

ABSTRACT BOOK

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PLENARY LECTURES

Plenary lecture 1.

Dendritic computation

Michael Hausser

Wolfson Institute for Biomedical Research, University College London, UK

Plenary lecture 2.

The earliest cortical circuits

Zoltán Molnár, Anna Hoerder-Suabedissen, Franziska Oeschger, Wei Zhi Wang

Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom

The subplate zone is a highly dynamic transient sector of the developing cerebral cortex that contains some of the earliest generated neurons, and the first functional synapses of the cerebral cortex. Subplate cells have important functions in early establishment and maturation of thalamocortical connections, as well as in the development of inhibitory cortical circuits in sensory areas. So far no role has been identified for cells in the subplate in the mature brain and disease association of the subplate-specific genes has not been analyzed systematically. Here we present gene expression evidence for distinct roles of the subplate across development as well as novel molecular markers to extend the repertoire of subplate labels. Performing systematic comparisons between different ages (E15, E18, P8 and adult) we reveal the dynamic and constant features of the novel markers labeling subplate cells during embryonic and early postnatal development and in the adult. This can be visualized using the online database of subplate gene expression at <http://subplate.jouda.cc>. We also

identify embryonic similarities in gene expression between the ventricular zones, intermediate zone and subplate, and distinct postnatal similarities between subplate, layer 5 and layer 2/3. The genes expressed in a subplate-specific manner at some point during development show a statistically significant enrichment for association with autism spectrum disorders and schizophrenia. Our report emphasizes the importance of the study of transient features of the developing brain to better understand neurodevelopmental disorders.

Plenary lecture 3.

Neural syntax: organization of cell assembly sequences by brain rhythms

György Buzsáki

NYU Neuroscience Institute, New York University, Langone Medical Center, New York, USA

Plenary lecture 4.

**From the nucleus to the synapse and outside in learning and memory:
From c-Fos to MMP-9**

Leszek Kaczmarek

Nencki Institute of Experimental Biology, Warsaw, Poland

c-Fos, a constituent of transcriptional regulator AP-1 was discovered in late eighties as driven by neuronal activity and then has been associated with plasticity, learning and memory. Next, we have identified

extracellular proteolytic system, composed of matrix metalloproteinase-9 (MMP-9) and its endogenous inhibitor, TIMP-1 (tissue inhibitor of matrix metalloproteinases) as being controlled by c-Fos/AP-1 in activated neurons. Over the last decade we have extensively investigated the role of MMP-9 in the learning and memory and underlying synaptic plasticity. In brief, we have found that MMP-9 plays a role in a contextual fear conditioning as well as in appetitive, but not aversive learning in the Intellicages. Furthermore, we have shown that MMP-9 specifically in the central amygdala is critical for the reward learning. We have also shown that MMP-9 is mandatory for the late phase of long-term potentiation (LTP) in the hippocampus, prefrontal cortex and central amygdala (but not in the lateral amygdala). Furthermore, we have localized MMP-9 mRNA, protein and enzymatic activity at the dendrites and dendritic spines, harboring excitatory synapses (but not at the inhibitory synapses). Locally available mRNA can be translated in response to enhanced synaptic activity. We have also shown that MMP-9 controls morphological plasticity of the dendritic spines and associated mobility of glutamate receptors mobility. In aggregate, we have shown that MMP-9 is important for synaptic plasticity, learning and memory, and its understanding may explain c-Fos involvement in those phenomena.

Plenary lecture 5.

Fejezetek az idegtudomány történetéből

Miklós Palkovits

Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology and the Human Brain Tissue Bank, Semmelweis University, Budapest, Hungary

SYMPOSIUM LECTURES

Symposium 1. Wiring principles of the cerebral cortex

S1.1.

Unravelling the Neuronal Knitting

Julian Budd

Department of Informatics, Sussex University, Brighton, United Kingdom

Mapping how neurons in cerebral cortex are interconnected – the cortical connectome – is widely expected to provide a foundation for explaining fundamental cognitive and perceptual functions. Yet the task of reconstructing the connectivity of even a single axon, for example, is a complicated and time consuming business. The cortical neuropil is composed of the dense interweaving of very many glial, neuronal, and vascular processes. Hence the trajectory of an axon and its branches must be carefully traced through a maze of cellular processes to identify its likely postsynaptic targets. When the vast number of distinct axons contained within even a modest volume of cortical neuropil is considered, the task of completely mapping axonal connectivity takes on an epic stature. However, a number of recent technical advances now offer the potential for much more mapping data at multiple spatial scales of cortical organization. Here I will consider the advantages and limitations of these recent advances towards achieving the goal of mapping the cortical connectome. The main focus of the talk will be to argue for the importance of discovering the nature and purpose of cortical wiring principles underlying the organization of cerebral cortex.

S1.2.

In vivo whole-cell recording, transfection and transsynaptic tracing in mouse neocortex

Ede Rancz, Charly Rousseau, Mateo Velez-Fort, Molly Strom, Christian Niedworok, Troy W. Margrie

Neurophysiology, MRC National Institute for Medical Research, London, United Kingdom

Relating structure and function is required to better understand the nervous system. To this end, several microscopy and tracing techniques have been devised and perfected to assess local and long-range connectivity in simple and more complex model organisms. I will describe a method that may be used to genetically label the presynaptic partners of a single, functionally characterized neuron. We combined in vivo whole-cell recording of synaptic and spiking receptive fields of mouse visual cortical neurons with delivering plasmids through the patch pipette. A high proportion of recorded cells survived for days and expressed exogenous proteins coded by several plasmids in the recording solution. By delivering plasmids coding for a fluorescent protein, TVA-receptor and rabies-virus glycoprotein, we made single, recorded cells exclusively susceptible to modified rabies virus infection. The modified rabies virus, expressing a fluorescent protein but lacking the glycoprotein from its genome, was then able to spread to monosynaptically coupled presynaptic partners but no further. This method thus allows us to ascertain the synaptic and anatomical receptive field of single neurons however results in large volumes of data, the acquisition and processing of which poses new challenges. I will describe some recent advances we have made on this front.

S1.3.

The Blue Brain Project: In silico Neuroscience – an integrative approach

Felix Schürmann

Blue Brain Project, EPFL, Lausanne, Switzerland

Many areas of science and engineering have adopted simulation-based research as a novel tool for discovery and insight. The Blue Brain Project has pioneered this approach for brain research. On the one hand, the heterogeneity of neural systems poses particular challenges: the data is multi-modal, multi-scale and often times incomplete, intricate workflows are required for model generation, mathematical formulations are volatile, and memory and compute requirements to cope with the heterogeneity are demanding. On the other hand, neurobiology has potentially a lot to gain: systematically accounting for the data and bringing it together in a unifying computer model provides an integration strategy capable of overcoming the fragmentation of data and identifying gaps in our knowledge. Attempting this ultimate integration is revealing novel design principles of the brain. These principles are in turn helping to predictively fill gaps in data and knowledge. This approach differs from computational neuroscience in that the models are zero tweak models - the brain is built bottom up from first principles and validated top down by comparing models with biological emergent properties. As a proof of concept, the Blue Brain Project built a facility comprised of many key technologies and workflows and used this facility to build and simulate the neocortical column of the somatosensory cortex of the young rat.

S1.4.

Connectome and hodology, structure and function

Ervin Berényi, András Jakab

Axonal tracing approaches have been extensively used in the domain of connectomics and they were found plausible in determining the large-scale connections of the mammalian cortex (=macroscale connectome). Emerging non-invasive neuroradiological approaches, like diffusion tensor MR imaging (DTI) and functional MRI (fMRI) are promising candidates to acquire such data in humans. Here we sought to portray the possible applications of these techniques with particular attention on the mathematical frameworks that interpret macroscale connections as a complex network. The principle behind DTI and tractography techniques is that the possible (structural) pathways of information transfer can be traced by depicting the voxel-wise directionality and profile of water diffusion, which is hindered by axonal membranes. Consequently, the probability of existing structural connectivity can be determined between brain regions. On the contrary, functional MRI depicts the blood-oxygen level dependent contrast, which is presumably coupled with neuronal activity. Generally, this was feasible in revealing low frequency synchronisation between remote brain areas (=functional connectivity). In this decade, both in vivo determinants of connectivity were used in network-based interpretations such as graph theoretical analysis. We demonstrate our developments in this domain and forecast the possible role of graph analysis in understanding brain pathologies.

Symposium 2. Unbiased strategies in drug discovery

S2.1.

Single cell transcriptome by sequencing all messages: reveals novel drug targets on neurons and adipocytes

¹James Eberwine, ²Tamás Bártfai

¹University of Pennsylvania, USA; ²The Scripps Research Institute, USA

We report on an 'unbiased' molecular characterization of individual, adult neurons, active in a central, anterior hypothalamic neuronal circuit, by establishing cDNA libraries from each individual, electrophysiologically identified warm sensitive neuron (WSN). The cDNA libraries were analyzed by Affymetrix microarray. The presence and frequency of cDNAs were confirmed and enhanced with Illumina sequencing of each single cell cDNA library. cDNAs encoding the GABA biosynthetic enzyme Gad1 and of adrenomedullin, galanin, prodynorphin, somatostatin, and tachykinin were found in the WSNs. The functional cellular and in vivo studies on dozens of the more than 500 neurotransmitters, hormone receptors and ion channels, whose cDNA was identified and sequence confirmed, suggest little or no discrepancy between the transcriptional and functional data in WSNs; whenever agonists were available for a receptor whose cDNA was identified, a functional response was found. Sequencing single neuron libraries permitted identification of rarely expressed receptors like the insulin receptor, adiponectin receptor 2 and of receptor heterodimers; information that is lost when pooling cells leads to dilution of signals and mixing signals. Despite the common electrophysiological phenotype and uniform Gad1 expression, WSN transcriptomes show heterogeneity, suggesting strong epigenetic influence on the transcriptome. Our study suggests that it is well-worth interrogating the cDNA libraries of single neurons by sequencing and chipping.

S2.2.

Cytoskeletal changes in suicide subject's brain: is there a possibility of biomarkers for suicide prediction?

Gábor Juhász, András Czurkó, Péter Gulyácssy, Attila Simor, Katalin Adrienna Kékesi

Laboratory of Proteomics, Eötvös Loránd University, Budapest, Hungary

Probing molecular brain mechanisms related to increased suicide risk is an important issue in biological psychiatry research. Gene expression studies on *post mortem* brains and the recently discovered ribosomal RNA gene promoter hyper-methylation indicate that there are extensive changes in protein expression prior to a successful suicide attempt; however, proteomic studies are scarce. Thus, we performed a DIGE proteomic analysis of *post mortem* tissue samples from the prefrontal cortex and amygdala of suicide victims to identify protein changes and biomarker candidates of suicide. We found 72 and 43 significant differences among our matched spots with the industry standard *t* test in the prefrontal cortex and amygdala, respectively. Because of the risk of false discoveries in these data we also made a more stringent statistical approach that reduced the number of significant spots to 39 and 9 respectively. From these we identified 67 proteins in the cortex and 7 proteins in the amygdala. These proteins are related to biological functions and structures such as metabolism, the redox system, the cytoskeleton, synaptic function, and proteolysis. Thirteen of these proteins (CBR1, DPYSL2, EFHD2, FKBP4, GFAP, GLUL, HSPA8, NEFL, NEFM, PGAM1, PRDX6, SELENBP1 and VIM,) have already been suggested to be biomarkers of psychiatric disorders at protein or genome level. From the statistically less stringent data pool we pointed out 11 proteins that changed in both the amygdala and the cortex, and from these, GFAP, INA, NEFL, NEFM, TUBA1 and VIM are interacting cytoskeletal proteins that have a functional connection to glutamate, GABA, and serotonin receptors. Moreover, ACTB, CTSD, GFAP and VIM displayed reverse changes that might be suitable for brain imaging studies. The reverse changes of ACTB, CTSD and GFAP in the two brain structures were validated by western blot analysis.

S2.3.

Development of novel drug targets in postpartum depression

Árpád Dobolyi

Postpartum depression occurring in 10-15% of births has the potential for significant impact on both the health of the mother and baby. Today's medications for depression target the brain's classical monoaminergic neurotransmitter systems and remain suboptimal, with many patients showing only partial remission or significant side-effects. Therefore, there is a large need to develop new therapeutic strategies designed to control postpartum depression. The relatively high ratio of mothers developing postpartum mood disorders suggests common mechanisms that could trigger the development of depression. Thus, proteins that selectively appear in the brain of mothers represent potential drug targets to treat postpartum depression. We describe tuberoinfundibular peptide of 39 residues expressed selectively in a posterior thalamic cell group in mother rats. It was demonstrated that this area conveys the suckling information towards different limbic and hypothalamic structures that in turn contain its receptor, the parathyroid hormone 2 receptor, a potential new drug target in postpartum depression. Another maternal neuropeptide, amylin, was identified in a microarray study by its selective expression in the maternal preoptic area, a region responsible for maternal motivation. The projection pattern of preoptic neurons in the brain overlaps with the distribution of amylin receptors, a potential drug target in postpartum depression. Changes in the maternal level of a number of other genes were also identified and confirmed by RT-PCR and in situ hybridization histochemistry. A cortical area involved in the etiology of depression, the medial prefrontal cortex was also examined by proteomics. Changes in protein levels were measured by 2-D gel electrophoresis followed by mass spectrometry identification of proteins. A number of genes and proteins whose expression change in the postpartum period were identified. Influencing the activity of some of maternally altered genes affects the behavior of animals in tests of anxiety and depression. Additional tests in mothers will provide targets for the development of new therapeutic strategies designed to control postpartum mood disorders. Support: OTKA K100319; Bolyai Fellowship of HAS.

S2.4.

Molecular complexity and molecular networks: novel trends in psychiatric drug research

György I Lévy

Gedeon Richter Plc., Budapest, Hungary

Symposium 3. Neurobiology of addiction

S3.1.

