMADA[1], M. MIZUNAKA[3], K. ARAI[3], M. E. LARKUM[4], A. MIYAWAKI[5], M. MURAYAMA[1]
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Abstract: Methods for monitoring neural activity from freely moving animals are vital for understanding a neural basis underlying animal behavior. For truely free behavior the recording system should not constrain the animal which is best achieved with wireless systems. While relatively common with electrical recordings, a wireless approach to optical recordings has become a priority, particularly given the recent explosion in optical methods. Here we describe a wireless system for recording fluorescence signals revealing intracellular calcium activity in freely moving mice. The system consists of two main devices: 1) an epifluorescence microscope with an LED, photo diode, excitation filter, dichroic mirror, emission filter and a gradient index (GRIN) lens (miniaturized with dimensions 10 x 10 x 7.75 mm and 2.0 gram). 2) A circuit board that divided into two parts because of stabilizing the center of balance: one with batteries, an amplifier, power supply circuitry and a light control IC with dimensions 20 x 22 mm. The other consists of a transmitter and a microcomputer with dimensions 20 x 20 mm. The total weight mounted on the back of the mouse is 7.7 g. For calcium recordings, the calcium-sensitive indicator (Oregon green 488 BAPTA-1 AM) was loaded into a glass pipette and pressure-injected into layer 2/3 of hindlimb area of the somatosensory cortex. The GRIN lens of the

epifluorescence microscope was positioned on surface of the injected site by referring to the fluorescence intensity, and then the microscope attached to skull with dental cement. The system can monitor population calcium activity from freely moving mice, and transmit data to a PC that also monitors mouse behavior with simple off-the-shelf ("web") cameras. If combined with transgenic animals expressing a genetically encoded calcium indicator, this system could also record the activity of specific cell types.

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Poster

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Swiss SystemsX.ch initiative (project 2008/2011-Neurochoice)

Title: 3D two-photon microscopy made simple: Rapid z-scanning with electrically tunable lenses

Authors: F. F. VOIGT, O. PFÄFFLI, M. VAN 'T HOFF, *F. HELMCHEN
Brain Res. Inst., Zurich, Switzerland

Abstract: Standard 3D microscopy methods rely on mechanical translation of the microscope objective, using either standard z-stages or piezoelectric actuators. Due to the inertia of the moving parts, such methods are not commonly employed for z-scanning rates exceeding 20 Hz. To allow faster imaging, especially for two-photon calcium imaging
of neuronal populations in vivo, optical focusing methods based on tunable optical elements are desirable. A simple and cost-effective way to achieve rapid z-scanning is the use of an electrically tunable lens (ETL) in the optical path. We will provide information on the best ways

to integrate an ETL (from Optotune) into two-photon microscopes with galvo-, resonant- or acousto-optical scanners. Depending on the performance requirements, implementations range from very simple (usually achieved by placing the ETL as close as possible to the scanners) to sophisticated setups (involving additional relay optics and optical feedback). ETLs can be used in a wide variety of imaging modes, ranging from the slow acquisition of z-stacks, essentially replacing piezo-electric actuators, to rapid (>20 Hz) multi-plane imaging or volume sweeps. Depending on the desired performance, care has to be taken to optimize the driving signal and to avoid drift effects. To this end, we have built an optical feedback module for ETLs (based on an astigmatic focus detector with a quadrant photodiode) which can be used in a two-photon microscope or as a stand-alone device. Using this system, we have characterized the dynamical properties of a range of tunable lenses, including next-generation ETLs. Such ETLs achieve step response times below 10 ms and resonance frequencies above 0.6 kHz.
Work is ongoing to combine ETL-based focusing with other microscopy methods.

Disclosures: F.F. Voigt: None. **O. Pfäffli:** None. **M. van 't Hoff:** None. **F. Helmchen:** None.

Poster

927. Novel Optical Methods

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:927.13/FFF82

Topic: G.04. Physiological Methods

Support: NIMH Grant 5R01MH083686-04

NSF Graduate Research Fellowship

Title: Optical instrumentation for simultaneous imaging and patterned photostimulation at cellular resolution in awake mice

Authors: *J. P. RICKGAUER, D. W. TANK
Princeton Univ., PRINCETON, NJ

Abstract: Recently developed methods allow optical imaging of calcium-dependent fluorescence transients at cellular resolution from populations of neurons in awake, behaving mice. For some studies, it could be valuable to complement these methods for optical detection of activity with technology additionally allowing optical perturbation of activity in patterns based on observed cellular response profiles. Here, we describe instrumentation that combines and controls two optical systems, one for high-resolution two-photon imaging and one for spatially localized two-photon stimulation. The instrument design is based on our biophysical findings

describing two-photon excitation (TPE) of channelrhodopsin-2 (Rickgauer & Tank, PNAS 106, 15025; 2009) and optical methods to generate spatially patterned TPE profiles using ultrashort pulse dispersion (Vaziri & Emiliani, Curr. Opin. Neurobiol. 22, 128; 2012).
The imaging system allows fast, high-resolution detection of green fluorescent indicator probes (e.g., GCaMP), and is designed to produce minimal collateral excitation of red-shifted opsins (e.g., C1V1). To achieve this, fsec excitation pulses from a source operating at 920+/-6nm are focused to a diffraction-limited spot in the sample plane. The spot can be scanned for either full-frame image acquisition or rapid random-access sampling using an XY scanner pair of large aperture acousto-optic deflectors pre-compensated for chromatic dispersion.
The photostimulation system is designed to allow rapid sequential excitation of red-shifted opsins at cellular resolution, and on a physiological timescale (msec), while producing minimal collateral excitation of fluorescence from green indicator probes. To achieve this, fsec excitation pulses from a second source operating at 1064nm+/-6nm are temporally focused to form a variable-diameter spot (5-20um) that can be adjusted to the projected dimension of a cell soma, and which has a thin axial TPE profile (FWHM=6um) in the perifocal region. This spot can be repositioned rapidly (>1kHz visitation rate) across the 200um square imaging field of view using galvanometer-driven mirrors.
The two optical systems are combined using a long-pass dichroic. Spatial registration and scanner synchronization are controlled using custom hardware and software written in LabView; during an experiment, visualized cells can be targeted for optical stimulation while imaging. The instrument employs a custom microscope head, toroidal projection screen, and sample stage that are designed to work with virtual reality-based behavior training in rodents (Harvey et al., Nature 461, 941; 2009).

Disclosures: J.P. Rickgauer: None. **D.W. Tank:** None.

Poster

927. Novel Optical Methods

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Topic: G.04. Physiological Methods

Support: AHA 11IRG5440002

NIH EB001954 R01

NIH R00NS067050

NIH EB00790 R01

NIH EB009118

NIH EB2066

NIH NS051188

Title: Investigating laminar specificity of neurovascular coupling using OCT angiography

Authors: *V. J. SRINIVASAN[1], A. DEVOR[2], H. RADHAKRISHNAN[3], D. A. BOAS[1], M. FRANCESCHINI[1]
[1]Martinos Ctr. / Dept of Radiology, Massachusetts Gen. Hosp. / Harvard Med. Sch., Charlestown, MA; [2]Dept. of Neurosciences, UCSD, La Jolla, CA; [3]Ctr. for Neural Engin., Pennsylvania State Univ., University Park, PA

Abstract: Depth-resolved Optical Coherence Tomography (OCT) angiography was used to investigate neurovascular coupling in the rat somatosensory cortex. In contrast to other optical microscopic imaging modalities, OCT affords sufficient penetration depth to image up to cortical layer V in the rat. We recently developed an angiography technique that enables simultaneous, depth-resolved imaging of hemodynamic changes. The goals of this study were (i) to establish theoretically and empirically that our OCT angiogram yields a depth-specific measurement of red blood cell (RBC) content under our experimental conditions, (ii) to investigate subtle timing differences in the RBC responses across cortical layers, and (iii) to characterize the RBC response across arteriolar, venular, and capillary compartments. Five Sprague-Dawley rats anesthetized with alpha-chloralose were used for these preliminary experiments. Imaging was performed through a closed cranial window during forepaw stimulation. The Spectral / Fourier domain OCT light source at 1310 nm consisted of two superluminescent diodes combined with a 50/50 fiber coupler, yielding an axial resolution of approximately 3.6 microns and a penetration depth of approximately 1 mm. Either a block stimulus paradigm (2 s or 4 s, 3 Hz) or parametric stimuli with varying stimulus frequencies (4 s, 1-5 Hz) were applied. For parametric stimuli, simultaneous surface potentials were recorded. Two-dimensional or three-dimensional scanning protocols were used to investigate laminar or compartment differences, respectively. Our data show that the hemodynamic response, as determined by RBC content, occurs earliest in layer IV, and is delayed in layers I-III and layer V, consistent with recent findings obtained using two-photon microscopy and BOLD fMRI. We observe responses (increases in RBC content) in all three vascular compartments, with the largest and earliest response found in the arterial compartment. Furthermore, our data shows virtually no evidence of “all or nothing” capillary recruitment, in agreement with earlier studies using confocal microscopy. We anticipate that these techniques we have developed will enable more detailed and comprehensive characterization of the neurovascular relationship across cortical layers than was previously possible.

Disclosures: V.J. Srinivasan: None. **A. Devor:** None. **H. Radhakrishnan:** None. **D.A. Boas:** None. **M. Franceschini:** None.