Physiological and anatomical properties of dopamine neurons

Paul J. Bolam

MRC Anatomical Neuropharmacology Unit, Department of Pharmacology, Oxford, United Kingdom

Midbrain dopamine neurons are involved in a variety of functions and are particularly associated with reward mechanisms and addictive behaviour. In order to understand the contribution of dopamine neurons it is necessary to understand their properties and connections. The aim of this presentation to summarize some of the physiological and anatomical properties of dopamine neurons. Burst firing of dopamine neurons is associated with reward mechanisms and inhibition of firing in response to failure of rewards or aversive stimuli. Recordings from dopamine neurons in the substantia nigra pars compacta (SNc) of anaesthetized rats reveal a marked heterogeneity of responses to aversive stimuli (Brown et al 2009 J. Neuroscience 29:2915-2925). Light and electron microscopic analysis has

revealed the structural correlate of the inhibitory response. Inhibitory responses are correlated with the relative extension of SNc neuron dendrites into the SN pars reticulata and the overall GABAergic innervation (Henny et al 2012 *Nature Neuroscience* 15:613-619). At the level of the striatum dopamine neurons provide a massive innervation. Dopaminergic axon terminals are in a position to modulate the flow of excitatory input derived from both the cortex and the thalamus although the quantitative electron microscopic analysis suggests that the innervation is non-specific (Moss & Bolam 2008 *J. Neuroscience* 28:11221-11230). Quantitative analyses suggest that individual SNc dopamine neurons give rise to 100-200 thousand synapses at the level of the striatum. The massive axonal arbour is likely to put SNc dopamine neurons under a high energy demand and this may underlie their selective vulnerability in Parkinson's disease (Bolam & Pissadaki 2012 *Movement Disorders* 12:1478-83; Pissadaki & Bolam 2013 *Frontiers in Computational Neuroscience*, in press).

S3.2.

Gene mapping opens up new avenues for treatment of addiction

Csaba Vadász

Laboratory of Neurobehavioral Genetics, New York University Medical Center, Nathan Kline Institute, 140 Old Orangeburg Road

Alcohol dependence (alcoholism) and other addictions are complex diseases in which both genetic and environmental factors affect susceptibility. The consistently demonstrated 40-60% genetic variability in alcohol dependence and our profound ignorance of the underlying mechanisms which lead to addiction, highlight the need for identifying the genetic basis of susceptibility. The "genetic revolution" of the turn of the millennium raised hopes that the genetic underpinnings of complex neuropsychiatric disorders, including addictions, can be quickly identified. However, as recent reports of "lost heritability", and divergence of opinion

over the hidden variance indicate, it is now becoming clear that genetic complexity has been underestimated. New strategies take advantage of exciting progress in sequencing technologies, and integrate studies of human populations and model organisms. "Genetic noise-reduction" in animal models is an example of novel Quantitative Trait Locus (QTL) mapping strategies. Using a large panel of recombinant congenic mouse strains developed in our laboratory we mapped the QTL Ethyl alcohol consumption 2 (Eac2) to mouse chr. 6, and identified the underlying gene as cis-regulated glutamate receptor, metabotropic subtype 7 (Grm7; mGluR7). The gene variant coding for lower mRNA expression in the brain predisposes for higher alcohol consumption, which is consistent with studies on knockout mice showing increased voluntary alcohol drinking. Preliminary animal model and human genetic studies indicate translational potentials of targeting mGluR7. Treatment with N, N'-dibenzylhydriethane-1,2-diamine dihydrochloride (AMN082, 4 mg/kg, i.p.), a positive allosteric modulator of mGluR7, reduced alcohol drinking, while AMN082 did not effect significantly consumption of sucrose which served as natural reward. mGluR7 is the most conserved subtype among metabotropic glutamate receptors, and we hypothesized that its modulatory function has also been conserved in the course of evolution. Preliminary results of our collaborative human genetic studies show a trend of association between a GRM7 variant and an alcohol drinking phenotype developed on the basis of our animal model. In conclusion, recent developments in complex trait genetics suggest that integration of unbiased genome-wide animal model and human genetic strategies will be important in understanding addiction susceptibility, and development of new treatment avenues for addiction.

S3.3.

The ventral tegmental area in drug reward and beyond

Christian Lüscher

Department of Basic Neurosciences and Clinic of Neurology, University of Geneva, Switzerland

S3.4.

Pathological gambling: wheel or wheelchair of Fortuna?

Zoltán Janka

University of Szeged, Department of Psychiatry, Szeged, Hungary

Pathological gambling is a medically defined category and classified among impulse control disorders. However, it resembles in many aspects compulsion as uncontrollable urges appear to wager money to win (i.e. to rotate symbolically the wheel of Fortuna). Nevertheless, if gambling habit is under control in a socialized manner and does not extend to the compulsive field it can provide pleasure source for the risk-taking individual. Typical symptoms of pathological gambling are preoccupation with the topic, increasing the amount of money wagered, unsuccessful efforts to cut back/stop gambling behaviour and irritability/restlessness when doing that, alleviation of dysphoric mood, an effort to get even after losing, lies to conceal the habit, illegal acts to finance it, begging for loans from others, jeopardizing or destroying important relationships, job, career, and the behaviour is not due to manic mood elevation. Comorbidity with other psychiatric disorders is high, like alcohol and other substance abuse, depression/dysthymia, manic episode, generalized anxiety, panic, specific/social phobias, post-traumatic stress-disorder. Cognitive psychological investigations showed impairments in working memory, mental flexibility, planning, inhibition, and time management of the affected individuals. In vivo neuroimaging techniques revealed altered activation in specific brain areas (e.g. ventrolateral prefrontal cortex) in association with neurocognitive tests (Stroop, Iowa Gambling). Neurotransmitter systems (DA, 5HT, NA, opioidergic) have been shown to participate in gambling behaviour. Evidences for dysfunctional mesolimbic DA pathway, nucleus accumbens, aberrant salience attribution, deficits in motivational reward have been suggested in the pathophysiology. Serotonergic mechanisms have also been implicated, and further, therapeutic efforts with serotonin reuptake inhibitors point to the

involvement of 5HT. Molecular genetic investigations suggested some DA receptor gene allelic associations (DRD2, DRD3, DRD1) with pathological gambling. Treatment (psycho -and pharmacotherapy) is difficult as less than 10% of gamblers seek medical or psychological help and the attrition rate during therapy is high. Consequently, the outcome for the majority with this addictive behaviour is devastating: a complete existential collapse, i.e. starting to rotate the wheel of Fortuna leads directly and irresistibly to a handicapped state in the wheelchair of Fortuna.

Symposium 4. Imaging techniques of the 21st century

S4.1.

Quantitative molecular differences in presynaptic active zones

Zoltan Nusser

Laboratory of Cellular Neurophysiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

Synapses of the central nervous system display remarkable structural and functional heterogeneities, the underlying mechanism of which is largely unknown. I will present our recent experiments obtained with two-photon imaging-based optical quantal analysis and correlated electron microscopic (EM) 3D reconstruction, showing that the probability of glutamate release (Pr) from local axon collaterals of hippocampal CA3 pyramidal cells scales linearly with the size of the active zone (AZ). Combined *in vitro* two-photon Ca²⁺ imaging from presynaptic boutons and correlated EM reconstruction of the imaged boutons revealed that the total fluxed Ca²⁺ following an action potential is also proportional with the AZ area. SDS-digested freeze-fracture replica immunogold labeling of the presynaptic

voltage-gated Ca^{2+} channel subunit Cav2.1 showed that this ion channel is confined to the AZ within the axon terminals. The density of Cav2.1 and that of other AZ proteins (e.g. Rim1/2) is uniform across AZs. The Pr and short-term plasticity patterns of axon terminals contacting parvalbumin or mGluR1 α immunopositive GABAergic interneurons also display great variability. I will also present some recent data demonstrating that the underlying mechanism might be a lower presynaptic Ca^{2+} channel density at the AZ of those terminals that synapse on mGluR1 α positive cells. Our results provide evidence for quantitative differences in the molecular composition of the presynaptic AZs of local axon terminals of CA3 pyramidal cells, with the consequence of allowing the fine tuning the release probability.

S4.2.

Fast two-photon in vivo and in vitro imaging in near-cubic-millimeter volume up to sub-millisecond temporal resolution

¹Balázs Rózsa, ¹Gergely Katona, ¹Gergely Szalay, ²Pál Maák, ¹Kaszás Attila, ²Máté Veress, ³Dániel Hillier, ¹Balázs Chiovini, ³Botond Roska

¹*Two-Photon Imaging Center, IEM HAS, Budapest, Hungary;* ²*Dept. of Atomic Physics, Budapest University of Technology and Economics, Budapest, Hungary;* ³*Neural Circuit Laboratories, Friedrich Miescher Institute for Biomedical Research, Basel, Hungary*

The understanding of brain computations requires methods that read out neural activity on different spatial and temporal scales. Following signal propagation and integration across a neuron and recording the concerted activity of hundreds of neurons pose distinct challenges, and the design of imaging systems has been mostly focused on tackling one of the two operations. We developed a high-resolution (PSF diameter in the center: 450 nm), fast (up to 55 microsecond temporal resolution), acousto-optic two-photon microscope with continuous three-dimensional trajectory and random-access scanning modes that reaches near-cubic-millimeter scan

range (over $700 \times 700 \times 1.400 \mu\text{m}^3$) and can be adapted to imaging different spatial scales. The system designed in ZEMAX and OSLO contains new design concepts: it physically separates the z-dimension focusing and lateral scanning functions to optimize the lateral AO scanning range; it allows the acoustic frequency chirps in the deflectors to be adjusted dynamically to compensate for astigmatism and optical errors; it involves a custom angular compensation unit to diminish off-axis angular dispersion introduced by the AO deflectors and it uses a high-NA, wide-field objective and high-bandwidth custom AO deflectors with large apertures. The resolution of the system allows simultaneous functional measurements in many fine neuronal processes, even in dendritic spines within a central core ($\sim 290 \times 290 \times 200 \mu\text{m}^3$) of the total scanned volume. Furthermore, the PSF size remained sufficiently low ($\text{PSF}_x < 1.9 \mu\text{m}$, $\text{PSF}_z < 7.9 \mu\text{m}$) to target individual neuronal somata in the whole scanning volume for simultaneous measurement of activity from hundreds of cells. With respect to the number of measurement locations on neuronal processes and cell bodies, we demonstrated fast 3D measurement of activity from 87 fine dendritic segments and over 500 cells included; however, the number of locations is not limited by the AO scanning method. The selection of active neurons in a volume that respond to a given stimulus was aided by the real-time data analysis and the 3D interactive visualization accelerated selection of regions of interest.

S4.3.

Diffusion tensor tractography in stereotactic functional brain surgery

István Valálik

Department of Neurosurgery, St. John's Hospital, Budapest, Hungary

There are a significant number of patients with movement disorders, Tourette-syndrome (TS) and obsessive-compulsive disorder (OCD) who have not benefitted from pharmaceutical treatment or psychotherapy. Stereotactic radiofrequency thermo lesion and reversible and controllable

deep brain stimulation (DBS) may offer further opportunities for certain neurological and psychiatric cases. The individual anatomy is only used to scale the targets based on the location of the third ventricular and other internal landmarks. While on conventional MR imaging the thalamic nuclei are indistinguishable, recently, it has become possible to identify individual thalamic nuclei using different connectivity profiles, as defined by MR diffusion tractography. So far only few cases have been reported in the literature, especially with the involvement of tractography during surgical planning.

Prior to surgery, an extensive MR imaging was performed in all cases. Two patients needed general anaesthesia during the investigation. All patients underwent diffusion MRI protocol with subsequent deterministic and probabilistic tractography. We investigated the inter-individual variation of the location of target nuclei for thalamotomy or DBS: the putative ventralis oralis posterior (Vop) and the ventral intermedius (Vim) nucleus. The optimal target positioning is in the region of Vo/centromedian/parafascicular nuclei in TS and the region of nucleus accumbens (NA) and ventral part of the anterior limb of the internal capsule (ALIC) for OCD patients. The target locations were defined based on connectivity profiles. For final target verification a 5-channel microelectrode recording and stimulation testing was applied intraoperatively. The quality control of proper electrode placement was performed with postoperative CT, preoperative MRI and tractography co-registration with visualisation of connectivities of the brain tissue near the activated electrode contacts.

After surgery all the patients showed substantial improvement. No surgical and device-related complications were observed. Besides, there were only slight stimulation-related complications which were successfully eliminated after refining the stimulation parameters. Tractography is a promising technique in individual target determination for the treatment of neurological and psychiatric disorders with stereotactic surgery.

S4.4.

Exploring the human brain using fMRI: from cortical areas to functional networks

Zoltán Vidnyánszky

*Kognitív Tudományi Tanszék, Budapesti Műszaki Egyetem,
Természettudományi Kar, Budapest, Hungary*

Functional magnetic resonance imaging (fMRI) is currently the most popular neuroimaging technique in human cognitive neuroscience. The goal of the talk is to provide an overview of the current state of fMRI, including the recent advances in understanding the neurobiological principles of the BOLD signal and the constraints they impose on the interpretation of the fMRI data. I will show that future development of multivariate analysis methods focusing on spatial patterns of brain activity rather than mean activation in a specific region promises to provide a better characterization of the neural representation of different sensory processes and cognitive functions. In the talk I will also give a short overview of the resting-state fMRI method, which allows to study functional brain connectivity by measuring spontaneous fluctuations in the BOLD signal during rest. I will conclude that future neuroimaging research aiming at understanding the brain's function and dysfunction must adopt a multimodal approach in which different fMRI methods are combined with other non-invasive techniques that directly assess the brain's electrical activity such as EEG and MEG.

Symposium 5. Selected free oral communications

S5.1.