Poster

927. Novel Optical Methods

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:927.15/FFF84

Topic: G.04. Physiological Methods

Support: NIH Grant R01 DC012060

Title: Real-time intracellular thermodynamics and calcium imaging upon pulsed infrared laser excitation *in vitro*

Authors: *H. A. HOLMAN[1], M. FRERCK[2], R. RABBITT[2]
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Abstract: The use of pulsed infrared lasers (IR) to stimulate neurons and other excitatory cells is thought to be primarily through non-equilibrium thermal excitation of trans-membrane proteins and lipid bilayers. Here we describe a method for imaging IR evoked mitochondrial Ca^{2+} cycling and intracellular thermodynamics. To investigate the origins of IR-evoked intracellular Ca^{2+} transients we used genetically encoded Ca^{2+} indicators (CMV-mito-GEM-GECO and G-GECO1 plasmids) in human immortal HeLa cells *in vitro*. Results provide additional evidence that pulsed IR modulates mitochondrial Ca^{2+} cycling and that this is the major mechanism responsible for fast IR phase-locked action on synaptic transmission. Confocal fluorescence and time-lapse imaging was used to track mitochondria-localized free and bound Ca^{2+} transients, and cytoplasmic Ca^{2+} transients in response to IR. Pulses of IR were delivered at ~1862nm using 200µm diameter optical fibers,100-500µs pulse widths, at a rate 1-80pps (Capella, Lockheed Martin Acculight). Low duty cycle pulsed IR generates relatively small changes in temperature (T), but substantial changes in the rate of change dT/dt. Our results show that mitochondrial Ca^{2+} cycling is particularly sensitive and implicate the uniporter mCU and exchanger mNCX as likely candidates. Energy delivered to cells by pulsed IR modulates the state of the plasma membrane currents, intracellular signaling and thermodynamics.
Temperature fluctuations can modulate thermally sensitive ion channels, while IR-evoked rates of change in temperature (dT/dt) may underlie rapid changes in plasma membrane capacitance and modulation of synaptic transmission through non-equilibrium thermodynamics. Pulsed IR also evokes controllable intracellular Ca^{2+} transients across numerous excitable cell types that persist in zero extracellular Ca^{2+}. Recent data led to the hypothesis that pulsed IR stimuli trigger uptake and extrusion of Ca^{2+} by mitochondria and that intracellular Ca^{2+} signaling underlies IR-

evoked responses. This study examines further the Ca^{2+} dynamics and intracellular temperature changes from pulsed IR in real-time.

Disclosures: H.A. Holman: None. **M. Frerck:** None. **R. Rabbitt:** None.

Poster

927. Novel Optical Methods

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:927.16/GGG1

Topic: G.04. Physiological Methods

Title: Neurophotonics: Optically controlled guidance, manipulation and stimulation of neurons

Authors: *S. MOHANTY, L. GU, B. BLACK, K. DHAKAL
Univ. of Texas, Arlington, TX

Abstract: Though photonics technologies are being used for imaging structure and function of neurons at the cellular to animal levels, it has just begun to be used to remotely manipulate, and control neuronal circuitry and activity at cellular as well as whole animal level. While new methods are being discovered to direct neuronal growth by light, precise surgery by ultrafast lasers is enabling transfection and nano-surgery of neurons. Recently, optogenetic stimulation has allowed modulation of activities of specific neuronal circuitry, which has enabled potential intervention of neurological disorders. We will discuss recent developments and work carried out in my group on Neurophotonics. These include highly efficient optical guidance of axons, ultrafast laser-assisted delivery of genes into retina, new repair and migration path of regenerated axons after laser-induced injury, and use of non-linear optogenetics for precise stimulation of neurons. Use of some of these technologies in intervention of diseases such as retinitis pigmentosa and chronic pain will also be described.

Disclosures: S. Mohanty: None. **L. Gu:** None. **B. Black:** None. **K. Dhakal:** None.

Poster

927. Novel Optical Methods

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:927.17/GGG2

Topic: G.04. Physiological Methods

Support: NIH Grant 5R43HL092682-03

Title: An automated sleep detection and deprivation system for fruit flies

Authors: M. CHAN[1], S. GABBERT[2], H. HARMON[2], E. L. AKERS[2], J. E. ZIMMERMAN[1], A. I. PACK[1], *D. A. JOHNSON[2]
[1]Ctr. for Sleep and Circadian Neurobio., Univ. of Pennsylvania, Philadelphia, PA; [2]Pinnacle Technol, Inc., LAWRENCE, KS

Abstract: Sleep is a fundamental biological process. Insufficient sleep leads to significant, wide-reaching changes in physiology, mental acuity, physical performance and mood. Because the fruit fly (*Drosophila melanogaster*) has demonstrated conservation of pharmacological processes, neurological control and behavioral characteristics of vertebrate sleep, this model system is used to identify and investigate the genes underlying sleep behavior. We have developed a scalable, modular, and fully enclosed video-based system capable of measuring and selectively disrupting the sleep state of 160+ fruit flies in a standard commercial incubator. Video data is processed in real-time and stored locally. Experiment control and video preview along with access to x-y position, sensor information (light intensity, temperature and humidity) and real-time video feeds are available for all underway experiments via a master monitoring computer that can reside locally or remotely. Measurement of sleep bout length was similar between this system and that of a previously validated video measurement system, confirming the ability of our design to use video tracking for the purpose of detecting sleep bouts in flies. In addition to simple sleep state monitoring, the use of sleep deprivation has been shown to be a powerful technique for understanding the molecular mechanisms underlying sleep. Our system is capable of delivering real-time sleep deprivation stimuli to individual flies based on the currently observed sleep/wake state. Using this system, we measured changes in total sleep and average sleep bout duration for the 12 hours following application of individual sleep deprivation stimuli. We found a significant increase in total sleep in the 12 hours following the automated stimulus (SD) for flies as compared to a second control group which were not sleep deprived (C) ($p=0.005$, t-test). In addition, there was a significant increase ($p=0.010$, t-test) in the average duration of sleep bouts after stimulation of the SD flies compared to the C flies. The increase in sleep and the increase in average sleep bout duration are both strong behavioral indicators that the SD flies were undergoing recovery sleep, the normal homeostatic response to loss of sleep. This instrument represents the first, validated method for automatically measuring, recording and selectively depriving individual fruit flies using video-based monitoring and presents a useable solution for very large scale genetic screens requiring the simultaneous monitoring of several hundred flies.

Disclosures: M. Chan: None. **S. Gabbert:** Employment; Pinnacle Technology, Inc. **H. Harmon:** Employment; Pinnacle Technology, Inc. **E.L. Akers:** Employment; Pinnacle

Technology, Inc.. **J.E. Zimmerman:** None. **A.I. Pack:** None. **D.A. Johnson:** Employment; Pinnacle Technology. Ownership Interest; Pinnacle Technology.

Poster

927. Novel Optical Methods

Location: Hall F-J

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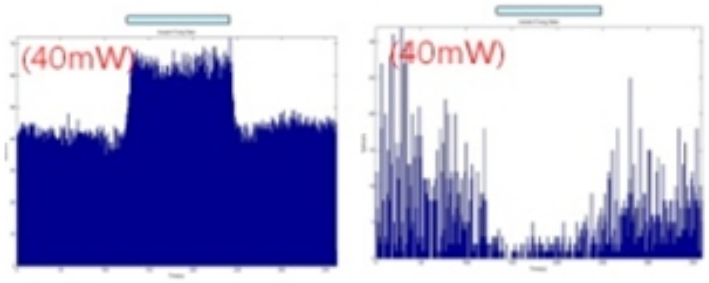
Program#/Poster#:927.18/GGG3

Topic: G.04. Physiological Methods

Title: Near infrared stimulation of globus pallidus and subthalamus

Authors: M. YOO[1], H. GOO[2], H. KIM[1], M. KIM[2], S. KIM[1], *H.-I. KIM[1]
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Abstract: Near infrared stimulation (NIRS) is an emerging technique to have the potential of replacing the conventional electrical stimulation method. The efficacy of NIRS in evoking action potentials has been demonstrated in different nervous tissues. However, few studies were performed to stimulate the globus pallidus (GP) and subthalamic nucleus (STN), frequent targets of deep brain stimulation for Parkinsonism.
Male Sprague-Dawley rats were randomly divided into three groups: GP stimulation (n=11), STN stimulation group (n=6), and sham-operated group (n=5). After introducing the optrodes sterotactically into GP or STN, we stimulated neural tissue for 2 minutes with continuous near infrared light of 808 nm varying the radiant exposure from 10 mW to 40 mW, and its effect was investigated with extracellular recording before, during, and after NIRS. Sham-operated group was not exposed to NIRS with implanted optrode. Recorded spikes were analyzed with MATLAB-based spike sorting program.
NIRS was found to elicit the excitatory response in 8/11 (73%) and inhibitory response in 3/11(27%) for GP stimulation group whereas NIRS predominantly evoke inhibitory response in 5/6 (83%) for STN stimulation group. Stimulation-response pattern was dependent on the intensity of radiation exposure. Only radiation above 30mW could elicit the statistically significant neural response ($p<0.05$).
It is concluded that NIRS can induce local neural response in excitatory or inhibitory pattern with more than 30 mW intensity. GP showed excitatory responses more frequently and STN predominantly showed the inhibitory responses.



Disclosures: M. Yoo: None. **H. Goo:** None. **H. Kim:** None. **M. Kim:** None. **S. Kim:** None. **H. Kim:** None.

Poster

927. Novel Optical Methods

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Topic: G.04. Physiological Methods

Support: NSF CAREER Award DBI-0953902

Kavli Institute

NIH Grant R01 EY018742)

Ludcke Foundation and Pierce Charitable Trust

Title: Chronic multiphoton microscopy of entire visual cortical columns in awake mice using microprisms

Authors: M. WOLFEL[1], G. GOLDEY[2], N. GILFOY[1], R. N. S. SACHDEV[3], D. A. MCCORMICK[3], C. REID[5], M. ANDERMANN[5], *M. J. LEVENE[1,4]
[1]Yale Univ., NEW HAVEN, CT; [2]Nothwestern Univ., Boston, MA; [3]Neurobio., [4]Neurol., Yale Sch. of Med., New Haven, CT; [5]Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Multiphoton microscopy of cortical brain activity has largely been restricted to superficial layers. Thus, conventional in vivo imaging techniques are not capable of monitoring activity of neurons across all cortical layers. Recent, advanced multiphoton methods have enabled imaging from as deep as layer 5, but only in sparsely labeled cortices and with relatively low resolution in the z axis. Further, these methods currently cannot provide simultaneous

recordings from neurons in superficial as well as in deeper layers.
We achieved simultaneous in vivo multiphoton microscopy of neuronal activity throughout all cortical layers using glass microprisms. A prism is inserted chronically into the neocortex such that it reflects a vertical image plane containing all 6 layers onto the horizontal image plane at the brain surface. The imaging face of the prisms used has side lengths of 1.0 mm or 1.5 mm, enough to span the entire neocortical depth. The calcium indicator dye GCaMP3 is expressed in various types of neurons, either using transgenic animals, or through GCaMP3 viral transduction. We successfully imaged neuronal activity in acute preparations, and in chronic preparations for several weeks after surgery. Simultaneous spontaneous and stimulus-evoked activity in hundreds of active neurons in layers 2/3, 5 and 6 could be recorded across days in primary visual cortex of awake, head-restrained mice.
Electrical recordings of neuronal spiking and local field potentials were obtained in order to evaluate the impact of the prism implant on electrophysiological measures of neural activity. Whisker-evoked responses could be recorded in barrel field somatosensory cortex in anesthetized, head-restrained mice within minutes after prism insertion, and for weeks thereafter. By combining Cre-dependent fluorescence expression with transgenic mice that express Cre in a cell type- or layer-specific manner, we can examine the response properties and correlated activity of identified neural populations across all layers of neocortex. We conclude that the simultaneous imaging of neuronal activity throughout the depth of the cortex is readily available through the use of microprisms.