Single molecule analysis of rapid non-classical estrogen action on live neurons

István Ábrahám

Center for Neuroendocrinology, Department of Physiology and Institute of Physiology, University of Otago, New-Zealand and Medical School, University of Pécs, Pécs, Hungary

The gonadal steroid estradiol (E2) regulates the function of cholinergic neurons. Beside the classical genomic action the E2 exerts rapid, non-classical effects via membrane receptors and the intracellular signal-transduction such as ERK1/2 signaling pathway. One of the key mechanisms in ERK activation is the transient immobility of surface movement and the dimerization of TrkA receptor (TrkAR). In this study we investigated the rapid non-classical effect of E2 on surface movement of TrkAR expressed in live adult cholinergic neurons. Quantum dot (Qdot) based in vivo labeling technology was applied to fluorescently label TrkAR in live adult basal forebrain cholinergic neurons obtained from cholinergic-GFP mouse brain. To follow the surface diffusion of Qdot labelled TrkAR a unique total internal reflection fluorescence hybrid microscopy system (TIRFM) was used that we have developed to allow super-resolution real-time imaging of membrane receptor surface trafficking in live neurons. TIRFM investigations demonstrated that TrkAR followed confined diffusion in control conditions in adult cholinergic neurons. The TrkAR had two distinct motility states on the membrane surface, characterized as mobile and immobile phases. The nerve growth factor, the primary ligand of the TrkAR, rapidly increased the frequency and time interval of the immobile periods as well the number of TrkAR dimers. E2 and membrane estrogen receptor agonist enhanced the time of immobile periods of TrkAR trafficking in adult cholinergic neurons. These results demonstrate for the first time the confined diffusion of TrkAR with transient immobile periods in the receptor trajectory in the neuronal membrane. Furthermore single molecule analysis showed a rapid NGF-like membrane effect of E2 on TrkAR mobility, suggesting the presence of membrane-initiated E2-induced TrkAR signalling in adult cholinergic neurons.

S5.2.

A TNF-alfa előkezelés elősegíti az embrionális sejtek neuronális érését és integrációját avulziós gerincvelősérülés modellben

¹Tamás Bellák, ¹Krisztián Pajer, ¹Gábor Márton, ²Karri Lamsa, ³József Maléth, ¹Antal Nógrádi

¹ Szemészeti Klinika, Neuromorfológiai Laboratórium, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary; ² Department of Pharmacology, University of Oxford, Oxford, United Kingdom; ³ I. sz. Belgyógyászati Klinika, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary

A gerincvelő sérülése vagy motoneuronokat érintő betegsége jelentős motoneuron károsodással jár. A károsodott motoneuronok pótlásának egyik ígéretes módja lehet embrionális motoneuron-graftok beültetése. Korábbi tanulmányaink szerint a transzplantált sejtek jól integrálódnak, de többségük gliális irányba differenciálódik. A tumor-nekrózis faktor alfa (TNF-alfa) neuroprotektív és neuronális érést elősegítő tulajdonságokkal is rendelkezik. Vizsgálataink célja, hogy a beültetett sejtek differenciálódását és integrálódását tanulmányozzuk TNF-alfa előkezelést követően. Sprague-Dawley patkányokon a lumbális 4-es (L4) gerincvelői szegmentumhoz tartozó ventrális gyökér avulzióját, majd annak motoneuronokban gazdag embrionális szövetrel együtt történő visszaültetését végeztük el. A transzplantált szövet 12,5 napos SD-eGFP patkányembriókból származott. Kontroll állatok esetében kezeletlen embrionális szövetet ültettünk be, a másik csoportban a gazdaállatok rhTNF-alfával (1 ug/ml, 1 óra inkubáció szobahőmérsékleten) előkezelt graftokat kaptak. A különböző túlélési időket (2-12 hét) követően kiterjedt morfológiai analízist végeztünk. A harmadik kísérleti csoportban SD graftokat ültettünk be SD-eGFP gazdaállatok gerincvelőjébe. Eredményeink szerint a beültetett sejtek jól integrálódtak és neuronális, valamint gliális irányba differenciálódtak. A graftolt terület belsejében nem találtunk gazda-eredetű sejteket, a gazdaszövet-eredetű erek viszont

teljesen átszőtték a graft területét. A kontroll csoportban a transzplantált sejtek döntően az asztroglia sorsot választották, csak néhány sejt mutatott érett neuronokra jellemző morfológiát. Ezzel ellentétben a rhTNF-alfa előkezelésben részesülő csoportban a sejtek többsége neuronális neurokémiai és morfológiai tulajdonságokkal rendelkezett, sőt jelentős számú motoneuronná differenciálódott graft-eredetű sejtet is találtunk. Utóbbiak egy része képes volt reinnerválni a perifériás célszerveket, amit retrográd jelölésekkel bizonyítottunk. Érdekes módon a differenciálódott sejtek eGFP expressziója jelentősen csökkent. Eredményeink szerint a TNF-alfa előkezelés drámaian növeli a transzplantált embrionális sejtek neuronális differenciációját és integrációját, valamint a visszaültetett gyökér innervációját. A fenti munkát a nagy-britanniai Élettani Társaság (The Physiological Society, UK) támogatta (International Junior Research Grant 2010).

S5.3.

Characterization of a non-human primate model for Parkinson's disease in common marmoset monkeys transplanted with neural progenitor cells

¹Boldizsár Czéh, ²Enrique Garea-Rodríguez, ³Sigrid C. Schwarz, ³Johannes Schwarz, ⁴Christina Schlumbohm, ²Jessica König, ⁵Gunther Helms, ²Cornelia Heckmann, ⁶Birgit Meller, ⁷Johannes Meller, ²Eberhard Fuchs

¹Laboratóriumi Medicina Intézet, PTE ÁOK, Pécs, Hungary; ²Clinical Neurobiology Laboratory, German Primate Center, Göttingen, Germany; ³DZNE, German Center for Neurodegenerative Diseases, Munich, Germany; ⁴Encepharm, Encepharm, Göttingen, Germany; ⁵Dept. Cognitive Neurology, University Medical Center, Georg-August-University, Göttingen, Germany; ⁶Dept. Nuclear Medicine, Martin-Luther-University Halle, Halle, Germany; ⁷Dept. Nuclear Medicine, University Medical Center, Georg-August-University, Göttingen, Germany

The common marmoset (*Callithrix jacchus*) is a small New World primate that has been increasingly acknowledged as a useful animal model system for biomedical and neuroscience research applications. Here, we characterize a marmoset model for Parkinson's disease, generated by unilateral injections of 6-OHDA into the nigro-striatal projection pathway. Besides the traditional behavioural and post-mortem histopathological analysis we demonstrate here the advantage of using in vivo imaging with single photon emission computed tomography (SPECT) to characterize dopaminergic brain structures. Our aim was to establish a protocol for imaging the dopamine transporter (DAT) using serial SPECT and structural magnetic resonance imaging (MRI). The combined usage of the radioligand ¹²³I-N-ω-fluoropropyl-2β-carbomethoxy-3β-{4-iodophenyl}nortropane (¹²³I-FP-CIT) with MRI enables the detection of small dopaminergic brain structures. Furthermore, we tested the survival capacity of neural progenitor cells transplanted into unilaterally lesioned marmosets. We used human midbrain neural progenitor cells derived from fetal tissue. Such cells are a promising source to generate human DA neurons for therapeutic approaches. Our data support the idea that the common marmoset monkey is a useful preclinical model system to study novel therapeutic approaches for neurodegenerative disorders like Parkinson's disease. Support: CMPB, GE Healthcare, Munich, SAB #10864/1669, EU-FP7 Brain Cav. EC, EU ERA-Net NEURON.

S5.4.

Mangán kontraszttal történő MRI vizsgálatok, mint új lehetőség a magatartás szabályozásában résztvevő agyi struktúrák feltérképezésére patkányban

¹Rita Gálosi, ¹Csaba Szalay, ²Mihály Arady, ²Gábor Perlaki, ³József Pál,

²Attila Schwarcz, ¹Zoltán Karádi, ¹László Lénárd

¹*Élettani Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;* ²*Idegsebészeti Klinika, Pécsi Diagnosztikai Központ, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;*

A feszültségfüggő Ca^{2+} csatornákon keresztül a neuronokba jutó Mn^{2+} akkumulálódik, csökkentve a T1 relaxációs időt ezzel lehetővé téve az aktívabb agyterületek megjelenítését MRI során. A magatartásvizsgálatokban alkalmazásának korlátja, maga a mangán toxicitása. Vizsgálatainkban választ kerestünk arra, hogy mely dózis alkalmazható az instrumentális válaszban résztvevő struktúrák megjelenítésére toxicitása figyelembevételével. 20 mg/kg MnCl_2 ismételt infúziójának hatását tanulmányoztuk a plazma GOT, GPT, teljes bilirubin, protein, albumin és glükóz szintet meghatározva, illetve neurológiai vizsgálattal és a szomjúság motivált, tartós figyelmet követelő operáns szignál diszkriminációs (OSD) tesztben. A tesztben résztvevő, valamint a szomjajztatás után itatott, illetve a szomjajztatás mellett a teszt kontextusában is résztvevő állatcsoportokon a Mn^{2+} beadás előtt és után, 3 T mágneses térerejű klinikai MR készülékkel készítettünk T1 térképeket. A T1 idő meghatározásához turbo spin echo inverziós visszatérési szekvenciát használtunk, majd a különböző inverziós időkhöz tartozó adatok 3-paraméteréhez illesztett görbéjével határoztuk meg. A Mn^{2+} 20 mg/kg i.p. egyszeri és kétszeri dózisa nem okozott kóros eltérést a plazmaszintekben, a szenzoros-motoros funkciókban és nem rontotta a helyes válaszok arányát OSD tesztben. A 2 x 20 mg/kg MnCl_2 dózis a második beadást követően már csökkentette a vigilanciát. A 3 x 20 mg/kg dózis májtoxikus volt. A 2 x 20 mg/kg dózist alkalmazva a képalkotás során szignifikánsan csökkent a T1 relaxációs idő az OSD tesztben részvett operáns választ adó állatok alábbi agyterületein: temporális asszociációs kéreg, laterális másodlagos vizuális kéreg, hippocampus, subiculum, centrális és mediális amygdala és tuberculum olfactorium. Az eljárás, tehát alkalmazható 3 T mágneses térerősség mellett is komplex magatartási folyamatokban résztvevő struktúrák feltérképezésére, így az operáns válasz során aktiválódott agyterületek elkülönítésére. Készült a TÁMOP 4.2.2/B-10/1-2010-0029 és 4.2.1/B-10/2/KONV-2010-0002 támogatásával.

Anatomy of sharp-wave ripples: pyramidal cells are responsible for initiation and PV interneurons for ripple generation and termination

AI Gulyás, D Schlingloff, S Káli, Z Kohus, Z., T Freund, N Hájos
Kíséleti Orvostudományi Kutatóintézet, Budapest, Hungary

Sharp-wave ripple (SWR) initiation and termination was examined in the mouse CA3 area in vitro. Excitatory activity increases 60msec before SWR peak. Inhibitory cells are activated 20msec later, quickly overshoot excitatory activity and start to ripple-phase modulate cellular firing. The initiation of SWRs can be explained by a mixed stochastic/refractory model. Local inactivation of inhibition in str. pyramidale by GABAzine or agatoxin proved that SWR field generation and ripple phase locking of cellular firing is driven by PV containing perisomatic cells (PVCs). As revealed by experiments, ripple oscillation is generated through the Inhibitory Neuron Fast Oscillation (INFO) mechanism: excitatory drive, that builds up in the recurrent CA3 pyramidal cell (PC) network drives mutually interconnected inhibitory neurons, that generate high-frequency oscillation. SWR termination might be the result of the strong depression observed in the inhibitory transmission of PVCs. Manipulating the firing pattern and the release probability of the PVCs by bath application of riluzol and DAMGO the properties of SPWs were modified according to the synaptic depression hypothesis. Supported by OTKA K83251.

S5.6.

Prion proteins in differentiating and mature neural cell types

¹Barbara Orsolits, ²Adrienn Borsy, ²Ervin Welker, ¹Emília Madarász

¹ *Idegi Sejt- és Fejlődésbiológiai Laboratórium, MTA - Kíséleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Institute of Biochemistry, Biological Research Centre, Szeged, Hungary*

Prion diseases include Creutzfeldt-Jacob Disease, Fatal Familial Insomnia, Chronic Wasting Disease and Bovine Spongiform Encephalopathy best known as mad cow disease. The infectious Prion protein involved in these diseases originates from a conformational change of the cellular Prion protein, which is part of the prion superfamily also including the glycoproteins Shadoo (Sho) and Doppel. Shadoo, a recently discovered member of the family shares several properties with the Prion protein. However, its physiological function and possible role in prion disease are not known. Initial experimental results suggest that Shadoo and Prion protein may complement each other. We investigated the expression of Sho at the mRNA and protein levels and examined its subcellular localization both during in vitro neural differentiation and in the adult mouse brain. The expression patterns of Sho were compared to those of the Prion protein.

S5.7.

A Brattleboro patkány, mint skizofrénia modell az EEG jelek tükrében

¹Kata Terstyánszky, ²Dóra Zelena, ²Nikolett Venczkóné Bakos, ²János Varga, ²Andrea Szabó

¹*Semmelweis Egyetem, Hungary;* ²*Magatartásélettan és Stressz Kutatócsoport, MTA Kísérleti Orvostudományi Intézet, Budapest, Hungary*

A skizofrénia gyógyszeres kezelése nem megoldott, a preklinikai kísérletekhez állatmodellekre van szükség. Néhány irodalmi adat arra utalt, hogy a vazopresszin hiányos Brattleboro patkányokban (BB) spontán kialakulnak a skizofréniasokra jellemző magatartászavarok, ezért ez a törzs megfelelő genetikai modell, de az igazoláshoz további vizsgálatok voltak szükségesek. Mivel a szubjektív magatartás-zavarok mellett objektíven mérhető EEG elváltozások is velejárói a betegségnek, ezért erre fókuszáltunk. A hím BB állatokat vad típusúakkal hasonlítottunk össze. A magatartást a “prepulse inhibition”, a tárgyfelismerés és a szociális

elkerülés tesztekben vizsgáltuk. Az EEG jeleket a fronto-frontális és fronto-parietális elvezetésekben 72h-n keresztül rögzítettük nyugalomban és tárgyfelismerési teszt során. A BB állatok a magatartási kísérletek során skizofrénia-szerű tüneteket mutattak, melyek antipszichotikum kezelésre javultak. Az EEG elemzés során kimutatható volt az alvászavar, a cirkadián ritmus zavara és az abszolút EEG teljesítménysűrűség csökkenés, mely összhangban van az irodalomban leírt skizofrénia-jellemző EEG elváltozásokkal. A skizofrénia-jellemző memória funkció romlásával összhangban a tárgyfelismerési kísérlet alatt a θ értékek csökkentek, alvás alatt a delta értékek alacsonyabbak voltak. Eredményeink a magatartási zavarokon túl jellemző EEG elváltozásokkal is bizonyították a BB patkányok természetes skizofrénia modellként való alkalmazhatóságát.