Disclosures: M. Wolfel: None. **G. Goldey:** None. **N. Gilfoy:** None. **R.N.S. Sachdev:** None. **D.A. McCormick:** None. **C. Reid:** None. **M. Andermann:** None. **M.J. Levene:** None.

Poster

927. Novel Optical Methods

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Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:927.20/GGG5

Topic: G.04. Physiological Methods

Support: CIHR

Title: Wide-field multiphoton imaging of cellular dynamics in thick tissue by temporal focusing and patterned illumination.

Authors: *O. DUPONT-THERRIEN[1], B. AUBÉ[2], P. DE KONINCK[2], D. CÔTÉ[2]
[1]Ctr. De Recherche Univ. Laval Robert-Giffard (CRULRG), Univ. Laval, Quebec, Canada; [2]Ctr. De Recherche Univ. Laval Robert-Giffard (CRULRG), Univ. Laval, Quebec, QC, Canada

Abstract: To understand circuit function, strategies to monitor the activity of a large number of neurons at high speed in vivo are necessary. Optical methods based on beam scanning, such as two-photon microscopy, have the capacity to access neurons within surface layers of the brain, but are less suitable for high speed network imaging. Wide-field temporal focusing is a technique that provides optical sectioning for imaging without the need for beam scanning. However, illuminating over large areas greatly reduces the photon density which limits the technique applicability to small regions, precluding functional imaging of cellular networks. Here, we report the first use of temporal focusing combined with spatial light modulation to create a fast imaging modality for functional neuronal activity measurements. Our strategy combines beam shaping and temporal focusing of amplified pulses for fast imaging of cells from the central nervous system in acute slices. We show multiphoton video-rate imaging with an optical sectioning under 10 μm at 800 nm, leading to imaging of calcium dynamics of multiple cells simultaneously in brain slices. It is also shown that out-of-focus fluorescence background rejection by temporal focusing of amplified pulses leads to an improved signal-to-background ratio when monitoring the spontaneous variations in free calcium ion concentration in cells from neuronal acute slices. With beam shaping, addressing of individual neurons is achievable and makes it possible to combine the sensing of activity with optical stimulation for analysis of network communication. The combined fast imaging and optical sectioning capabilities of this approach open the way to also probe voltage fluctuations within neural networks in brain slices or in vivo over large regions simultaneously.

Disclosures: O. Dupont-Therrien: None. **B. Aubé:** None. **P. De Koninck:** None. **D. Côté:** None.

Poster

927. Novel Optical Methods

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Program#/Poster#:927.21/GGG6

Topic: G.04. Physiological Methods

Support: United States Department of Education P116Z080112

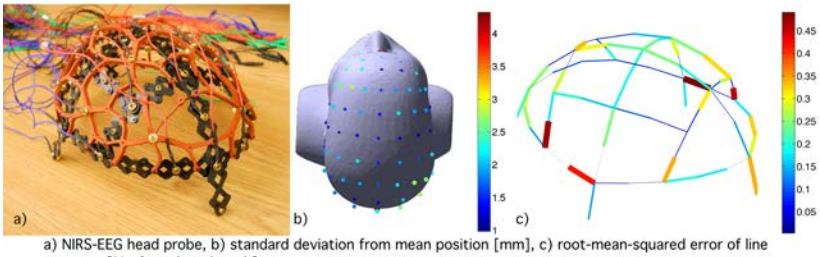
NIH National Institute of Aging R21AG033256

Rogue Research, LLC

Title: Head probe for combined near-infrared spectroscopy and electroencephalography

Authors: P. GIACOMETTI, *S. G. DIAMOND
Thayer Sch. of Engin., Dartmouth Col., Hanover, NH

Abstract: We present a new noninvasive head probe for concurrent measurement of neural and hemodynamic signals related to human brain function. This device is needed to support neuroimaging research that aims to advance our understanding of the neurovascular relationship. Multimodal research on the neurovascular coupling is important to fundamental neuroscience and biomedical research on neurovascular diseases.
This head probe combines near-infrared spectroscopy (NIRS) optodes and electroencephalography (EEG) electrodes into a single cap with whole-head coverage. It is designed with a low profile and assembled without ferromagnetic materials for compatibility with magnetic resonance imaging (MRI) and magnetoencephalography (MEG). The design accommodates normal head shape variations and a wide range of head sizes. Standard EEG 10-10 electrode positioning and 10-5 optode placement layout are used to facilitate clinical interpretation of results. Important evaluation criteria for the probe design are NIRS and EEG data quality, reduction of motion artifact, accuracy of electrode and optode positioning, electrical and optical coupling, ease of use, and durability.
The current prototype of the NIRS-EEG head probe has been successfully built and tested. It contains 65 electrodes and 64 optodes. Its design is based on an expandable linkage structure that follows the primary contours of the scalp. Electrodes serve as linkage joints to allow size expansion to fit the desired head size while maintaining equidistant spacing. Electrodes placed at the inion, nasion, preauricular, and central fiducial positions determine the location of all optodes and electrodes by mechanically following the 10-10 coordinate system and ensuring accuracy in the head probe positioning.
An electrode positional accuracy test was performed on 13 subjects to evaluate the head-surface positioning accuracy. Measurements were obtained for test-retest accuracy and with respect to the geometry of the 10-10 system. The probe had a mean positional error of 1.9 (±0.6mm) and an error with respect to 10-10 of under 0.5%.



a) NIRS-EEG head probe, b) standard deviation from mean position [mm], c) root-mean-squared error of line segment [% of total arc length]

Disclosures: P. Giacometti: Ownership Interest; Patent pending. **S.G. Diamond:** Ownership Interest; Patent pending.

Poster

928. Bioinformatics

Location: Hall F-J

Time: Wednesday, October 17, 2012, 1:00 PM - 5:00 PM

Program#/Poster#:928.01/GGG7

Topic: G.05. Bioinformatics

Support: 1 RC4 NS073008-01

Title: A framework for remote voxel-level access to distributed neuroimaging data

Authors: *B. N. NICHOLS[1,2], R. F. DOUGHERTY[4], L. T. DETWILER[1], G. SCHAEFER[4], R. J. FRANK[2], J. F. BRINKLEY[1,3], B. A. WANDELL[4], T. J. GRABOWSKI[2]
[1]Biomed. Informatics and Med. Educ., [2]Integrated Brain Imaging Ctr., [3]Biol. Structure, Univ. of Washington, Seattle, WA; [4]Ctr. for Cognitive and Neurobiological Imaging, Stanford Univ., Stanford, CA

Abstract: Scientific discovery about the human brain will be accelerated by neuroinformatics services on the programmable web. Just as bioinformatics databases provide services for molecular data, a scalable service-oriented framework is needed to take advantage of the large number of human neuroimaging data sets now available online. We have developed a specification, NIQuery, for remote access to observation-level data in distributed and heterogeneous image-specialized databases. NIQuery integrates emerging open source standards for metadata description, access & query, and provides investigators with computational access to remote voxel-level data.
The protocol supporting this functionality consists of: 1) a persistent Session object that wraps databases (e.g. XNAT[1], NIMS[2], Allen Institute[3]), exposes the NIQuery application programming interface, and serves objects and requests; 2) a Query object that provides a mechanism to interrogate databases with user defined and/or predefined web-accessible queries; 3) a Data object conforming to a supported 'image' data model (e.g., DICOM, NIfTI, etc.) that provides a mechanism to return pixel data to an application; and 4) a Workflow object through which a server provides a computational service on a Data object. A registry service (www.niquery.org) provides an index of available NIQuery servers, as well as the query, data, and workflow objects available on each server.
NIQuery enables client applications to discover shared neuroimaging data using metadata-level distributed queries and then execute image processing workflows on discovered data at their source, on a cached copy in the cloud, or locally. A sample implementation of this framework involves exporting a snapshot of XNAT and NIMS databases into XCEDE XML files, indexing the snapshots with the NIQuery registry service, and remotely calculating quality control metrics on resting-state fMRI data. These informatics tools will support agile exploration and reuse of open access neuroimaging data.
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3. Zeng H, et. al. 2012. Large-Scale Cellular-Resolution Gene Profiling in Human Neocortex Reveals Species-Specific Molecular Signatures. Cell.

Disclosures: B.N. Nichols: None. **R.F. Dougherty:** None. **L.T. Detwiler:** None. **G. Schaefer:** None. **R.J. Frank:** None. **J.F. Brinkley:** None. **B.A. Wandell:** None. **T.J. Grabowski:** None.

Poster

928. Bioinformatics

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Program#/Poster#:928.02/GGG8

Topic: G.05. Bioinformatics

Support: NIH Neuroscience Blueprint HHSN271200800035C via NIDA

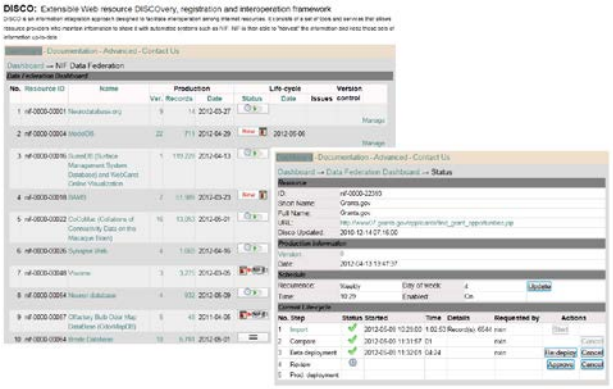
NIDCD Grant 5R01DC009977

Title: DISCO: Building a robust semi-automated data integration pipeline for the neuroscience information framework

Authors: *L. MARENCO[1], R. WANG[1], P. L. MILLER[1], G. M. SHEPHERD[2], L. LUI[3], A. BRANDOWSKI[3], J. S. GRETHE[3], A. GUPTA[4], M. E. MARTONE[3]
[2]Neurobio., [1]Yale Univ. Ssch MED, NEW HAVEN, CT; [3]Ctr. for Res. in Biol. Systems, UCSD, San Diego, CA; [4]SDSC, San Diego, CA

Abstract: In the past five years the Neuroscience Information Framework (NIF) has provided increasing data integration services to the Neuroscience community. NIF currently provides data integration at many levels, including information in databases from over 150 resources making NIF the largest collection of Neuroscience information on the web. In addition, NIF provides Entrez LinkOut broker services to more than 30 resources. This service allows these resources to have data links from NCBI and PubMed. To help in accomplishing these tasks, at the resource interoperation level, NIF utilizes and continues to enhance DISCO, an extensible framework for managing the integration of data from disparate Internet resources.
DISCO uses a proprietary format specification written in XML to define how and when the information is retrieved from each resource. DISCO uses this information to build a dynamically robust federated data warehouse environment. In addition, DISCO includes other components

within NIF that operate on this data to index, semantically map, interrelate and link this content to resources in the federation as well as external national resources such as NCBI. NIF makes available all resource information via several applications at the NIF portal (http://neuinfo.org).A key component of the DISCO implementation within NIF is a comprehensive management dashboard that allows curators to coordinate all portions of the data ingestion and integration process. We will show how this information pipeline is currently implemented in the



NIF.

Disclosures: L. Marenco: None. **R. Wang:** None. **P.L. Miller:** None. **G.M. Shepherd:** None. **L. Lui:** None. **A. Brandowski:** None. **J.S. Grethe:** None. **A. Gupta:** None. **M.E. Martone:** None.

Poster

928. Bioinformatics

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Support: EPSRC Grant EP/E002331/1

BBSRC Grant BB/I001042/1

HEFCE University Modernisation Fund: shared services pilot project

BBSRC Grant BB/H023569/1

Title: Services and workflows to share and analyse multielectrode array data on the CARMEN web platform

Authors: *C. D. INGRAM[1], E. SERNAGOR[1], M. WEEKS[2], M. JESSOP[2], J. AUSTIN[2], L. S. SMITH[3], T. JACKSON[2]

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Abstract: Developments in multielectrode arrays (MEAs) now permit simultaneous recording of up to several thousand channels of data. Sharing and analysing these rich data sets can provide considerable opportunity for discovering the properties of neural networks. CARMEN (www.carmen.org.uk) is a virtual (web-based) collaboration environment open to all neuroscientists for the analysis and management of electrophysiological data. A number of groups are using CARMEN to share MEA data derived both from established networks (eg retina) and from evolved networks (eg hippocampal cultures), and here we present some of features of the CARMEN platform that facilitate sharing and analysis of MEA data. A key function of CARMEN is the ability to undertake analysis using community-derived software, including algorithms to explore network properties derived from MEA data. CARMEN provides the infrastructure to allow software code to be deployed, via a lightweight wrapping process, as interactive services that run on the cloud resource. Working on files of up to 4096 channels, analysis routines deployed on CARMEN enable visualisation of activity patterns in a variety of formats, including movies of heat maps, and user groups have deployed a variety of bespoke analysis software. Whilst this software can be configured to run on proprietary data formats, we have developed a common format (NDF) and deployed a number of services in CARMEN to convert MEA files into NDF. By supporting users to configure their code to run on NDF, analysis routines can achieve universal format compatibility. NDF supports ‘n’ channels of recording with options to visualise and select specific channels for analysis. Undertaking analysis on CARMEN using NDF offers many benefits, including the efficient re-use of software applications, the ability to compare processing algorithms, and access to hardware resource for high intensity computing tasks. This has particular advantages when scaling up for analysis of MEA data, or when combining analysis services in an orchestrated processing pipeline, or workflow. To facilitate this multi-step analysis we have developed a workflow generation and execution system which satisfies the requirements relating to the CARMEN cloud execution model and the NDF data format. The CARMEN Workflow Tool is Java-based and supports both data and control flow, and allows parallel execution of services. The complete workflow tool consists of a graphical design tool, a workflow engine, and access to a library of CARMEN services and common tasks in analysing MEA data. We will present a number of analysis algorithms and workflows that can extract key features of network activity from MEA data.

Disclosures: C.D. Ingram: None. **E. Sernagor:** None. **M. Weeks:** None. **M. Jessop:** None. **J. Austin:** None. **L.S. Smith:** None. **T. Jackson:** None.

Poster

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Support: INCF

RR00166

Title: Making the NeuroNames brain structure ontology available in multiple formats

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Abstract: NeuroNames began twenty years ago as a standard nomenclature for classical structures of the primate brain. We have gradually expanded it with the goal that it become: 1) a comprehensive neuroanatomical nomenclature, 2) in multiple languages, 3) with detailed descriptions of relations among brain structures, 4) in the species most studied by neuroscientists. For ten years NeuroNames has provided the ontological structure for BrainInfo, a portal to neuroanatomy on the Web (http://braininfo.org).
The purpose of this project is to make the standard terminology and definitions of NeuroNames more accessible to operators of large databases who need a standard nomenclature and ontology for indexing and interoperability of massive neuroscientific data storage and retrieval systems. Important features of NeuroNames include:
--- A standard vocabulary of unique, commonly used, short, pronounceable English terms for some 3,000 structures found in the brains of humans, macaques, rats or mice.
--- Bottom-up definitions of most structures indicate their location relative to classical structures in brain atlases of the four species, the method by which each structure is defined (stain for Nissl substance, myelin, a neurotransmitter, a receptor, expression of a gene, etc.), and which of the four species possess it.
--- Top-down modelling (parent-child relations) of each structure in multiple hierarchical models, such as the NeuroNames' Classical Brain Hierarchy (structures grouped by proximity), a Functional Hierarchy (based on Swanson's grouping by function and connectivity as well as proximity), neurochemical hierarchy (e.g., grouped by neurotransmitter), etc.
--- 12,000 synonyms: English, French, German, Italian, Indonesian, Latin, Russian, and Spanish
The NeuroNames standard nomenclature is or will be available in:
--- Excel and XML at http://braininfo.rprc.washington.edu/Nnont.aspx
--- OWL at website of Neuroscience Information Framework (NIF)
--- UMLS as a source vocabulary of the Unified Medical Language System of the National Library of Medicine.

Disclosures: D.M. Bowden: None. **E. Song:** None.

Poster

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Olle Engkvist Foundation

Harald Jeansson Foundation

Segerfalk Foundation

Medical Faculty at Lund University

Title: A distributed light-weight database for the organization of electrophysiological data

Authors: *B. R. LJUNGQUIST[1], S. GERHARD[2], A. J. JOHANSSON[1], J. SCHOUENBORG[1], M. GARWICZ[1]
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Abstract: When sharing neural data, both electrophysiological and image data as well as other forms of data, there are some requirements which should be met: 1) Different acquisitions system have different data formats for offline and real time data, which needs to be accessed and processed in a common way by applications; 2) Data need to be stored in a database system directly in connection with the recording facility in order to facilitate data organization and sharing; 3) Data must be accessible for data-intensive applications. In this work, we present a distributed database system which allows for storing and analysis of electrophysiological data in real time simultaneously across multiple locations, addressing the problems identified above. The database is light-weight as it stores only the electrophysiological data itself, and may be complemented by databases of meta data, whose format is more likely to change from experiment to experiment and may not be easily standardized across labs. We also present a case study which shows how data both from simulations and experiments, the latter taken form

multiple types of acquisition systems, can reside in the same data base and be shared in real time, thus allowing for direct and parallel computational analysis of electrophysiological data.

Disclosures: B.R. Ljungquist: None. **S. Gerhard:** None. **A.J. Johansson:** None. **J. Schouenborg:** None. **M. Garwicz:** None.

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Topic: G.05. Bioinformatics

Title: Neuroinformatics database (NIDB): a portable database for storage, pipeline processing, and sharing of neuroimaging data

Authors: G. A. BOOK[1], *P. SKUDLARSKI[1,2], M. STEVENS[1,2], G. PEARLSON[1,2]
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Abstract: An OHBM article from 2001 described an estimated 1500 studies collected per year globally[1]. Now, a single MR scanner can easily be used to collect 1500 studies in a year. This capacity coupled with an increasing number of imaging centers creates a huge amount of neuroimaging data and presents a challenge to storing and sharing. Databases including xNAT, COINS, and Redcap are available to the neuroimaging community. xNAT can be hosted on site but requires a significant time investment to install and configure and as of version 1.4 does not have series level search or processing. COINS is comprehensive, but allows only remote access to neuroinformatics data and access is granted by invitation only. Redcap is IRB compliant, but does not store neuroimaging data. We propose to fill the gaps in existing databases by creating a data-up style system with these features: a) local hosting of data b) series level searching and processing c) ease of use/install d) automatic pipeline processing e) easy sharing of data.
Methods: NIDB defines the primary objects in the database as subjects, studies, and series. Subjects can belong to multiple projects and can have multiple studies, independent of project. Studies can have multiple series of a single modality. Each subject is randomly assigned a unique ID (S1234ABC). This string format allows 175 million unique IDs. A person is unique based on their name, DOB, and gender. DICOM data is parsed and stored in NIDB within 1m after data is uploaded from the MRI scanner, and QA parameters are calculated within 5m.
Results: The development site has collected 10,010 MRI studies, 1,041 EEG studies, and smaller numbers of other modalities in 9 years. An additional 2000 MR studies from other sites have been stored in NIDB. We currently host 118,000 MRI series, and 22,200,000 files. Average

search times for 20 series: 0.05s, 2000 series: 0.5s, and 15000 series: 1.5s. Import of DICOM data from the MR scanner requires no human intervention, while importation of PAR/REC and EEG requires some manual processing. An audit of the database found no orphaned data and 117 file count errors due to unique MR sequences.
Discussion: Our open-source database met the requirements set forth, with fast, granular access to data, allowing highly specialized automated pipeline processing of data. We felt NIDB filled the gaps left by other databases. Data from existing databases is easily imported into NIDB and the system provides simple search, statistics, and pipelining. Additionally, the structure of NIDB allows easy packaging of data for export to projects such as 1000 functional connectomes.
1. OHBM, Neuroimaging Databases, Science 292, 1673 (2001)

Disclosures: G.A. Book: None. **P. Skudlarski:** None. **M. Stevens:** None. **G. Pearlson:** None.