S5.8.

Abeta1-42 induces impairment of LTP and spiking rate in the CA1: role of glutamate reuptake inhibition

¹Edina Varga, ¹Gábor Juhász, ¹Zsolt Bozsó, ¹Botond Penke, ²Viktor Szegedi

¹ *Orvosi Vegytani Intézet, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary;* ² *Szegedi Biológiai Központ, Biokémiai Intézet, Magyar Tudományos Akadémia, Szeged, Hungary*

Abeta1-42 impairs long-term potentiation (LTP), a form of neuronal plasticity. The precise temporal control of spiking activity is crucial in synaptic plasticity. Pre- and postsynaptic neuronal discharges separated by few milliseconds induce modification of synaptic strength, referred to as spike-timing dependent plasticity (STDP). The effect of Abeta1-42 on LTP is well documented, however, data about Abeta1-42 impact on neuronal firing connected to synaptic plasticity is still lacking. Here, we applied Abeta1-42 onto murine hippocampal slices, and recorded field excitatory postsynaptic potentials (fEPSPs) and spiking activity from the CA1. LTP was induced by theta-burst stimulation (TBS), and neuronal discharges

were recorded before, 1.5 h and 3 h after TBS. We have found that firing rates correlate with the level of LTP, except in the Abeta1-42 treated groups. Spiking activity increased after TBS in the control slices, but similarly to LTP, it returned to baseline in the Abeta1-42 treated group after 1.5 h. When Abeta1-42 was applied after TBS, there was no LTP impairment; however, spiking rate was enhanced 3 h after TBS compared to slices having received only TBS. Abeta1-42 induced a permanent increase in firing rate 1.5 h and 3h after treatment, without affecting fEPSPs amplitude in slices not subjected to TBS. Inhibiting glutamate reuptake mimicked the effects of Abeta1-42 both on LTP and spiking rate. Based on these results we suggest that the LTP impairment by Abeta1-42 might be the consequence of altered spiking activity, which is probably caused by the blockade of glutamate reuptake.

POSTER PRESENTATIONS

Session 1. Neurophysiology, P1.1 – P1.43.

P1.1.

Reliable synaptic transmission from CA3 pyramidal cells requires unusual recording conditions

¹Tibor Andrási, ¹János Brunner, ¹János Szabadics

¹ *Farmakológia, MTA KOKI, Budapest, Hungary*

The current theoretical models, which describe the hippocampal functions, consider the auto-associative mesh of CA3 pyramidal cells to be fundamental for the acquiring and retrieval of memories. This elementary assumption derives from anatomical studies that showed that the extensive axonal arbors of CA3 pyramidal cells frequently form synaptic junctions with dendritic spines of pyramidal cells. Intriguingly, our knowledge about this all-important connection is limited due to the extremely low reported connectivity rates (1-2%) using simultaneous paired recordings of CA3 pyramidal cells in acute slice preparations, which technique was used in numerous cases to reveal the fundamental synaptic properties of identified neurons such as failure rates, unitary amplitudes and kinetics. On the other hand, the connections between CA3 pyramidal cells are easily accessible with other experimental arrangements, such as extracellular stimulation of the CA3 fibers and paired recordings in organotypic slices. However, these methods are not capable of providing several of the crucial properties of the connections. Therefore, considering the differences between the various methods, we hypothesized that the apparent lack of monosynaptic connections between CA3 pyramidal cells is due to specific alterations of the CA3 pyramidal cell functions in acute slice preparation. To address this issue we performed large number of simultaneous paired recordings from

CA3 acute slice in various recording conditions. We found that omitting ATP and GTP, which are contained by the conventional intracellular solution, dramatically changed the probability of finding connected CA3 pyramidal cells (ATP/GTP-free solution: 18 connection out of 366; conventional solution: 2 out of 497) in acute slices suggesting that leaking ATP and/or GTP from the patching pipette affects the output of CA3 pyramidal cells. Application of P2X7 antagonists, A438079 (5 μ M) and A804598 (1 μ M) to prevent the constitutive P2X7 activity in the slice, did not increase further the probability of finding mono-synaptically connected CA3 pyramidal cells (4 connected pairs from 186 tested). These results reveal reliably monosynaptic connections between CA3 pyramidal cells in acute slices and highlight the possibility that conventional recording circumstances may not be optimal for each neuron type.

P1.2.

Hippocampal Adult-Born Granule Cells Form Two Functionally Distinct Populations

¹János Brunner, ²Susan Van-Weert, ¹Máté Neubrandt, ¹Tibor Andrási, ³Felix Kleine Borgman ³Sebastian Jessberger, ¹János Szabadics

¹ *Celluláris Neurofarmakológia, MTA-KOKI, Budapest, Hungary;* ² *Cellular and Developmental Neurobiology, Institute of Experimental Medicine-HAS, Budapest, Hungary;* ³ *Institute of Cell Biology, Swiss Federal Institute of Technology, Zürich, Switzerland*

Dentate gyrus granule cells are continuously generated in the adult hippocampus and the active and passive membrane properties of these adult-born granule cells (ABGC) undergo gradual changes. Recent studies suggested that the distinct integrative properties of young and old cells allow them to contribute to distinct physiological functions. However, there is a contradiction between the two above observations. How is it possible forming two distinct populations if the underlying properties do not show bimodality, but these properties are continuously changing with

the age of the ABGCs? The bimodal distinction between ABGCs as two homogeneous populations is crucial to understand whether and how young and old ABGCs are capable of fulfilling the proposed two distinct functions, namely participating in the pattern separation or pattern completion, respectively. To address this apparent contradiction we analyzed the integrative properties of 13-72 days old ABGCs, whose age was determined by temporally precise virus vector labeling (RFP labeled cells were born within 24 hours). Specifically, we measured the ability of ABGCs to transform inputs to action potential outputs using sinusoidal current injections with increasing amplitude at physiologically relevant frequencies (5-80 Hz). We found that the input-output function of 20-47 days old ABGCs showed sigmoid shape, which were steep in a restricted input amplitude range; whereas old cells (72 days) continuously and only incrementally increased their output rates with input intensity. Thus, 20-47 days old ABGCs were capable of large changes of their output in response to small changes in the input suggesting that they are indeed ideally suited to perform pattern separation. Surprisingly, the results showed that this high sensitivity period in the input-output function were very similar throughout the populations of 20 to 47 days old ABGCs indicating that during this relatively long period ABGCs capable of performing similar input-output computations. Our detailed electrophysiological analysis confirmed that all measured membrane properties changed gradually with the age of the cells. However, our observation suggest that interaction of multiple properties, some of which have opposing effect on the excitability of neurons, allows ABGCs to respond with similar characteristics during a relatively long period of their early life and they form two homogeneous populations, which perform distinct neuronal computations.

P1.3.

Selective silencing of individual dendritic branches by an mGlu2-activated potassium conductance in dentate gyrus granule cells

¹János Brunner, ²Jeanne Ster, ³Susan Van-Weert, ¹Tibor Andrási, ¹Máté Neubrandt, ⁴Corrado Corti, ⁴Mauro Corsi, ⁵Francesco Ferraguti, ⁶Urs Gerber, ¹János Szabadics

¹ *Celluláris Neurofarmakológia, MTA-KOKI, Budapest, Hungary;* ² *Brain Research Institute, University of Zürich, Zürich, Switzerland,* ³ *Cellular and Developmental Neurobiology, Institute of Experimental Medicine-HAS, Budapest, Hungary;* ⁴ *Molecular and Cell Biology, Aptuit Srl., Verona, Italy;* ⁵ *Department of Pharmacology, Innsbruck Medical University, Innsbruck, Austria;* ⁶ *Brain Research Institute, University of Zürich, Zürich, Switzerland*

Group II metabotropic glutamate receptors (mGlu-IIs) modulate hippocampal information processing through several presynaptic actions. Using subcellular patch clamp recordings from the axonal, somatic and dendritic membrane of dentate gyrus granule cells (GCs) we found that that pharmacological activation of mGlu2 recruits an inwardly-rectifying potassium conductance in the dendrites whereas axonal and somatic regions are unaffected. In contrast to the inhibition of axonal calcium influx, mGlu2 activation slightly enhanced spike-triggered calcium signals in the proximal dendrites suggesting a restricted localization for this mGlu2 response. Somatic hyperpolarization and GABAB activation had similar effects on the dendritic calcium signals but their effects were restricted to the proximal and distal dendritic segments, respectively. Dual soma-dendritic recordings from granule cells suggested that input resistance-dependent changes in the time course of the local action potentials are likely to be important for the unusual, spatially restricted enhancement of calcium signals. Next, we examined the potential functional consequences of the spatial confinement of dendritic mGluR2 activated conductance using glutamate uncaging and multicompartmental simulations. Our results indicate that the mGlu2-activated potassium conductance effectively and uniformly reduces the peak amplitude of synaptic inputs arriving in regions distal to the shunt, whereas inhibition is minor for proximal inputs. Thus, the perforant path inputs onto the distal two-third of the dendritic tree of GCs are inhibited uniformly within individual branches. Furthermore, because of the weak perisomatic shunting and the unique dendritic organization of GCs, neighboring

branches are not affected when mGlu2-mediated shunting is activated in a single dendrite. This selective glutamatergic inhibitory process enables dendritic branch-specific modulation of inputs, consistent with the theoretically predicted independent operation of GC dendrites.

P1.4.

Robust transient rearrangement of the strength of synaptic outputs of hippocampal granule cells

¹Máté Neubrandt, ¹János Szabadics

¹ *Celluláris Neurofarmakológia, MTA-KOKI, Budapest, Hungary*

Dentate gyrus granule cells (DG GC) are unique among cortical principal cells due to several fundamentally different properties, including unusual axonal and dendritic morphology and synaptic properties. Here, we show a novel exceptionality of GCs at the level of plasticity of their synaptic output. Granule cells can be found not only in the dentate gyrus, but several GCs exist within the CA3 region (CA3 GC), which share the basic synaptic properties of DG GCs. Thus, CA3 GCs are reliable and easily accessible models for studying the properties of synaptic connections of GCs to CA3 neurons. Our results show a robust enhancement (8.3 ± 2.1 -fold average relative increase) of the unitary synaptic responses in various types of CA3 neurons that lasted for several seconds following a short burst (15 action potentials at 150Hz) of single presynaptic CA3 GCs. This strong amplification was not permanent as the amplitudes of the synaptic responses returned to their initial levels within one and a half minutes. Large majority of the trials (34 out of 38) at the tested monosynaptic connections from CA3 GC to CA3 interneurons showed enhancement within 6.5 seconds following the presynaptic burst, but the amount and the time course of the amplification of the responses showed clear postsynaptic cell type dependency. Intriguingly, in several cases (n=10) the unitary synaptic responses enhanced even beyond the level of the maximal amplitudes, which occurred during the high frequency presynaptic burst.

The data that demonstrated decreased failure rates and more pronounced depression or less prominent facilitation during the augmented period suggested that the amplitude enhancement was due to mainly presynaptic changes. Altogether, these results highlight a novel form of extremely robust synaptic plasticity that rearranges the synaptic impact of single granule cells within the CA3 network for several seconds.

P1.5.

Different availability of potassium currents distinguishes two functionally distinct populations within CCK-expressing perisomatic-targeting interneurons in the CA3 area

¹Viktor János Oláh, ¹Tibor Andrási, ¹János Brunner, ¹János Szabadics

¹ *Celluláris Neurofarmakológia, Magyar Tudományos Akadémia, Kísérleti Orvostudományi Kutató Intézet, Budapest, Hungary*

The GABAergic cells show great diversity in terms of morphology and functionality, which allow them to control distinct aspects of the activity of hippocampal circuitry through complex interactions. In the hippocampus, more than twenty GABAergic cell types have been described based on their distinct morphology and molecular composition. Additionally, cell type-specific expression of ionic channels provides a further aspect to this functional complexity as they are fundamental in the determination of the excitability of neurons. Here we report heterogeneity in voltage-gated potassium currents among CCK-expressing GABAergic neurons of the CA3 area that results in two functionally distinct firing properties within morphologically homogeneous groups. When activated by current injections from hyperpolarized membrane potential (-80 mV) action potentials were transiently inhibited in a subpopulation of CCK expressing neurons. However, this transient outward rectification was not present in the firing pattern of the same neurons from more depolarized membrane potential (-64 mV). Cells that showed this membrane potential-dependent transient outward rectification included both of the two major

morphological subpopulations of CCK neurons in the CA3, the basket cells and the mossy fiber associated neurons. However, not all cells within these anatomical populations showed transient outward rectification in their firing suggesting functional diversity among these morphologically well-defined groups. Voltage-clamp recordings revealed that CCK cells with outward rectification exhibited a large amplitude, low voltage-activated, inactivating potassium current, which was activated at more hyperpolarized membrane potential than the action potential threshold and showed significant inactivation at -60 mV. In contrast, the inactivating potassium current component was smaller in cells with regular firing pattern and these currents were activated only at relatively depolarized membrane potentials. Analysis of other functional properties, such as the kinetics of the synaptic connections, morphology of the dendritic tree, did not show differences between the cells with different firing characteristics. Altogether, our results revealed functional differences within the anatomically defined populations of CCK-expressing neurons in the CA3 area due to the different availability of distinct potassium currents.

P1.6.