Poster

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Topic: G.05. Bioinformatics

Support: NIH R01 39600

ONR MURI 14101-0198

Title: Neuromorpho.org - What's next?

Authors: *R. PAREKH, K. SUBRAMANIAN, J. SAXENA, S. POLAVARAM, G. A. ASCOLI
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Abstract: NeuroMorpho.Org was introduced to the neuroscience community in 2006 as a collection just shy of 1,000 digital reconstructions of 3D neuron morphologies. Six years later, the repository now contains nearly 8,000 curated reconstructions, reflecting over 200,000 human tracing hours from 19 species, 89 cell types, 59 brain regions, and 86 laboratories worldwide. Millions of downloads of these shared digital reconstructions continue to result in new published discoveries from morphometric and stereological analyses, statistical quantification of potential synaptic connectivity, computational models of axonal and dendritic development, and biophysically realistic electrophysiological simulations of neuronal activity. The repository also maintains a literature database providing a comprehensive coverage of publications that report 3D digital reconstructions.

The steady increase in the number of reconstructions in the repository reflects a change in attitude of authors towards data sharing. Furthermore, advancements in high-throughput data acquisition and automated digital reconstructions will result in an unprecedented wealth of data. As a consequence, NeuroMorpho.Org is on a projected trajectory of including over 25,000 reconstructions by 2013. Reaching this critical mass will shift scientific investigations to the next phase of large-scale comparative studies across metadata categories. Combining metadata and morphometry searches may allow more effective identification of relevant data.
The expected data upsurge will also expand the database scope, requiring appropriate representation of multiple neurons within the same circuit and of time series. Both of these aspects, the relative spatial arrangement of different reconstructions and the temporal sequence of the same reconstruction, will dictate alterations of the database infrastructure, as well as distinct curation and annotation protocols.
This poster is intended as a platform to discuss, design, and stimulate feedback on future NeuroMorpho.Org directions with users (such as computational modelers), data contributors, software developers, and anyone interested in digital reconstruction of neuronal morphology. Community interaction has integrated NeuroMorpho.Org into a synergistic "ecological niche" around digital reconstructions that includes resources and tools for data acquisition, 3D visualization, morphometric analysis, and biophysical modeling. NeuroMorpho.Org plays an important role in the curation, annotation and standardization of reconstruction data and can contribute to establishing standards and norms for data description in the field.

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Poster

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Topic: G.05. Bioinformatics

Title: The zebrafish neurophenome project (znp) database: Developing use cases and terminology standardization

Authors: *E. J. KYZAR[1,2], J. CACHAT[2], S. GAIKWAD[2], J. GREEN[2], A. ROTH[2], C. COLLINS[2], A. STEWART[2], A. V. KALUEFF[2,3]
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Abstract: The Zebrafish Neurophenome Project (ZNP) database (ZNPdb) was developed to complement the available repositories for zebrafish genetic information by providing a dynamic open-access data repository for behavioral and related physiological data obtained from zebrafish experiments (Kyzar et. al. 2012). Currently, the ZNPdb represents the most comprehensive curated collection of results from zebrafish neurobehavioral experiments. As of May 2012, it contains over 4,500 experimental results, from over 75 unique physiological and behavioral tests and 330 different drug treatments, comprehensively covering published zebrafish literature. The ZNPdb is available online at http://www.kaluefflab.com/znpindex.html. The ZNPdb is actively growing based on the use cases and requests from the research community. In its current state, one of the most applicable use cases is determining if a drug has been previously tested in zebrafish models, and what doses (if any) were effective. As with any novel animal model, determining this can be a painstaking process, requiring large-scale meta-analysis of published literature. However, the ZNPdb can provide insight to the drug/dose question rather quickly and easily. From the homepage, the drug or treatment of interest can be selected from the Treatment dropdown menu, and searched. This will return all entries within ZNPdb containing that treatment, along with the administration procedure, dose, and reported effects (both significant and non-significant) in respective experimental designs. Additionally, through the continued addition of zebrafish experiments to ZNPdb, we have gathered a diverse, but comprehensive lexicon of the terminology used to describe zebrafish behaviors. Within this lexicon, we have observed that the same behavior or behavioral endpoint is often labeled with similar, but variable terminologies. For example, the amount of time spent in a particular region of a testing arena could be labeled ‘time spent in X’, ‘duration in X’, ‘time in X’, ‘preference (time spent) for X’, ‘X duration’ etc, all of these referring to the same basic measurement. This observation suggests that a standardized, zebrafish behavioral ontology would be useful for the field. Given the fact that ZNPdb has already collected most lexical variations, our lab has recently initiated efforts to create zebrafish behavior ontology, the Zebrafish Behavioral Phenotype Ontology (ZBPO). Upon completion, ZBPO will be made open-source and available to the community (via ZIRC and NCBO) for future research and integration with larger biomedical ontologies.

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Poster

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Support: NIH Neuroscience Blueprint HHSN271200800035C via NIDA

Title: A linked open data resource for the neurosciences: The Neuroscience Information Framework's (NIF) unified semantic framework and associated tools for integration and utilization of biomedical data and resources

Authors: *J. S. GRETHE[1], A. BANDROWSKI[1], J. CACHAT[1], A. GUPTA[2], F. IMAM[1], S. LARSON[1], L. MARENCO[3], P. MILLER[3], G. SHEPHERD[3], M. MARTONE[1], N. I. F. CONSORTIUM[1]
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Abstract: Neuroscience, as with other biological sciences, has become a data intensive science where new insights and discoveries are critically dependent on researchers ability to integrate and synthesize related biological data. Neuroscience is perhaps the most information-rich of all biological sciences. The Neuroscience Information Framework (NIF; http://neuinfo.org) was launched in 2008 to address the problem of finding and integrating neuroscience-relevant resources through the establishment of a semantically enhanced framework. To enable researchers to gain access to data and information they may not have been aware of, the NIF has deployed a discovery portal that connects neuroscientists and biomedical researchers to available resources. These sources include the: (1) NIF Registry: A human-curated registry of neuroscience-relevant resources; (2) NIF Literature: A full text indexed corpus derived from the PubMed Open Access subset as well as an entire index of PubMed; (3) NIF Database Federation: A federation of independent databases that enables discovery and access to public research data, contained in databases and structured web resources (e.g. queryable web services) that are sometimes referred to as the deep or hidden web. Over the past year, NIF has continued to grow significantly in content, providing access to over 4800 resources through the Registry, and more than 160 independent databases in the data federation, making NIF the largest source of neuroscience information on the web.
To further enable the utilization of this vast collection of information, the NIF is applying semantic web technologies to its holdings. By defining a set of standards and best practices for describing and representing data, semantic web and linked data technologies eliminate the barriers between database silos and foster the evolution of the Web into a Web of data. Such technologies are being successfully applied as integration engines for linking data and information in many domains. The publication of this content relies on a coordinated suite of tools and infrastructure that manage the integration of data from disparate Internet resources. In addition, the semantic underpinning for this linked data is NIF's comprehensive ontology (NIFSTD) that covers major domains in neuroscience, including diseases, brain anatomy, cell types, subcellular anatomy, small molecules, techniques and resource descriptors. Exposing NIF's content as Linked Open Data will enable further integration with the growing amount of information available from the linked open data cloud - thereby providing a much richer resource for the neuroscientist.

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Poster

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Support: INCF

Title: Creating INCF digital atlas hubs through registration workflows

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Abstract: The International Neuroinformatics Coordinating Facility (INCF, http://incf.org/) Digital Atlasing Program is working towards a digital atlasing framework that will make multidimensional data of the rodent brain more widely accessible and usable to the research community (Hawrylycz et al, 2011, *PLoS Comput Biol* 7[2]: e1001065. doi:10.1371/journal.pcbi.1001065). The framework requires standard spatial reference systems (principally Waxholm Space, WHS) and a supporting data sharing infrastructure (Digital Atlasing Infrastructure, DAI); each of which is the main focus of an Atlasing Task Force. User data are primarily shared through atlas hubs, specialized servers available as part of the infrastructure or that may be installed and managed locally. Hubs register users' data to a spatial reference system, publish the data and services with INCF Central and share data via web services. At this time, the focus of both groups is on integrating image registration workflows to the underlying infrastructure with the aid of metadata, provenance, and ontology standards. Data sharing use cases drive our workflow development. At this time, we are focusing on 2D

brain slice images (sampling varies from sparse to densely sampled). The goal is to create tools, recommendations, and standard operating procedures to aid in the registration of data to a known standard atlas space and creation of new atlas hubs. With workflows that allow the creation of atlas hubs, new datasets will be able to be queried and compared to data in other atlas hubs, such as EMAGE and Allen Brain Atlas.
The two task forces are working on multiple related project areas, including:
•Production of registration fiducials and landmarks
•Standards for registration transformations
•Data management and handling
•Metadata
•Provenance
•Atlas hub in a Box
•WHS for the rat
•PONS pan mammalian delineations of WHS dataset
These areas are either needed to integrate other components with the INCF DAI, or are integrated with the infrastructure as they are developed.
This program welcomes input from the community, and requests expert recommendations in these project areas. Please contact any author for more information.
This work was conducted within the WHS and DAI Task Forces of the INCF Program on Digital Brain Atlasing.

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Poster

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NIH 5R21NS062254-02

Title: Resting-state fMRI determination of optimal target locations for non-invasive brain stimulation

Authors: *F. S. SALINAS, S. NARAYANA, L. JIMENEZ-CASTRO, P. T. FOX
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Abstract: Resting-state fMRI (rs-fMRI) covariance patterns reflect inter-regional functional connectivity (e.g. resting-state networks (RSNs)). Applying independent components analysis (ICA) to these datasets provides a set of archetypal independent components (ICs), which are similar in appearance to both resting-state and task-dependent data. We, therefore, propose the use of rs-fMRI to determine the optimum site of stimulation for non-invasive brain stimulation protocols_i.e. repetitive transcranial magnetic stimulation (rTMS). We demonstrate this approach in the dorsolateral prefrontal cortex (DLPFC), a brain area connected to subgenual cingulate (SGC)_with both locations being implicated in the neural circuitry of depression via the default mode network (DMN).
rs-fMRI was acquired in human (N=20) and non-human primates (NHP; N=11) using previously developed rs-fMRI acquisition protocols. The rs-fMRI group datasets were (separately) pre-processed and analyzed with the FMRIB's FSL toolkit. ICA was performed on each group's rs-fMRI dataset using FSL's MELODIC program with multi-subject temporal concatenation (#ICs=20). The SGC nodes identified from the group ICAs were then used for region-seeded analysis (RSA) with regression analysis performed using the general linear model in FSL's FEAT program, resulting in the optimized DLPFC target site for SGC connectivity. We then applied suprathreshold rTMS (at 10 Hz) to each group's DLPFC site during positron emission tomography (PET) to demonstrate successful activation of the SGC. All procedures were performed with institutional approval for both the human and animal studies.
We successfully localized the DLPFC node connected to the SGC in both human and NHP groups using the ICA/RSA approach and delivered rTMS to the optimized target site (in human and NHP experiments), producing remote network modulations in the SGC_i.e. non-invasive, efficient delivery of a depression therapy. The rs-fMRI targeting approach has great potential since task-dependent targets are difficult to obtain in animal models and patient groups.

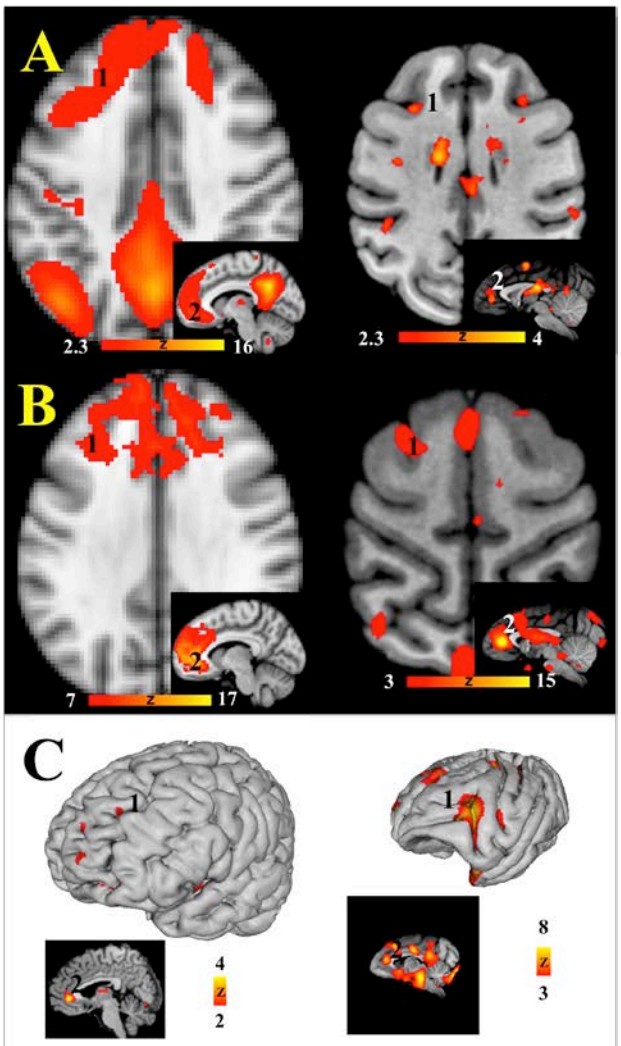


Figure 1. ICA/RSA Analyses and rTMS/PET application to DLPFC. **A.** The default mode network (DMN) IC is shown for the group ICA in humans (left) and baboons (right). **B.** Region-seeded connectivity from SGC (label #2) to DLPFC (label #1) for a single human (left) and a single baboon (right). **C.** TMS/PET brain activations from targeting the DLPFC node of the DMN for humans (left) and baboons (right). Each image shows the DLPFC target location (#1) and the SGC node (#2).

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Poster

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Title: Model-free reconstruction of excitatory neuronal connectivity from calcium imaging signals

Authors: *O. STETTER[1,2,3], D. BATTAGLIA[1,3], J. G. ORLANDI[4], J. SORIANO[4], T. GEISEL[1,2,3]
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Abstract: A systematic assessment of global neural network connectivity through direct electrophysiological assays has remained technically infeasible, even in dissociated neuronal cultures. We introduce an improved algorithmic approach based on Transfer Entropy to reconstruct approximations to structural connectivity from network activity monitored through calcium imaging. We focus in this study, as a first step, on the inference of excitatory synaptic links. Based on information theory, our method requires no prior assumptions on the statistics of neuronal firing and neuronal connections.
The performance of our algorithm is benchmarked on surrogate time series of calcium fluorescence generated by the simulated dynamics of a network with known ground truth topology. We find that the functional network topology revealed by Transfer Entropy depends qualitatively on the time-dependent dynamic state of the network (bursting or non-bursting). Thus by conditioning with respect to the global mean activity, we improve the performance of our method. This allows us to focus the analysis to specific dynamical regimes of the network in which the inferred functional connectivity is shaped by the underlying excitatory connections, rather than by collective synchrony.
Our method can discriminate between actual causal influences between neurons and spurious non-causal correlations due to light scattering artifacts, which inherently affect the quality of fluorescence imaging. Compared to other reconstruction strategies such as cross-correlation or Granger Causality methods, our method based on improved Transfer Entropy is remarkably more accurate. In particular, it provides a good reconstruction of the excitatory network clustering coefficient, allowing for discrimination between weakly and strongly clustered topologies.
Finally, we present the applicability of our method to real recordings of in vitro cortical cultures.

We demonstrate that excitatory connections in these networks are characterized by an elevated level of clustering compared to a random graph (although not extreme) and can be markedly non-local.

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Support: NIH/Neuroscience Blueprint contract HHSN271200800035C

Title: Integrating brain connectivity data from multiple sources with the nif platform

Authors: *A. GUPTA, A. E. BRANDOWSKI, S. LARSON, A. GUPTA, V. ASTAKHOV, C. CONDIT, X. QIAN, M. E. MARTONE
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Abstract: Brain connectivity refers to a pattern of anatomical links ("anatomical connectivity"), of statistical dependencies ("functional connectivity") or of causal interactions ("effective connectivity") between distinct units within distinct brain regions. Brain connectivity can be studied at multiple spatial scales; individual synaptic connections that link individual neurons, networks connecting neuronal populations, brain regions linked by fiber pathways - are all valid ways of studying the local and global properties of the connectivity network. Over the last decade, significant research effort has been devoted to the construction, analysis and comparative studies of the graph-theoretic properties binary directed anatomical network, the weighted undirected functional network and the weighted directed effective network. However, no generic, publicly accessible platform has been developed so far for integrating and analyzing connectivity information. The Neuroscience Information Framework (NIF), with its ability to store large, heterogeneous sets of connectivity information, provides the first platform of this nature. In addition to storage and semantic search abilities, the NIF platform now provides an analysis layer that combines NIF's software with 3rd party tools to perform different kinds of analysis. To demonstrate this, we have performed connectivity analysis by integrating mammalian brain connectivity data sets from partonomic relationships in the Neurolex semantic data store, the BrainMaps Atlas, the Temporal Lobe Atlas, the CoCoMac Project, ABCD for avian brains, BAMS and the functional data from the UCLA Multimodal Connectivity Database. The system

reconciles the variation of nomenclature across these data sets by mapping brain region names into the NIFSTD ontology. The user of the system can create different views of the network by selecting metadata properties like organism and technique, the spatial scale of brain structures based on the ontological hierarchy of anatomical structures, network node properties like betweenness and closeness centrality, and network edge properties such as strength of connection and edge-betweenness properties. The network features like measures of resilience and modularity are precomputed every time the system gets updated with new data sets, thus enabling a user to explore any part of the network by features through a simple query interface created for exploring connectivity information. However, users may also compute these functions on the fly using the feature library built from the Brain Connectivity Toolbox, and estimate the statistical bias of the analyses.

Disclosures: A. Gupta: None. **A.E. Brandowski:** None. **S. Larson:** None. **A. Gupta:** None. **V. Astakhov:** None. **C. Condit:** None. **X. Qian:** None. **M.E. Martone:** None.

Poster

928. Bioinformatics

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Program#/Poster#:928.14/GGG20

Topic: G.05. Bioinformatics

Support: HHSN271200900425P

Title: AntibodyRegistry.org & Domeo: A toolset for robust identification and linking of specific antibody catalog information to the scientific literature.

Authors: P. N. CICCARESE[1,2], *T. W. CLARK[1,2,3], A. BANDROWSKI[4], V. ASTAKHOV[4], J. S. GRETHE[4], M. MARTONE[4]
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Abstract: Repeatability of experiments is undermined by the lack of pointers to appropriate current, and specific, descriptive information about reagents such as antibodies, in published literature. The Neuroscience Information Framework (NIF; http://neuinfo.org) and the MIND Informatics group at Massachusetts General Hospital have joined forces to build a set of tools that can be used to address this pressing need.
The NIF Antibody Registry (AR, http://antibodyregistry.org/) is a database of over 890,000 individual antibody records, covering almost 80% of the commercial antibodies used in Journal

of Comparative Neurology articles over the last 5 years. AR links these published articles with the related reagent-specific descriptors, including vendor catalog identifiers. The AR is searchable by the general NIF search engine, accessed from a Web browser or through a web service API. Individual records can be added and edited by members through a public browser interface.
Identification and curation of antibody information from research publications poses several challenges. To meet them, NIF is working to implement a textmining-assisted (semi-automatic) curation process. When catalog numbers are listed in articles and those catalog numbers match the AR, text-mining software would detect and record the match, aligned with its location in the article's text. Curators could then evaluate the results and confirm or reject the entries. When catalog numbers are not present, information such as the clone number and the supplier can help the curator to select one or more possible registry candidates.
To efficiently enable this curation process, Domeo (http://annotationframework.org) a web application for producing and managing annotation of any online documents and document fragments, has been extended to interact with the AR. A software plug-in dedicated to antibodies was created to allow visualized, in-context annotation of the document text with an antibody match from the AR. The curator specifies which methods have been applied, on what species. A free-text comment may be attached to the annotation item. The resulting annotation can be exported in RDF format according to the Annotation Ontology [1] model, and augmenting the information natively provided by the NIF framework. As Domeo integrates with text mining services, we now aim to leverage software for recognizing catalog numbers, display the results on the document and collect curator feedback enabling the literature to be robustly linked to the reagents used therein.
1. Ciccarese P, Ocana M, Garcia Castro LJ, Das S, Clark T: An open annotation ontology for science on web 3.0. J Biomed Semantics 2011, 2 Suppl 2:S4.