Cholinergic receptor activation differently controls the input-output properties of distinct types of neurons in the basolateral amygdala

¹Boglárka Barsy, ¹Gergely G. Szabó, ¹Norbert Hájos

¹ *Hálózat-Neurobiológia, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary*

The basolateral amygdala (BLA) plays a key role in processing fear learning and anxiety, which is strongly controlled by cholinergic transmission. In the BLA, parvalbumin (PV)-containing fast-spiking basket cells (FSBCs) and axo-axonic cells (AACs) as well as the cholecystokinin (CCK) and CB1 cannabinoid receptor-expressing regular-spiking basket cells (RSBCs) innervate the perisomatic region of projection neurons. In this study we have investigated how cholinergic receptor activation

modulates the perisomatic inhibition which could shape the activity of BLA projecting cells (PC). To specifically target FSBCs and AACs, we used a transgenic mouse expressing eGFP controlled by PV promoter, while the RSBCs were sampled in mice expressing DsRed under the control of CCK promoter. Using whole-cell patch-clamp technique, we recorded miniature excitatory postsynaptic currents (mEPSCs) in amygdalar slices to investigate the cholinergic modulation of glutamatergic inputs. In PCs and RSBCs, bath application of an acetylcholine receptor agonist carbachol (CCh) significantly decreased the frequency of mEPSCs, whereas no change was found in PV-expressing interneurons. The effect in former cell types was reduced by muscarinic receptor antagonist atropine. To reveal the modulation of synaptic inhibition originated from different types of perisomatic inhibitory cells by cholinergic receptors, paired recordings were obtained from presynaptic inhibitory cells and postsynaptic PCs. Bath application of CCh significantly reduced the amplitude of inhibitory currents in all pairs. While this reduction was reversed by muscarinic receptor type 2 antagonist AFDX116 in the FSBC-PC and AAC-PC pairs, similar effect was observed by CB1 receptor antagonist AM251 in the RSBC-PC pairs. These data suggest that cholinergic receptor activation (i) can regulate the transmitter release from glutamatergic terminals in the BLA, an effect that varies among different types of amygdalar neurons; (ii) reduces the efficacy of perisomatic inhibition in PCs. Thus, acetylcholine by altering synaptic transmission could contribute to the regulation of BLA output.

P1.7.

Characterization of single-unit activity recorded with high channel count electronic depth control probes in different thalamic nuclei of the anesthetized rat

¹Rita Patrícia Beregszászi, ²Richárd Fiáth, ³László Acsády, ⁴István Ulbert

¹*Elektrofiziológiai Labor, Pázmány Péter Katolikus Egyetem, Információs Technológiai Kar, Budapest, Hungary;* ²*Institute of Cognitive*

Neuroscience and Psychology, HAS Research Centre for Natural Sciences, Budapest, Hungary; ³*Laboratory of Thalamus Research, HAS Institute of Experimental Medicine, Budapest, Hungary;* ⁴*Faculty of Information Technology, Pázmány Péter Catholic University, Budapest, Hungary*

During natural slow-wave sleep and anesthesia thalamic neurons switch from tonic firing mode typical to the awake state to burst firing activity and exhibit membrane potential levels alternating between depolarized and hyperpolarized states at slow frequencies (0.5-4 Hz). During bursting the information is transferred via fast action potential trains to the target structures. Differences between the burst properties of neurons located in first order and higher order thalamic nuclei could possibly imply distinct intrinsic or network mechanisms among the investigated structures. Literature data about the comparison of the firing patterns of cells in different thalamic nuclei during anesthesia are scarce. Therefore, to examine the differences, we recorded the local field potential, multiple- and single-unit activity from the thalamus of ketamine-xylazine (KX) anesthetized rats with a high-channel count silicon electrode. The probe consists of four 8 mm long shafts with a total of more than 1000 potential recording sites. We can select 32 contacts electronically (8 channels/shaft) through the software of the electrode (electronic depth control; EDC). The EDC makes it possible to adjust recording site positions independently (with some restrictions) inside the brain without physical movement of the probe. Thus the advantages of EDC lie in simultaneous recording from different thalamic areas and minimalizing the brain damage. Action potentials of cells from several first order somatosensory (VPL, VPM) and motor (VA, VL) thalamic nuclei, higher order sensory (Po, LDVL) thalamic nuclei and from the nucleus reticularis thalami (nRt) were recorded together with spikes from additional brain structures (e. g. somatosensory cortex, capsula interna). Spike sorting was performed on the recorded data and the clustered cells were grouped to the corresponding thalamic nuclei based on histological analysis. Several spike and firing properties (firing rate, intraburst frequency, average number of spikes in the bursts etc.) of the recorded neurons were calculated and compared between the different cell groups. According to our preliminary results there are significant differences between the firing characteristics of the investigated thalamic nuclei during KX anesthesia. The research was

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P1.8.

Electroanatomy of the rat hippocampus: input-output relations in single neurons

¹Antal Berényi, ²Zoltán Somogyvári, ³Gautam Agarwal, ¹Anett Nagy, ⁴Erik Schomburg, ¹Attila Nagy, ⁵György Buzsáki

¹ *Élettani Intézet, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary;* ² *Department of Biophysics, KFKI RIPNP of the Hungarian Academy of Sciences, Budapest, Hungary, Hungary;* ³ *Redwood Center for Theoretical Neuroscience, University of California, Berkeley, Ca, Usa, Hungary;* ⁴ *Physics Department, California Institute of Technology, Pasadena, Ca, Usa, Hungary;* ⁵ *Neuroscience Institute, New York University, School of Medicine, New York, Ny, Usa, Hungary*

Knowledge of how afferent inputs are converted to spiking outputs of single neurons in the behaving animal is a requisite for the understanding of neuronal computation. In principle, this can be achieved by monitoring all inputs to the dendritic tree and the spike output of single neurons. Recording the extracellular currents with sufficiently high density in the extracellular space surrounding a neuron should bring us closer to this goal. The orderly cytoarchitecture and the known afferents to neurons in different hippocampal layers facilitate this process. We recorded local field potential (LFP) and spikes at 512 locations in two 1.5 mm X 2.1 mm grids covering parts of dentate gyrus and CA3-CA1 region, using a high density silicon probe (8 shanks with 32 sites at 50 μ m intervals each). The equally spaced and dense recording sites allowed a smooth and detailed reconstruction of the hippocampal neuroanatomy based purely on electrophysiological data. The spatial distribution of high frequency (200-400 Hz) power faithfully identified the cell body layers of pyramidal cells and granule cells. Cross-correlation and coherence of the gamma band (30-

90 Hz), calculated across all recording site combinations, reliably determined the boundaries between the major afferent layers of each hippocampal region. The position of the rat was correlated with the clustered spikes of classified principal cells and interneurons as well as the various bands and combinations of the LFP signal. Many principal cells had well-defined place fields so that the firing rates and the phase of the spikes relative to the theta cycle well defined the position of the rat on the track. Spikes of all place cells faithfully tracked the position of the animal. The power of the high-frequency bands (>300 Hz) could also predict the direction of run on the track. We used the relative power changes in the CA1 str. radiatum and str. lacunosum-moleculare to assess the contributions of the CA3 region and the entorhinal input to the discharge of CA1 place cells in different parts of the place field. When the rat entered the place field, the dominant afferent drive was the CA3 input. In contrast, in the outbound part of the place field, the contributions of CA3 and the entorhinal input were similar in most neurons. In general, our experiments show that high-density monitoring of the extracellular space is a powerful method to reveal the major afferents driving single neurons in the behaving animal.

P1.9.

High frequency oscillations in the human hippocampal formation using single pulse cortical electrical stimulation

¹Virág Bokodi, ¹Emília Tóth, ²Zsófia Maglóczky, ³László Entz, ³Loránd Erőss, ⁴István Ulbert, ⁵Dániel Fabó

¹ *Élettani Intézet, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary;* ² *Department of Biophysics, KFKI RIPNP of the Hungarian Academy of Sciences, Budapest, Hungary, Hungary;* ³ *Redwood Center for Theoretical Neuroscience, University of California, Berkeley, Ca, USA,* ⁴ *Physics Department, California Institute of Technology, Pasadena, Ca, USA,* ⁵ *Neuroscience Institute, New York University, School of Medicine, New York, USA*

Cortical electrical stimulation (CES) and electrically evoked potentials (eEP) are widely used investigational methods to study neural connections and networks both in rodent and human brain. High frequency oscillations (HFO) (ripples and fast ripples) participate in memory consolidation and epileptogenic processes predominantly in and around the hippocampal formation (HcF). There are sparse and not focused publications about HFOs activated by CES. We examined the effect of CES under general anesthesia in the HcF of five temporal lobe epilepsy (TLE) patients. We used laminar multielectrodes to record local field potential (LFP), and spectral activation of iEPs elicited by brief, single pulse, subdural CES (0.1ms; 5- 15mA; 0.5Hz). HFO packages, ripples, were detected by semi automatic methods. Hippocampal regions were reconstructed based on histological assessment of the removed HcFs. Bi- or tri-phasic iEPs were elicited from the HF stimulating the temporo-basal areas. HFO activity was measured during the initial sink in the region of high intensity MUA. The central frequencies of different ripples were 100 and 250Hz. HFO activity was elicited with high probability (60-98%). As a general rule both the subiculum (SUB) and the dentate gyrus (DG) generated HFOs, with highest amplitude in the distal part of the SUB. SUB had lower stimulus amplitude threshold (5mA) than DG (10mA). Stimulation closer to the temporal pole evoked HFOs with higher probability. We conclude that CES evokes stimulus strength dependent response in the HF with high fidelity. The iEPs contain abundant amount of ripples and fast ripples. There are ripple and fast ripple generators both in the SUB and DG of the epileptic human HcF. EPs and evoked HFOs may harbor fundamental information on the epileptogenic properties and plasticity of the human HcF contributing to the understanding of the epileptic transformation of the human brain, and also has the potential to develop an easy to assess diagnostic tool during surgery. The research was supported by the following grants: OTKA PD101754, OTKA K81354, OTKA PD77864, ANR-TÉT Neurogen, ANR-TÉT Multisca, TÁMOP-4.2.1.B-11/2/KMR-2011-0002

P1.10.

Hippocampal sharp waves associated dendritic calcium transients revealed by three dimension acousto-optic imaging in parvalbumin positive interneurons

¹Balázs Chiovini, ²Gergely F Turi, ¹Attila Kaszás, ³Pál Maák, ¹Gergely Katona, ⁴Gábor Szabó, ⁵Dénes Pálfi, ¹Gergely Szalay, ¹Miklós Madarász, ¹Balázs Rózsa

¹ *Bioinformatikai Tanszék, Pázmány Péter Katolikus Egyetem, Budapest, Hungary;* ² *Cellular and Network Neurobiology Dept, Institute of Experimental Medicine, Budapest, Hungary;* ³ *Funkcionális Idegsebészeti Részleg, Országos Idegtudományi Intézet, Budapest, Hungary;* ⁴ *Összehasonlító Pszichofiziológiai Részleg, Institute of Cognitive Neuroscience and Psychology, Budapest, Hungary;* ⁵ *Neurológiai Osztály, Országos Idegtudományi Intézet, Budapest, Hungary*

Sharp wave (SPW) oscillations, the most synchronous activity in the hippocampus, are generated by complex activity of the pyramidal cells and interneurons. Parvalbumin expressing, fast spiking (PV-FS) interneurons are one of the key members in feed forward and feedback inhibitions during SPW activity, however their excitatory dendritic input pattern and signaling during SPW has remained elusive. In order to study dendritic computation under close to physiological conditions a double perfusion recording chamber were used to generate spontaneous SPW activities in vitro (Hájos et al 2009, Katona et al. 2011). We combined whole cell patch clamp electrophysiology with local field recording (LFP) and three dimension fast acousto-optical (3D-AO) imaging to measure the integration of excitatory inputs in PV-FS interneurons (Katona et al, 2012) during SPW oscillations. 3D Ca²⁺ responses were simultaneously measured in the majority of the dendritic arbors during SPW activity. We found SPW associated dendritic Ca²⁺ signals accompanied by somatically recorded supra- and subthreshold responses. SPW associated Ca²⁺ transients were initiated in dendritic hot spots and propagated along the apical dendritic segments both in the presence and absence of somatic action potentials while the basal dendrite of the interneuron remained nearly inactive. The amplitude of the bAP-induced 3D Ca²⁺ responses increased as a function of the distance from the soma when AP triggered

by SPW. Similar distance dependent increase was found during somatically subthreshold events. In addition, there was a positive correlation between the amplitude of the Ca²⁺ transients, LFP and somatic voltage signals. Our results indicate that SPW oscillations can actively induce Ca²⁺ transients in distal dendritic segments of PV-FS interneurons.

P1.11.

Activity of VGluT3-immunopositive median raphe neurons

¹Andor Domonkos, ¹Gábor Nyiri, ²Zsolt Borhegyi, ¹Balázs Hangya, ¹Edit Papp, ¹Tamás F. Freund, ¹Viktor Varga

¹ *Agykéreg Kutatócsoport, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Center for Brain Research, Medical University of Vienna, Vienna, Austria*

The median raphe nucleus (MR) gives rise to the main serotonergic input to hippocampus. In addition, non-serotonergic projecting neurons were also described in the MR. The vesicular glutamate transporter type 3 (VGluT3) was detected in both the serotonergic and non-serotonergic neuron populations, and was shown to facilitate the synaptic transmission in the serotonergic terminals. Glutamate transported by VGluT3 into vesicles in these terminals may be directly involved in synaptic communication. The non-serotonergic, but VGluT3-containing cells might provide a subcortical glutamatergic input to the hippocampus. However, the features of this newly identified glutamatergic pathway are unknown. Formerly, we demonstrated a fast and spatiotemporally focused, ionotropic glutamate receptor dependent activation of hippocampal interneurons following the electric or optical stimulation of MR-fibers. This novel type MR-hippocampal communication is supposed to be involved in shaping the network activity, when rapid response is required. To unravel the function of the glutamatergic MR-hippocampal pathway, we aimed to characterize the firing pattern and coupling to hippocampal network states of juxtacellularly recorded serotonergic and non-serotonergic MR cells,

including those expressing VGluT3. Since the ventromedial prefrontal cortex (vmPFC) is the main cortical input to MR, we analyzed the relationship between recorded cells' firing and vmPFC activity, as well. Recordings were carried out in urethane-anesthetized rats; the recorded cells were filled with Neurobiotin for further anatomical identification and morphological analysis. VGluT3-immunopositive non-serotonergic MR cells exhibited fast and more complex firing than serotonergic MR neuron populations and changed their activity during cortical state transitions. Serotonergic cells fired in a slow and regular manner whereas neurons containing neither serotonin nor VGluT3 were mostly fast spiking and received activation from vmPFC. These preliminary results suggest that VGluT3-expressing neurons are capable of tuning their target network oscillation at high temporal resolution.