Disclosures: P.N. Ciccarese: None. **T.W. Clark:** None. **A. Bandrowski:** None. **V. Astakhov:** None. **J.S. Grethe:** None. **M. Martone:** None.

Poster

928. Bioinformatics

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Program#/Poster#:928.15/GGG21

Topic: G.05. Bioinformatics

Title: Behavior Informatics: Integration, sharing and mining of behavioral data, models and tools

Authors: *A. R. KOENE
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Abstract: Various Neuroinformatics platforms have in recent years been established to facilitate a more coherent and integrated understanding of the brain (e.g. INCF, NIF). The primary focus of most of these efforts has been a bottom-up approach focusing on neuroanatomy (e.g. Allen Brain Atlas) and high-fidelity modeling of neural ensembles (e.g. Blue Brain project). To complement these Neuroinformatics efforts we propose a Behavior Informatics platform that will focus on the accumulation and sharing of behavioral data, and related analysis tools.
Behavior Informatics will integrate behavioral information across multiple scales and disciplines to establish a clear ontology of behaviors and discover new links between behaviors at micro and macro scales.
The pillars upon which the Behavior Informatics platform will be constructed are:
1. databases of behavior observations from field/real-world observation, laboratory studies and Virtual World behavior logs (information gathering from behavior in social platforms (e.g. Second-Life) and games (MMOGs));
2. literature mining, i.e. identification of relationships between published literature on the basis of commonalities in described behavior, experimental design, analysis method or model type;
3. analysis methods for: behavior classification, identifying spatio-temporal relationships between behaviors and describing their dynamic evolution during development and execution;
4. databases of models relating to behavior initiation/selection/execution.
To connect these pillars and establish a solid platform upon which to build an integrated understanding of behavior, we propose to:
1. support development of a standardized comprehensive ontology of behavior;
2. maintain a wiki for sharing of experimental methods;
3. support/develop standards for describing experiments and observed behaviors;
4. develop improved methods for visualizing multi-scale behavioral data.
Projected benefits and primary uses of Behavior Informatics:
1. establishing new links between data from different sources, scales and disciplines;
2. identify the most important gaps in our knowledge;
3. multi-scale integration of behavior models, e.g. integration of agent based and population based behavior models, validated using rich virtual world behavior logs.
Here we present the tentative road-map for the creation of a Behavior Informatics platform.

Disclosures: A.R. Koene: None.

Poster

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Topic: G.05. Bioinformatics

Support: NIH Blueprint for Neuroscience Research with Federal funds from the NationalInstitute on Drug Abuse, National Institutes of Health, Department of Health and Human Services: Contract Number HHSN271200577531C

Title: Fusion of heterogeneous neuroscience data; a method that combines a myriad of web services and the Kepler workflow engine

Authors: *A. E. BANDROWSKI[1], V. ASTAKHOV[2], A. GUPTA[3], J. GRETHE[2], M. E. MARTONE[2]
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Abstract: We report on progress of employing the Kepler workflow engine and service oriented architecture (SOA) to prototype application integration workflows that integrate data and web services developed by the Neuroscience Information Framework (NIF; http://neuinfo.org). One prerequisite of the scientific enterprise consists of searching for effective and useful data and resources, i.e., reagents, neuro-anatomy features, genes, or proteins. Finding relevant resources is becoming not a challenge of scarcity, but one of overabundance; in fact relevant data can be found anywhere among thousands of neuroscience-relevant information resources created by a range of information providers including, research groups, funding agencies, vendor groups, and public data initiatives.
NIF provides a graphical user interface, GUI, to locate and access ontologically aligned and semantically fused heterogeneous federated information. NIF also atomized the various functions that serve the user interface and put them out as services that can be used like “Lego blocks” to query the data, build entirely new interfaces or tools. Currently, we use Kepler to orchestrate communication among various NIF services and provide a transparent layer for data fusion. Kepler combines data and processes into a configurable, structured set of steps that helps to implement semi-automated workflows. Kepler provides a development environment with a graphical user interface for designing workflows composed of a linked set of components called Actors, which can be executed under different Models of Computation. In this work, we report on specific workflows that perform data fusion and orchestration of diverse web services. This “Brain data flow” outputs categorized counts of information from 150 data sources about brain regions. Obtaining a similar set of data from the NIF GUI, requires manually writing down result counts that are the result values for each database for each query. Kepler, unencumbered by the current configuration of the user interface can be asked to pull a different set of data from the result set, in this case the number of results, and place that into a table. This table can then be easily turned into a graphic that helps users see which databases are information rich given a particular query. In this example, Kepler loops and recovers the same set of information for all of

the brain parts and all databases, producing a massive matrix (http://www.neuinfo.org/NIF_Federated_Data_Heatmap.html).

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Poster

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the State of Arizona

Banner Alzheimer's Foundation

Title: Prediction of mci progression to ad based on regional hypometabolic convergence index: A fdg-pet study for Alzheimer’s disease neuroimage initiative (adni) project

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Abstract: **Background:** We previously developed a Hypometabolic Convergence Index (HCI) (Chen *et al.* 2011) reflecting the extent to which the pattern and magnitude of cerebral hypometabolism in an individual's fluorodeoxyglucose positron emission tomography (FDG-PET) image corresponds to that in dementia due to Alzheimer's disease (dAD) over the entire brain volume. As magnitudes at which different brain regions are preferentially affected vary at different stages of AD, this study introduces regional HCI (RHCI), a region-of-interest based HCI to better capture such pattern and magnitude variability among various brain regions and examines its use in identifying MCI patients who are close to progression to dAD in comparison with the original HCI, MetaROI means (Landau *et al.* 2009) and Magnetic Resonance Imaging (MRI) hippocampal volume.

Methods: RHCIs for frontal, posterior cingulate/precuneus, lateral temporal, lateral parietal cortex and a combination of some of these as mean cortical were calculated for baseline FDG-PET data from 207 MCI patients enrolled in ADNI. 42 participants subsequently progressed to dAD within 24 months from baseline. RHCIs, the original global HCI and MetaROIs from left and right angular, posterior cingulate, left and right temporal, composite ROI and MRI hippocampal volume were entered into stepwise Cox proportional hazard model for prediction comparison.

Results: Using RHCI predictors, MCI patients with higher RHCIs in both mean cortical and posterior cingulate/precuneus cortex are 6.56 times (model R^2=0.45) more likely to progress to AD within 24 month, compared to 4.59 times (model R^2=0.39), 5.51 times (model R^2=0.38) or 2.73 times (model R^2=0.22) for those with either higher global HCI, higher MetaROI means in both left angular and posterior cingulate cortex or lower hippocampal volume, respectively. MCI patients who have higher mean cortical RHCI (p=2.7e-5), lower metaROI means in left angular (p=0.01) and posterior cingulate (p=0.008) cortex and hippocampal volume (p=0.004) are at even higher risk, 56.06 times (model R^2=0.69) more likely, to progress to dAD within 24 month than those who do not.

Conclusion: RHCIs, alone or in combination with other biomarkers, may help predict the subsequent rate of decline in MCI patients. The results may depend on the subject cohorts, progression time, relationship of predictors analyzed, and prediction algorithm implemented.

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Poster

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Topic: G.05. Bioinformatics

Support: NSF Grant DBI-1146949

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Title: A neuroscience gateway for high performance computing

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Abstract: The goal of this project is to build a Neuroscience Gateway (NSG) that will reduce administrative and technical barriers to the use of High Performance Computing (HPC) resources by neuroscientists. Similar gateways have been successfully developed for other fields such as nanotechnology, phylogenetics, bioinformatics, chemistry, weather prediction etc., and allow efficient access to HPC resources by a large number of users.

The need for an NSG emerges from the tremendous expansion of computational neuroscience in recent years, as attested by the growing numbers of articles in leading journals that report work involving computational modeling. This has motivated development of domain-specific software for parallel simulation, e.g. NEURON, GENESIS3, MOOSE, NEST, and PyNN, which in turn has enabled investigators to address increasingly complex problems such as large-scale network models and optimization or exploration of high dimensional parameter spaces. These problems require access to cyberinfrastructure resources including HPC machines, fast networks, next generation data and storage technologies and software that can create and manage complex workflow, automatically run parallel simulations, and handle transfer and retrieval of data and output. While such resources exist, administrative and technical barriers to entry remain high for neuroscientists, due to the need to write convincing allocation proposals, understand complex HPC architectures, master complex OS/software environment, optimally install simulation software, learn policies and batch system environments, manage data transfer and retrieval, and understand the remote authentication process.

The NSG will provide optimally installed parallel neuronal software, such as those listed above, on HPC resources. We expect that, following the success of other science gateways, the NSG will allow a large number of neuroscientists to easily and efficiently use these programs on HPC resources and enable, enhance and broaden computational neuroscience research and education.

Disclosures: N.T. Carnevale: None. **S. Sivagnanam:** None. **K.K. Yoshimoto:** None. **V. Astakhov:** None. **A.E. Bandrowski:** None. **M.E. Martone:** None. **A. Majumdar:** None.

Poster

928. Bioinformatics

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Topic: G.05. Bioinformatics

Support: NIH Blueprint HHSN271200800035C via NIDA

Title: A strategy for reporting brain structures to promote interoperability for databases and literature

Authors: *M. E. MARTONE[1], F. IMAM[2], S. D. LARSON[2], D. BOWDEN[3], G. M. SHEPHERD[4], G. ASCOLI[5]
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Abstract: The brain is perhaps unique among major organ systems in the multiplicity of naming schemes for its major and minor regions. The brain has been divided based on topology of major features, cyto- and myelo-architecture, developmental boundaries, supposed evolutionary origins, histochemistry, gene expression and functional criteria. The gross anatomy of the brain reflects the underlying networks only superficially, and thus any parcellation reflects a somewhat arbitrary division based on one or more of these criteria. Furthermore, many of these parcellations were developed before we had the ability to image the brain in 3 dimensions, leading to many arbitrary divisions. The lack of coherence in naming schemes is major impediment in developing automated means of extracting and comparing information from databases and the literature. As we now have the capability to generate data on gene expression, regional and cellular connectivity, and functional activation on a large scale, it is time to consider a strategy for naming brain regions that is designed to work better with information systems. One of the core aims of the Neuroscience Information Framework (http://neuinfo.org) is to establish an interoperable semantic framework for searching and integrating across data contained in diverse information systems. NIF and several other influential neuroinformatics projects, e.g., Neuronames, BAMS, have worked to establish the means to translate among these different nomenclatures. Interoperability is also a key goal of the INCF Program on the Ontologies of Neural Structures (PONS). The NIF project, in collaboration with PONS, has established the Neurolex Wiki, a semantic wiki for developing a collaborative knowledge base around the core concepts of neuroscience. As part of the Neurolex, we have worked with to define a reasonable

strategy for defining brain structures that is useful for implementation within information systems like the NIF. Through work with the Neurolex and PONS programs, we describe a strategy for naming and defining brain regions that promotes interoperability of brain regions across information systems, species and spatial scales. The strategy relies on the distinction between a set of reference brain structures, defined largely from a subset of common mammalian brain structures in Neuronames and several other information sources, and parcellation schemes produced by individual authors in the form of atlases or manuscripts. The strategy does not seek to impose a standard definition of brain regions; rather, it provides a means of constructing neuroanatomical concepts in a more machine processable form.

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Poster

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Topic: G.05. Bioinformatics

Support: BMBF Grant 01GQ0801

Title: Integrating data management into the laboratory workflow using a common data API for electrophysiology

Authors: A. SOBOLEV[1], A. P. LEONHARDT[1], C. KELLNER[1], P. L. RAUTENBERG[1], *S. CARDOSO DE OLIVEIRA[2], A. V. HERZ[1], T. WACHTLER[1]
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Abstract: Progress in neuroscience has lead to rapid proliferation of data. The problem of efficiently organizing and annotating this information while allowing effortless access from different platforms becomes crucial if scientists are to fully exploit recorded experimental data. At the German INCF Node (G-Node, www.g-node.org), an infrastructure for cellular and systems neuroscience is being developed to improve key ingredients of neuroscientific research: data access, data storage and exchange, and data analysis. The G-Node services provide data management in the cloud, including tools for organization, annotation, search, and backup. These services are accessed through an application interface (API), which supports a common data model for electrophysiology and flexible data annotation. Native client libraries in commonly used programming languages, including Matlab and Python, enable researchers to perform

computations in familiar analysis environments while retaining the advantages that cloud storage entails. Thus, scientists may select whatever technology is most suitable for their current research with their data being available at all times and from all locations. The interface also features powerful search and query capabilities from simple full-text search to specialized query and filter mechanisms such as data slicing or recording channel selection. Collaborative research is facilitated by fine-grained data sharing mechanisms. Moreover, all the changes made to any object in the system are being tracked. Thus, scientists can to go back in time to any revision, which makes the reproduction of individual analysis steps effortless and transparent.
Using the G-Node tools and services, data organization and data exchange is easily integrated into the day to day laboratory workflow, facilitating scientific progress and reproducible research.

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Poster

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Support: NIH Grant 5R01NS050792

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Title: The neural systems language: Systematic and unambiguous description and communication in connectomics and neuroinformatics

Authors: *R. A. BROWN, L. W. SWANSON
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Abstract: Among the unresolved challenges - ancient and modern - faced by the neuroanatomy and connectomics communities, none are more fundamental than the systematic description and unambiguous communication of findings. Without these, no effective mass-scale integration of anatomical and connectional data can be achieved. The accelerating volume of novel neuroanatomical data generated worldwide vastly outpaces the rate at which it is curated into analytic frameworks to synthesize systems-level understanding of the nervous system. To help address these challenges, we propose the Neural Systems Language, NSyL. NSyL is a modeling language for systematically representing and communicating neural connectivity. NSyL may be

used irrespective of the animal taxon described, experimental or observational technique implemented, anatomical nomenclature referenced, or computer system used for storage and analysis. NSyL is internally consistent, concise, and readable by both humans and machines. NSyL's power to express connectivity asserted from any connectomic technique in any taxon or species using any nomenclature grants it high utility and flexibility while providing a universal framework for efficient and unambiguous communication between anatomists and experimentalists. Additionally, NSyL's machine-readable nature takes advantage of cutting-edge technology and automation to help bridge the biocuration gap between the producers and consumers of connectomic information. Here we present precedent like-systems, how NSyL synergizes with existing frameworks, the reasoning behind NSyL's development as a language and framework, how NSyL is to be used, and NSyLs potential impact in neuroanatomy, neuroinformatics, and systems neuroscience. NSyL is an intuitive yet nonetheless breakthrough step forward for both systematizing discourse of neural circuitry and streamlining the cycle of scientific inquiry.

Disclosures: R.A. Brown: None. **L.W. Swanson:** None.

Poster

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Topic: G.05. Bioinformatics

Support: Dirección de Investigación – Sede Bogotá -DIB Universidad Nacional de Colombia. Grant: 9373

Title: Analyzing clinical and genetic data of alzheimer's disease in a colombian population

Authors: *H. A. GRANADOS, Y. GUZMAN, J. ORTEGA, L. MORALES, L. SEGURA, L. LOPÉZ, A. MEJIA, D. FORERO, R. PARDO, J. YUNIS, G. ARBOLEDA, L. NIÑO UNIVERSIDAD NACIONAL DE COLOMBIA, Bogotá. D.C, Colombia

Abstract: Alzheimer's disease (AD) is a degenerative brain disorder that results in memory loss, difficulty to find the correct words when speaking and changes in personality. The institute of Genetics of the Universidad Nacional de Colombia has been performing association studies about the Alzheimer's disease (AD) in Colombia for about ten years. In this study, socio-demographic and genetic information from a colombian population of 48 controls and 98 AD cases are analyzed. This research aims at identifying genetic and clinical characteristics associated with the presence of the AD. Also, some classifiers based on computational

intelligence techniques to help the diagnosis of AD were designed and implemented. Particularly, some classifiers for the AD disease based on neural networks, support vector machines and Bayesian networks were implemented. A multilayer perceptron obtained a 73.9 % cassification rate, while a Support Vector Machine, using a polynomial kernel, obtained a 78.76 % classification rate. On the other hand, a Bayesian Network with simple estimator and search algorithm TAN obtained a 82.87% classification rate. This research also used the a priori algorithm to identify some association rules related to the diagnosis of AD, thus, some association rules were obtained. Some of such rules are: i) family history of dementia is related to AD diagnosis; ii) diagnosis of patients older than 65 and with a family history of dementia are related to AD; and iii) an individual whose age is in group G2 (older than 65) and with genotype APOE 34 is related to AD.

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Poster

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Topic: G.05. Bioinformatics

Title: Towards practical brain-computer interfaces using high frequency steady-state visual evoked potentials

Authors: *A. HATAKEYAMA[1], J. USHIBA[2], S. HONDA[3]
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Abstract: Patients suffering from severe motor disabilities have difficulties in communicating with external environments. Brain-computer interface (BCI) is known as a technology for providing alternative way of communication for these patients by directly measuring brain activity, independently to their peripheral neuromuscular functions. Steady state visual evoked potential (SSVEP), which is categorized as an evoked electroencephalogram, is one considerable resource for BCI system. SSVEP is induced by flickering light stimuli and is obtained from the scalp over the primary visual cortex. In a standard SSVEP-based BCI system, several stimuli flickering at different frequencies are presented to the subjects simultaneously. By focusing on one of the stimuli, it is observed the increase of SSVEP responses at the corresponding frequency

to the stimulus. In SSVEP-based BCI, flickering frequencies in 5-30 Hz range are mostly used since large amplitude of SSVEP is obtained. However, these perceptible flickering light stimuli easily cause irritation of eyes and stress. Moreover, flicker light with frequency around 8-16 Hz is known as a potential trigger of photogenic seizure . For these reasons, such low flickering frequencies seem not to be adequate for practical SSVEP-based BCI. The present study therefore proposed to use flickering light with frequencies beyond critical fusion frequency (CFF) to overcome these issues. The frequencies beyond CFF is known to evoke insufficient SSVEP responses so that we proposed a measure to increase the amplitude of SSVEP by means of stochastic resonance (SR) by applying noisy flickering light in peripheral vision. Moreover, application of cross spectrum analysis was proposed to detect weak but time-locked component to the flickering light in recorded electroencephalogram, and construct the feature set from the calculated coefficients. Five healthy subjects were participated to the proof-of-concept experiment. In the experiment, the flickering frequencies were set higher than CFF. As a result, averaged accuracy over the subjects was 78.5, 82.2, and 85.5% with normal power spectrum analysis, power spectrum analysis with stochastic resonance, and cross spectrum analysis with stochastic resonance, respectively. In conclusion, our proposed method allows all subjects participating in the study to obtain high accuracy.

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Poster

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Title: Enabling new research and developing methods to improve sharing of electrophysiology data using CRCNS.org

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Abstract: Progress in neuroscience requires experimentation and the gathering of data, as well as analyzing and modeling of data to test and form hypotheses. Usually, researchers within a laboratory do both of these steps and the data are not publicly available. It would be potentially much more efficient if the data were available because other researchers could then analyze it in new ways that are outside the scope of the original investigation.

The resource CRCNS.org was setup to enable the effective sharing of electrophysiology data between experimentalists and theorists through an online repository that hosts data sets and related documentation. The repository currently has 16 data sets. The data sets have been used for research that would not have been possible if the data were not shared. To date, this has resulted in 11 Journal publications and various conference presentations and posters. We seek now to grow the repository both through individual contributions and by having experts curate collections of data sets in their area of specialization.

In addition to being used for new research projects, the variety of data sets at CRCNS.org is very interesting as a test case to address main challenges of sharing electrophysiology data. The challenges are both cultural and technical. A major cultural challenge is that neuroscience researchers are typically reluctant to share data. To help overcome this reluctance, we are developing mechanisms at CRCNS.org to simplify the contribution process and to reward experimentalists for contributing data. These include: making data sets citable, so contributors can list data sets in their CV and get citations for published data; allowing contributors to specify conditions for usage of the data; setting up an infrastructure to make data contribution easy; and enabling self-supporting online communities, which can reduce the demands on contributors to support data users.

The technical challenges stem from the great heterogeneity of the data and metadata. We are developing methods to store neural data as well as stimuli and other metadata using HDF5 as a storage container. This work builds on other efforts in this area including CARMEN NDF, NEO and Neuroshare. Where possible, metadata will reference standard ontologies such as those being developed by the NeuroLex project. Metadata useful for locating relevant data sets will be provided to the Neuroscience Information Framework for indexing. Both data and stimuli in data sets will be made available for online browsing and for automated tools of machine learning.

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