P1.12.

Characterization of ubiquitin ligases involved in the regulation of thyroid hormone activating enzyme type 2 deiodinase

¹Péter Egri, ¹Balázs Gereben

¹ *Endokrin Neurobiológia Kutatócsoport, MTA KOKI, Budapest, Hungary*

The thyroid hormone (TH) plays an essential role in brain development and function. The thyroid gland produces predominantly a stable prohormone, thyroxine (T₄) that cannot bind TH nuclear receptors thus activation of T₄ by type 2 deiodinase (D2) is a key element in TH-action in the brain. D2 is an endoplasmatic-reticulum (ER) resident protein that undergoes substrate-mediated ubiquitination allowing a rapid regulation of its activity. This process is mediated by the WSB-1 and Teb4 ubiquitin ligases that are coexpressed with D2 in tanycytes, the main T₃-producing cells of the hypothalamus. In previous work using fluorescence resonance energy transfer method (FRET) we showed the interaction between N-terminus of Teb4 and D2 and we also found this interaction is T₄-sensitive. In the present study we aimed understand molecular mechanisms

underlying D2 ubiquitination in cells, like the self-renewing tanycytes that coexpress D2/WSB-1/Teb4. First we focused on the Sonic Hedgehog (Shh) mediated regulation of the ubiquitin ligases because the WSB-1 is known to be under Shh-mediated positive regulation. Therefore we cloned the 3.5 kb 5'-flanking region (5'-FR) of human Teb4 gene which is GC-rich and lacks a TATA-box. Using Dual Luciferase promoter assay we showed that the deletion of predicted Sp-1 binding sites-containing 130-bp region upstream to TSS results a ~50% decrease in basal promoter activity. We found that the 5'-FR of hTeb4 is not upregulated by the Shh effector transcription factor Gli2 in HeLa cells while the ~3 kb WSB-1 promoter used as positive control was readily induced. In the second part of our studies we tried to mimic the situation in tanycytes where the D2, WSB-1 and Teb4 are coexpressed. We set up a 3-FRET system in HEK293 cells using CFP-Teb4/D2-YFP/WSB1-mCherry fusion proteins allowing the parallel detection of interactions between Teb4/D2/WSB1. We tested the effect of T4 on these interactions and similar increases were measured indicating the equal implication of both ligases in the substrate-mediated degradation of D2. As summary, we found that the T4-sensitivity of D2 ubiquitination acts via both ligases while the Shh-induced downregulation of D2 has ligase specificity. This complex regulatory machinery ensures a rapid fine-tuning of TH action and allows a precise regulation between the proliferative Shh and differentiative TH on cellular level. This could be critically important to regulate cell proliferation in tanycytes.

P1.13.

Anatomico-functional parcellation of the brain based on human electrical stimulation data

¹László Entz, ²Emília Tóth, ³Corey J. Keller, ⁴Stephan Bickel, ⁵Dániel Fabó, ⁶Lajos R. Kozák, ¹Loránd Erőss, ⁷István Ulbert, ⁸Ashesh D. Mehta

¹ *Funkcionális Idegsebészeti Osztály, Országos Idegtudományi Intézet, Budapest, Hungary;* ² *Információs Technológiai Kar, Pázmány Péter Katolikus Egyetem, Budapest, Hungary;* ³ *Department of Neuroscience,*

Albert Einstein College of Medicine, Bronx, N.Y., United States; ⁴ Department of Neurology, Albert Einstein College of Medicine, Bronx, N.Y., United States; ⁵ *Epilepszia Részleg, Országos Idegtudományi Intézet, Budapest, Hungary;* ⁶ *MR Kutató Központ, Semmelweis Egyetem, Budapest, Hungary;* ⁷ *Kognitív Idegtudományi és Pszichológiai Intézet, MTA TTK, Budapest, Hungary;* ⁸ Department of Neurosurgery, NSLIJ, Manhasset, N.Y., United States

Cortico-cortical evoked potential (CCEP) mapping using single pulse electrical stimulation (SPES) in patients undergoing seizure monitoring with invasive intracranial electrode arrays is one means to delineate functional networks and at the same time localize them to distinct brain regions. In order to study the connectivity of the brain across subjects, we mapped each electrode location to a Brodmann's area (BA). By co-localizing each electrode to its respective BA, we are able to study anatomico-functional regions across subjects and create an electrical connectivity-based map of brain areas. 29 patients were enrolled in the study in two major epilepsy surgical centers. Patients were implanted with intracranial electrodes as required for localizing epileptogenic areas prior to surgery. The implanted electrodes were visualized and plotted on the preoperative surface reconstructed MRI to be able to assign each electrode to the underlying BA. SPES (0,5Hz, PW: 0,2ms, 10ma) was performed consecutively on every adjacent electrode pair, and CCEP was recorded. Significant CCEPs (peak exceeding 6SD of baseline) revealed intracortical connections which was analyzed using graph theoretical approaches. Most of the BAs, excluding insular cortex, and some medial areas were covered with electrodes. CCEPs reliably revealed functional networks, Z-score of the amplitude of the CCEP showed a moderate reduction with increasing the distance from the stimulation electrodes. A Z-score based connectivity matrix was created which show typical Z-scores between every BA. We created a 3D map of the BAs with the directed connections. The average degree of connections of each BA was calculated to create a reference for comparisons with pathological areas. The research was supported by the following grants: OTKA K81354, OTKA PD77864, ANR-TÉT Neurogen, ANR-TÉT Multisca, TÁMOP-4.2.2.B-11/2/KMR-2011-0002, NKTH-ANR 3DINVIVO.

P1.14.

Altered kinetic properties of GABAergic synaptic responses after the deletion of the GABAAR $\gamma 2$ subunit

¹Mark Eyre, ¹Katalin Kerti, ¹Zoltan Nusser

¹ *Laboratory of Cellular Neurophysiology, HAS Institute of Experimental Medicine, Budapest, Hungary*

Clustering of GABAA receptors (GABAAR) containing the $\gamma 2$ subunit at GABAergic synapses is thought to be essential for phasic synaptic inhibition. Genomic deletion of the $\gamma 2$ subunit causes neonatal lethality, limiting the usefulness of classical $\gamma 2$ knock-out animals for experimental analysis of mature synapses. To overcome this limitation, we injected adeno-associated viral vectors expressing a Cre-GFP fusion protein into the cortex of young adult mice in which the GABAAR $\gamma 2$ subunit gene was flanked by two loxP sites, allowing spatially and temporally controlled gene deletion. Inhibitory synapse properties of layer 2/3 cortical pyramidal cells (L2/3 PCs) were investigated using whole-cell patch-clamp recordings in acute slices. Miniature IPSCs were still observed in Cre-expressing, $\gamma 2$ subunit-deleted cells, but with reduced frequency and an increased mean weighted decay time constant (τ_w) relative to wild-type and non-Cre-expressing cells. Paired recordings from fast-spiking interneurons and L2/3 PCs indicated a reduced connection probability within the zone of infection. The evoked IPSCs showed a reduced peak amplitude for both Cre-infected and non-Cre-infected cells. However, the τ_w of the evoked currents was only increased in Cre-infected cells. Immunofluorescent labelling indicated a dramatic reduction in the $\gamma 2$ subunit, and a moderate reduction in the $\alpha 1$ subunit within the infection zone. To study quantitatively the distribution of different GABAAR subunits on the infected cells' plasma membrane we carried out immunogold labelling using the SDS-digested freeze-fracture replica labelling (SDS-FRL) method. Using neuroligin-2 to identify GABAergic synapses on the P-face, we found on the E-face a robust reduction of the $\gamma 2$

subunit in perisomatic synapses, but no change in synaptic $\alpha 1$ subunit immunogold labelling relative to controls. Pharmacological experiments indicated that the neuron-specific GABA transporter inhibitor NO711 increases τ_w only in Cre-expressing cells. Preliminary data indicate that low concentrations of the neurosteroid THDOC, a selective positive allosteric modulator of δ subunit-containing GABAAR, had no effect on mIPSC properties. Our results indicate that synaptic inhibitory currents persist after GABAAR $\gamma 2$ subunit removal, but have altered kinetic properties, suggesting that a different receptor population is activated by the released GABA.

P1.15.

Investigation of the thalamocortical slow oscillation in the anesthetized rat with high channel count electronic depth control probes

¹Richárd Fiáth, ²György Karmos, ²Domonkos Horváth, ²Bálint Péter Kerekes, ³Arno Aarts, ⁴Patrick Ruther, ⁵Hercules P. Neves, ⁶László Acsády, ²István Ulbert

¹ *Kognitív Idegtudományi és Pszichológiai Intézet, MTA Természettudományi Kutatóközpont, Budapest, Hungary;* ² *Institute of Cognitive Neuroscience and Psychology, HAS Research Centre for Natural Sciences, Budapest, Hungary;* ³ *ATLAS NeuroEngineering, Leuven, Belgium;* ⁴ *Freiburg, Németország, IMTEK, Freiburg, Germany;* ⁵ *IMEC, Leuven, Belgium;* ⁶ *Laboratory of Thalamus Research, HAS Institute of Experimental Medicine, Budapest, Hungary*

The slow (< 1 Hz) oscillation (SO) emerges during the slow-wave sleep and in certain types of anesthesia and is present over the whole cortical mantle and in most of the thalamic nuclei. The SO is characterized by rhythmic alternation of two phases: the so-called „up-state” or active phase with strong synaptic activity, cell firing and depolarized membrane potential, and the „down-state” or silent phase with neuronal silence and hyperpolarized membrane potential. The exact mechanisms underlying the

SO are still unclear. So far its generation was thought to be mostly of cortical origin however it also requires the dynamic interplay between the cortex and the thalamus. High density simultaneous mapping of thalamic and cortical areas during SO is still lacking. To investigate thalamocortical dynamics, we recorded the local field potential, multi-unit activity (MUA) and single-unit activity simultaneously from various thalamic and cortical areas of ketamine/xylazine anesthetized rats with a high channel count microelectrode array. The array consists of four 8 mm long shafts, each with 257 recording sites arranged into two columns, from which eight contacts can be selected simultaneously per each shaft. The electronic depth control makes it possible to adjust recording site positions independently inside the brain without physical movement of the probe and record simultaneously from several distant thalamic and cortical areas. To obtain a measure of thalamocortical dynamics, we characterized the up-state onset timing difference (USOTD) indexed by MUA between different cortical areas and thalamic nuclei. Compared to somatosensory and association cortices, higher order visual nuclei in the thalamus showed very small and reliable USOTD (LMPR) or were lagging behind the cortical onset activation (LDVL). The average USOTD in case of the first order somatosensory thalamic nuclei (VPL, VPM) depended on the cortical position compared with. The examined higher order somatosensory thalamic nucleus (Po) was leading compared to the cortex on average, with a large up-state onset variability. Our preliminary data indicate systematic differences between different thalamic nuclei in the thalamocortical up-state onset dynamics. Moreover, a large portion of the up-states may be originated in the thalamus under these experimental conditions. The research was supported by the following grants: OTKA K81354, ANR-TÉT Neurogen, ANR-TÉT Multisca, TÁMOP-4.2.1.B-11/2/KMR-2011-0002

P1.16.

Age-related alterations in resting state functional modular structure of neural networks

Networks of anatomically distinct brain regions that show a high level of functional connectivity during rest, including the so-called default mode network (DMN) is assumed to be exclusively crucial for the maintenance of cognitive functioning and presumably is altered in normal aging. The study aimed to investigate resting state network alterations in relation to normal aging. EEG data of young (18-26 years, N=14) and (60-71 years N=16) healthy elderly subjects were recorded during eyes closed and eyes open resting conditions. General cognitive performance was measured by Wechsler Adult Intelligence Scale. Functional brain connectivity was investigated based on the measurement of phase synchronization (phase lag index) in different frequency bands. By application of graph theoretical analysis the spatially non-overlapping functional modular structure of EEG data was determined. We investigated the modular structure of these networks and tested the hypothesis that physiological aging might be associated with changes in modularity of distributed neural networks. Topological roles were assigned to brain regions depending on their specific contributions to intra- and inter-modular connectivity. Both young and older neuronal networks demonstrated significantly nonrandom sub-network characteristics. In the young, the delta oscillatory network was decomposed into 4-7 major modules consisting fronto-central, centro-parietal, bilateral temporal and parieto-occipital regions. The overall modular pattern and the number of modules changed significantly as a result of aging. The analysis of inter-and intra-modular connections suggested an extensive loss of connectivity strength of frontal brain regions characterizing the elderly group. Positive correlation was found between inter-and intra-modular connectivity strength and cognitive performance indicating that the age related loss of communication between different functional brain regions reflects age-related cognitive decline. In summary our results show that the optimal modular pattern of adult age is gradually replaced by a more random topology (disconnection of sub-networks) at higher ages which presumably serve compensatory functions.

P1.17.

Calcium dynamics, release probability and active zone size of hippocampal glutamatergic axon terminals depend on the target cell types

¹Noémi Holderith, ¹Zoltan Nusser

¹ *Celluláris Neurofiziológia, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary*

It is known for decades that synaptic contacts display large diversity in their morphological and functional properties throughout the CNS. Yet relationship between ultrastructural and functional parameters has remained elusive. Recently we have demonstrated that release probability (Pr), action potential (AP)-evoked [Ca²⁺] transient and the number of the docked vesicles scales linearly with the active zone (AZ) area of CA3 pyramidal cell (PC) boutons that contact other CA3 PCs. Here we examined whether this principle holds for those CA3 PC axon terminals that establish functionally different synapses on parvalbumin (PV) and mGluR1 α expressing interneurons. Using two-photon [Ca²⁺] imaging, and post hoc triple immuno-fluorescent labeling we found that the peak amplitude of AP-evoked [Ca²⁺] transients in boutons that contact PV cells (PV-B; G/GMAX=0.16 $\pm$ 0.03, n=10) were 33% higher than in those that synapse on mGluR1 α cells (mG-B; 0.12 $\pm$ 0.04, n=17). Using correlated electron microscopic 3D reconstruction we measured the volume of PV-Bs and mG-Bs and calculated the total amount of fluxed Ca²⁺ per AP. In boutons with large initial Pr (e.g. PV-Bs) the total amount of fluxed Ca²⁺ was more than 2-times higher than in their low Pr (mG-B) counterparts (0.059 $\pm$ 0.027, n=6 and 0.025 $\pm$ 0.01, n=9, respectively). Electron microscopic 3D reconstruction of the two types of terminals in optimally fixed immunolabeled tissue showed that the AZ area of the PV-Bs is 34% smaller than that of the mG-Bs (0.06 $\pm$ 0.02 μ m², n=43 and 0.08 $\pm$ 0.05 μ m², n=46, p=0.0008, respectively) while there is no significant difference in their volume (0.21 $\pm$ 0.09 μ m³, n=43 and 0.23 $\pm$ 0.17 μ m³, n=46, p=0.19,

respectively). Assuming the same set of calcium channels with similar functional properties and active zone-confined distributions at both types of terminals, our data suggest that the Ca^{2+} channel density within the AZ of PV-Bs is ~ 2.2 times higher than that in mG-Bs. Our results are in agreement with a prediction indicating that differences solely in the AZ Ca^{2+} channel density can underlie the differences in the Pr and short-term plasticity of these two axon terminal populations.

P1.18.

Mechanisms of sharp wave-ripple generation and autonomous replay in a hippocampal network model

¹Szabolcs Káli, ¹Eszter Vértes, ¹Dávid G. Nagy, ¹Tamás F. Freund, ¹Attila I. Gulyás

¹ *Agykéreg Kutatócsoport, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary*

The hippocampus displays several distinct patterns of population activity *in vivo*, depending on the behavioral state of the animal. These dynamical patterns include theta-modulated gamma oscillations and low-level irregular activity with periodically occurring large-amplitude sharp wave-ripple complexes (SWRs). During SWRs, neuronal populations in the hippocampus have been found to “replay”, on a faster time scale, activity recorded during theta-gamma activity in the exploring animal, which may be important for long-term memory consolidation. Our aim was to develop a mechanistic understanding of cellular and network mechanisms underlying the generation of SWRs in general, and spatio-temporal sequence replay during SWRs in particular, based on *in vitro* and *in vivo* experimental observations. A recently developed hippocampal slice preparation, in which SWRs arise spontaneously, has allowed the collection of a large and diverse set of data regarding the properties of SWRs, as well as the characterization of several cell types and synapses which are critical in their generation. Based on these data, we developed a

large-scale network model of the hippocampal CA3 region. We found that our model based on measured cellular and synaptic parameters could faithfully reproduce the experimentally observed SWR activity as long as we included an appropriate slow feedback mechanism which was responsible for the termination of SWR bursts. Our model allowed us to rule out several potential candidates for the slow feedback process, suggesting that either slowly activating interneuronal feedback or short-term synaptic plasticity of connections within CA3 might terminate SWRs. Statistical analysis and fitting of the inter-event interval distribution of SWRs in vitro suggested that, following an initial 'refractory period' after each SWR, the next SWR is initiated stochastically, requiring the simultaneous activation of a threshold number of pyramidal cells. When we implemented the changes in cellular and synaptic properties measured in the slice following the activation of cholinergic receptors, our model replicated the experimentally observed transition from SWR activity to gamma oscillations. Finally, applying a spike-timing-dependent plasticity rule to the recurrent excitatory weights during simulated exploration, the emerging weight structure led to the spontaneous replay of sequences of place cell representations during simulated SWRs. Supported by OTKA K83251.

P1.19.

Age-related topological changes of EEG spectral power during working memory task

¹Zsófia Kardos, ¹Brigitta Tóth, ¹Roland Boha, ²Bálint File, ¹Zsófia Anna Gaál, ¹Márk Molnár

¹ *Pszichofiziológiai Osztály, MTA Természettudományi Kutatóközpont Kognitív Idegtudományi és Pszichológiai Intézet, Budapest, Hungary;* ² *Információs Technológiai Kar, Pázmány Péter Katolikus Egyetem, Budapest, Hungary*

Age related changes in memory functions can be captured by the study of memory control mechanisms, such as inhibition or maintenance. These mechanisms are relevant for the successful encoding, retention and retrieval of task-related information. One of the well-known correlates of these control processes is the frontal-midline/anterior theta oscillation, which is thought to be a good predictor of performance in memory tasks. In order to test the hypothesis of the impaired control mechanisms in old age, we compared healthy young adult (n=20) and elderly (n=16) groups in a visual delayed match to sample task, where the memory load was varied across trials (3 or 6 items = easy vs. difficult task). To differentiate between sustained attention and working memory activity, a simple visual odd-ball task was used as a control condition. 33 channel EEG-recording was applied during task execution. In the elderly group, task performance was significantly lower than in the young group in both conditions. In addition, EEG spectral power showed decreased theta activity in the elderly group. Compared to the control task, only the young adults produced more expressed frontal-midline theta synchronisation according to the higher memory load and successful performance. In the elderly group there was also a relative increase of theta activity during task execution but it was not dependent upon task difficulty. The present results indicate that age related differences manifested by lower task-related theta reactivity is a reliable correlate of declining behavioral performance. The present findings support the theory that the organisation properties of memory functions are deteriorating in aging.

P1.20.

Fast two-photon in vivo imaging with three-dimensional random access scanning in large tissue volumes

¹Gergely Katona, ¹Gergely Szalay, ²Pál Maák ¹Attila Kaszás, ²Máté Veress, ¹Balázs Chiovini, ¹Dénes Pálfí, ³Klaudia Spitzer, ⁴Dániel Hillier, ⁴Botond Roska, ¹Balázs Rózsa

¹ Two-Photon Imaging Center, IEM HAS, Budapest, Hungary; ² Dept. of Atomic Physics, Budapest University of Technology and Economics, Budapest, Hungary; ³ The Faculty of Information Technology, Pázmány Péter Catholic University, Budapest, Hungary; ⁴ Neural Circuit Laboratories, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

In order to understand neuronal signal integration, activity should be simultaneously recorded at many spatial locations within the dendritic and axonal tree of a single neuron or at the level of neuronal circuits. Therefore the systematic understanding of brain function requires methods that allow recording neuronal activity at different spatial scales in three dimensions at high temporal resolution. We have developed a high-resolution, acousto-optic two-photon random-access scanning microscope that reaches near-cubic-millimeter scan range (up to $700 \times 700 \times 1.400 \mu\text{m}^3$), with a high scanning speed (up to 500 points/kHz in 3D trajectory scanning mode), with $470 \times 490 \times 2.490 \text{ nm}^3$ resolution in the center core, and less than $1.9 \times 1.9 \times 7.9 \mu\text{m}^3$ resolution throughout the whole scanning volume. The improved performance of the microscope presented here can be explained by a number of factors: Detailed, diffraction-based optical modeling predicted an optimal arrangement of passive and active optical elements. Furthermore, modeling helped to optimize angular dispersion compensation. In contrast to previous arrangements, the four AO deflectors were grouped into two functionally different subunits in order to increase the lateral field of view of scanning. Random-access positioning in the x-y plane was restricted only to the second group of deflectors. In addition, not only deflector driver signals, but also deflector geometry, manufacturing, bandwidth, and TeO₂ orientation differ between deflectors of the two groups. In contrast to previous realizations of AO scanning, we dynamically compensated for optical errors during measurements by adding corrections to the AO deflector driver functions at each imaged point before starting the measurement: this increased spatial resolution in the whole scanning volume. We show 3D optical recordings of AP backpropagation at sub-millisecond temporal resolution. We also show volumetric random-access scanning calcium imaging of spontaneous and visual stimulation-evoked activity in hundreds of neurons of the mouse visual cortex at 80 Hz in vivo.

P1.21.

Analysis of event-related electric activity on 128 channels-EEG using finger-tapping paradigm

¹Zsófia Kelemen, ²György Kozmann, ²Péter Cserti, ³Dániel Fabó, ¹Zoltán Nagy

¹ *Bioelektromos Képalkotó Részleg, Országos Idegtudományi Intézet, Budapest, Hungary;* ² *Villamosmérnöki és Információs Rendszerek Tanszék, Pannon Egyetem, Veszprém, Hungary;* ³ *Neurológiai Osztály, Országos Idegtudományi Intézet, Budapest, Hungary*

Organization of voluntary movement by sequential activation of different cortical areas is studied mostly with BOLD fMRI methods, however the low temporal resolution is a serious limitation of these imaging techniques. Our bioelectric brain imaging approach based on high-density EEG with a 0.5 msec temporal resolution promises new tool to study in details the cortical organization of motor activity in healthy and diseased subjects using finger tapping paradigm. The method relies on 128 channel visually cued index finger tapping EEG data. Major steps of processing include artefact rejection, high-pass and low-pass filtering, trigger synchronized averaging, computation of surface-Laplacian, trigger-synchronized baseline-noise data-window definition, channel-by-channel determination of baseline noise standard deviation (SD), Karhunen-Loeve eigenvector based smoothing, determination of intervals with absolute amplitude significantly surpassing baseline-noise at a user defined significance-level. Results are presented by channel activity graphs, spatial-temporal activation sequence maps either on the scalp or back-projected to the cortical surface. Key words: bioelectric imaging, surface-Laplacian, cortical activation map The study was supported by the TÁMOP-4.2.2-08/1/2008-0018, TÁMOP-4.2.2/B-10/1-2010-0025 and OTKA ⁶9240 projects.

Multimodal analysis of the human cortical synchronous population activity in vitro

¹Bálint Péter Kerekes, ²Attila Kaszás, ²Kinga Tóth, ²Balázs Chiovini, ²Gergely Szalay, ²Dénes Pálfi, ²Klaudia Spitzer, ³István Ulbert, ³Lucia Wittner, ²Balázs Rózsa

¹ *Kognitív Idegtudományi és Pszichológiai Intézet, MTA TTK, Budapest, Hungary;* ² *Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary;* ³ *Institute of Cognitive Neuroscience and Psychology, Hungarian Academy of Sciences, Budapest, Hungary*

Spontaneous synchronous population activity (SPA) emerges from the cortical slices of epileptic and non-epileptic tumor patients maintained in physiological medium in vitro. SPA was recorded until now by our group using sharp intracellular and laminar extracellular methods to analyze the neural mechanisms giving rise to population synaptic/trans-membrane and spiking activity. In order to gain additional spatial information about the network mechanisms involved in the SPA generation, we introduced the two-photon Ca-imaging technique on human in vitro slice preparations. The excellent spatial coverage and resolution of this technique supplements the lower spatial resolution but higher temporal resolution of laminar extracellular, sharp intracellular and whole cell patch recording techniques. Human slices were maintained in a dual superfusion chamber of high flow rate physiological incubation medium and otherwise conventional submerged technique to elicit SPA in a two-photon microscope. The population activity was recorded by laminar extracellular electrodes and an extracellular patch electrode. After identifying the active regions of the slice using electrophysiology techniques, bolus loading of OGB-1 and SR101 was applied on the tissue. The neuronal and glial cells took up these dyes, thus we were able to image the SPA related Ca-transients in pyramidal cells with two-photon technique, simultaneously with extracellular and whole cell patch measurements. Combining high spatial resolution two-photon Ca-imaging technique and high temporal resolution extra- and intracellular electrophysiology techniques may permit

a deeper understanding about the network properties of SPA in the human cortex.

P1.23.

Ca²⁺ transients recorded with femtosecond two-photon laser microscopy in single GABAergic varicosities of hippocampal parvalbumin- and cholecystokinin containing interneurons

¹Máté Kisfali, ²Tibor Lőrincz, ²Katalin Eszter Sós, ²E. Sylvester Vizi

¹*Laboratory of Drug Research, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary;* ²*Department of Pharmacology, Institute of Experimental Medicine - HAS, Budapest, Hungary*

Over the past several years the rapid development in confocal and two-photon microscopies and the introduction of new, highly sensitive fluorescent probes have allowed us to study Ca²⁺ dynamics of cell bodies, dendrites and boutons. Using acute slice preparation in which structural and functional integrity is maintained and the application of femtosecond two-photon laser microscopy provided a possibility to record Ca²⁺ transients in varicosities of hippocampal GABAergic interneurons on a millisecond-time scale with high resolution. We studied calcium dynamics associated with action potentials in single GABAergic boutons of the axonal arbour of fast-spiking parvalbumin-containing (PV+) GABAergic interneurons located in stratum pyramidale and non-fast-spiking cholecystokinin-containing (CCK+) GABAergic interneurons present in the stratum radiatum. Using a somatic patch electrode we delivered high-(OGB-1) or low-affinity Ca²⁺ indicator (OGB-6F and OGB-5N) dye depending on the stimulation parameters and on the expected concentrations of [Ca²⁺]_i. The action potentials (AP) propagated without failures from axon hillock into the axonal arbour and resulted in Ca²⁺ transients. We observed that Ca²⁺ transients recorded in a given bouton could be seen repeatedly without failures or obvious rundown. Therefore

any failure in transmission in response to drug application must originate downstream of AP invasion. The $[Ca^{2+}]_i$ and Ca^{2+} transients in response to somatic single stimulation in CCK⁺ interneurons were much higher and longer lasting than those seen in PV⁺ interneurons. When more than two action potentials were consecutively applied low-affinity fluorescent Ca^{2+} indicators had to be used to accurately track the kinetics of spatially averaged $[Ca^{2+}]$. The $[Ca^{2+}]$ at rest was found to be 50 nM. One AP (OGB-6F) increased $[Ca^{2+}]_i$ by 387 ± 109 nM. When 60 Hz stimulation was applied in the presence of OGB-6F with 25 APs the $[Ca^{2+}]_i$ easily reached 17 μ M. Post hoc immunocytochemistry was used to identify interneurons. Since GABAergic interneurons generate oscillatory activity, synchronize pyramidal cell activity and regulate synaptic integration we will be able to study the role of key players, presynaptic Ca^{2+} transients in GABAergic transmission.

P1.24.

Modulation of neural oscillations during human visual cortical response habituation

¹Balázs Knakker, ²Béla Weiss, ¹Petra Hermann, ²István Kóbor, ³Zoltán Vidnyánszky

¹ *Információs Technológiai Kar, Pázmány Péter Katolikus Egyetem, Információs Technológiai Kar, Budapest, Hungary;* ² *Magnetic Resonance Research Center, Szentágothai J. Knowledge Center, Semmelweis Univ., Budapest, Hungary;* ³ *Department of Cognitive Science, Budapest University of Technology and Economics, Budapest, Hungary*

Visual cortical responses to subsequently presented, repeated stimuli are attenuated. Traditionally, in human electrophysiological research visual cortical response habituation is characterized and quantified by measuring the amplitude of the early components of the averaged ERP responses. As a result of this, we know very little about the changes in the neural oscillations during visual cortical response habituation. Here we addressed

this question by recording EEG while participants were viewing uninterrupted series of six face stimuli. For stimuli 2.-6. (the response to the first stimulus was excluded from the analysis), our ERP results show a small, but progressive N1 habituation effect. In accordance with this, we found a similar right-hemisphere lateralized gradual decrease in the power and inter-trial phase coherence of evoked theta and alpha oscillations at 100-250 ms after stimulus 2 to 6. This suggests that average ERP amplitude decreases arise from both within-trial amplitude and between-trial timing differences of evoked oscillations in these frequency bands. In contrast, the power of ongoing alpha oscillations – measured before each stimulus onset – gradually increased and saturated by the fourth stimulus. These results, in addition to explaining the findings of the traditional ERP approach, yield further insights into how oscillatory processes are modulated by visual cortical response habituation.

P1.25.

Frequency and firing pattern dependent short-term plasticity of IPSCs on CA3 pyramidal cells mediated by perisomatic targeting interneurons

¹Zsolt Kohus, ¹Mária Rita Karlócai, ¹Dániel Schlingloff, ¹Tamás Freund, ¹Attila Gulyás

¹ *Agykéreg Kutatócsoport, MTA KOKI, Budapest, Hungary*

Perisomatic inhibitory inputs to pyramidal cells (PCs) originate from three types of interneurons (INs) in the hippocampus: PV+ fast-spiking basket cells (FSBCs) and axo-axonic cells (AACs), and also CCK+ regular-spiking basket cells (RSBCs). They effectively control the output of PCs and contribute to network activity generation, but their recruitment differs among cell types. During sharp-wave ripples (SWRs) in the CA3 region of mouse hippocampal slices, perisomatic-targeting interneurons (PTIs) show cell-type specific behavior: most active are FSBCs firing 3-5, whilst AACs and RSBCs fire single or double action potentials. In pathological, high K+

induced epileptic-like events all INs increase their firing. To reveal inhibitory synaptic transmission between FSBCs, AACs, RSBCs and their PC targets, we performed whole cell pair recording of PTIs and PCs. We measured the changes of postsynaptic inhibitory currents (IPSCs) in response to presynaptic action potentials triggered with two stimulation patterns. First, we applied 4 trains of APs with 180 Hz (RIP protocol) comparable to the firing behavior of FSBCs during SWRs. Then presynaptic INs were stimulated by a train of 30 APs with 150 Hz followed by 4 APs with 300 Hz (EPI protocol), similar to firing pattern of PV+ INs during high K⁺ induced epileptic-like events. Both protocols were done in control conditions and in presence of high K⁺. Finally, to separate the action of PTIs on PCs, we applied DAMGO (pharmacological tool to selectively modulate PTI release) during RIP protocol. RIP protocol evoked IPSCs showed cell-type specific short-term plasticity (STP). PV+ FSBCs and AACs showed short-term depression, whilst CCK+ RSBCs displayed short-term facilitation. These templates of STP were also observed during EPI protocol, but prominent changes occurred between FSBCs and AACs: while each AP of AACs could evoke an IPSC, the release probability of FSBCs decreased to 0 after 10-15 APs. In high K⁺, FSBCs and AACs showed significantly decreased amplitude of IPSCs, but GABA release of FSBCs was intact. Eventually, we found, that DAMGO differentially modulated transmission in examined groups, it strongly depressed FSBC transmission. Our results highlight the differences of synaptic transmission between among different PTIs and PCs. We suggest that these interactions could be generally important in generation and termination of physiological and pathological oscillations. Supported by: OTKA K83251, NNF 85659

P1.26.

Obestatin enhances the acute analgesic effect of morphine and prevents analgesic tolerance in mice

¹Nándor Lipták, ¹Júlia Szakács, ¹Krisztina Csabafi, ¹Gyula Szabó

The aim of this study was to examine the effect of obestatin (1-23) on the acute and chronic antinociceptive actions of morphine. The heat-radiant tail-flick assay was used to investigate analgesic actions of morphine. CFLP male mice were treated twice a day with 10 mg/kg morphine or saline (s.c.) and once a day with obestatin (1.5 µg/2µl, i.c.v.) or artificial cerebrospinal fluid for five days. Antinociceptive effect was measured on 1st, 3rd and 5th days. In acute experiments, the 1.5 µg/2µl dose of obestatin had a mild analgesic effect 15 minutes after peptide administration compared to control mice. Obestatin maintained the analgesic effect of morphine 90 and 120 min after morphine injection in mice treated with morphine receiving obestatin compared to mice treated with morphine. In tolerance studies, obestatin diminished the analgesic tolerance to morphine on the 5th day in morphine tolerant mice receiving obestatin compared with morphine tolerant mice. In conclusion, obestatin enhanced the morphine-induced analgesia during both acute and chronic morphine treatment. These data suggest that obestatin may have a role in opioid-induced analgesia. This study was supported by ETT-Grant (355-08/2009); TÁMOP 4.2.1./B-09/KONV-2010-0005; TÁMOP-4.2.2/B-10/1-2010-0012 and TÁMOP 4.2.2-A-11/1/KONV-2012-0052.

P1.27.

Effects of two mitochondrial toxins on evoked and spontaneous cortical activity in rats in acute and repeated application

¹Katalin Mikite, ¹András Papp

¹ *Népegészségtani Intézet, SZTE ÁOK, Szeged, Hungary*

Neurons are extremely sensitive to influences impeding normal mitochondrial energy production. One of the mitochondrial toxins tested, manganese (Mn) is a neurotoxic heavy metal the toxic mechanisms of which involve oxidative neuronal damage. 3-nitropropionic acid (3NP), the

other test substance, is also known to cause CNS damage in humans. In previous studies both substances caused alterations in the evoked potentials, and to a lesser extent in the spontaneous activity, of the somatosensory cortex in rats. In some experiments presented here, Mn (50 mg/kg b.w.) and 3NP (20 mg/kg b.w.) was given to adult male Wistar rats acutely during recording in sequential combination, while in others Mn was given to similar rats subacutely, for 5-6 weeks, in the drinking water (7.5 mg/ml, ca 50 ml/day); and 3NP was applied during the recording sessions repeated weekly. The aim was to see possible interactions of the two agents, having similar mechanisms of action, on CNS electrical activity. In the acute electrocorticogram (ECoG) spectrum, 3NP decreased delta, but increased theta activity, especially when given before Mn which had no such effect. In rats with subacute oral Mn exposure, similar effect – no decrease of delta activity, in contrast to Mn-free rats – was seen. Evoked potentials in the somatosensory area had increased amplitude and decreased latency. Acutely, in the Mn-3NP sequence, additive effect was seen but the 3NP-Mn sequence was dominated by 3NP. In the rats receiving Mn via drinking water, the amplitude increase by 3NP was stronger than in Mn-free rats. The interaction of the two mitochondrial toxins tested was more complicated than simple addition, suggesting that more than one mechanism of action was responsible for the CNS effects observed.

P1.28.

Changes in the expression of c-fos in cerebellar granule cells following 4-aminopyridine induced convulsions in rat

¹Zoltán Tóth, ¹Beáta Krisztin-Péva, ¹Gergely Molnár, ¹Marietta Morvai, ¹András Mihály

¹ *Anatómiai Intézet, Szegedi Tudományegyetem, ÁOK, Szeged, Hungary*

Synaptic glutamate release plays an important role in the pathomechanism of acute convulsions. If prolonged, it induces the expression of c-fos in

postsynaptic neurons. Thus, the level of c-fos protein can be used to show the activation of the neuronal network. As previously described, immunoreactive cell nuclei appear in the brain after convulsions with highest numbers in the somatosensory cortex and striatum 60, in the hippocampus 180 minutes after injection. We investigated the expression of the c-fos protein following 4-aminopyridine (4-AP) induced motor convulsions by immunohistochemistry and immunoblotting. Adult male Wistar rats were used, 4-AP was injected intraperitoneally. Convulsions appeared 20 minutes after injection and continued for 40 minutes. At 1.5, 2, 3 and 4 hours following injection the animals were anesthetized and either total protein was purified for immunoblotting or they were fixed for immunohistochemistry, then sagittal frozen sections of the cerebellar vermis were stained with c-fos antibody and peroxidase. C-fos reactive nuclei of the granular layer were counted. Granule cells were significantly activated at 1.5 hours in all lobuli. Highest cell counts were seen at 3 hours which is remarkably different from neocortex. Immunoblotting showed us the same results. Differences in the neuron activation in neocortex and cerebellum can be explained by the existence of an inhibitory neuronal grid. Supported by TÁMOP-4.2.1/B-09/1/KONV-2010-0005.

P1.29.

A BV-2 mikroglia sejtvonal metabolikus szubsztrát-preferenciájának vizsgálata az oxigénfogyasztás mérésével

¹Ádám Miklós Nagy, ¹Veronika Ádám, ¹László Tretter

¹ *Neurobiokémia Munkacsoport, Semmelweis Egyetem, Orvosi Biokémiai Intézet, Budapest, Hungary*

A neurodegeneratív betegségek patomechanizmusában kiemelkedő szerepük van a mikroglia sejteknek. Fontos kérdés azonban, hogy ezek a mobilis sejtek az agy különböző kompartmentjeiben az eltérő metabolikus körülményekhez hogyan tudnak alkalmazkodni, a jelenlévő oxidatív szubsztrátokat milyen mértékben képesek energiatermelésre fordítani.

Jelen kísérleteink célja egérből származó immortalizált BV-2 mikroglia sejtvonal szubsztrát-preferencia vizsgálata a sejtek oxigénfogyasztásának mérésével. Tripánkék festékkel vizsgáltuk a sejtek viabilitását. A BV-2 sejtvonal és a primer mikroglia sejtek általános jellegzetessége, hogy rövid ideig sejtszuszpenzióként is életképesek. Így lehetőségünk adódik oxigénfogyasztásukat mind letapadt formában a fluoreszcens elven működő Seahorse készülékkel, mind szuszpenzió formában Clark elektródás módszerrel mérni. Annak érdekében, hogy a sejtek méréseink előtt endogén szubsztrátjaikat elhasználják, a kísérletek előtt csak fiziológias sóösszetevőket és puffert tartalmazó ACSF folyadékban inkubáltuk a sejteket két órán keresztül, majd ugyanezen ACSF-ben mértük oxigénfogyasztásukat a két műszerrel különböző hozzáadott szubsztrátok (glukóz, piruvát, laktát, ketontestek, aminosav-keverék) jelenlétében. A Seahorse készülékkel követtük továbbá az extracelluláris médium pH csökkenését is, amelyből következtethetünk a sejtek glikolízisének mértékére. A viabilitás vizsgálat alapján a két órás éheztetés nem befolyásolta a sejtek életképességét. Méréseink során megfigyeltük, hogy az alaplégzést a glukóz csökkentette, és ezt a csökkenést az extracelluláris pH jelentős csökkenése is kísérte, míg az egyéb mitokondriális szubsztrátok (laktát és aminosav-keverék) a légzést kis mértékben emelték. Az ATP szintézist gátolva a sejtek oxigénfogyasztása nagymértékben csökkent, amely a mitokondriumok funkcionális kapcsoltságára utal. Szétkapcsolószerezrel az endogén szubsztrátok oxidációját stimuláltuk, így elértük az energiatartalék részleges kimerülését. Az ezt követően hozzáadott szubsztrátok hatására a respiráció jelentősen megnőtt. A legerőteljesebb légzésfokozódást glutamin jelenlétében tapasztaltuk. Kísérleteink alapján a mikroglia sejtek metabolikus sokszínűséget mutatnak, képesek ketontestet, glukózt, laktátot, piruvátot és aminosav-keveréket felhasználni oxidatív metabolizmusuk során. Megállapítottuk továbbá, hogy a glikolízisbe be nem kapcsolódó tápanyagok közül leginkább a glutamint képesek felhasználni oxidatív anyagcseréjük táplálására.

Three-dimensional population measurement in complete hippocampal preparations during rhythmic oscillations

¹Róbert Nagy, ²Attila Kaszás, ¹Gergely Szalay, ¹Gergely Katona, ³Pál Maák, ³Máté Veress, ¹Klaudia Spitzer, ¹Dénes Pálfı, ¹Balázs Chiovini, ⁴Christophe Bernard, ¹Balázs Rózsa

¹ *Kétfoton Képzalkotó Központ, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Two-Photon Imaging Laboratory, Pázmány Péter Catholic University - ITK, Budapest, Hungary;* ³ *Department of Atomic Physics, University of Technology and Economics, Budapest, Hungary;* ⁴ *Faculté de Médecine de la Timone, INSERM UMR1106, Marseille, France*

Neurons receive thousands of inputs from other neighboring and distant neurons, and it has long been a question how these connections form assemblies or neuronal networks of synchronously working units. This assembled function has long been proposed of being a key component in how neurons compute in the neuronal networks. GABAergic interneurons are presumed to control the synchronization of pyramidal cell activity. In this study, we aimed to investigate network activity during spontaneous rhythmic oscillation in complete (in toto) hippocampal preparations. We have developed a high-resolution, acousto-optic (AO) two-photon microscope with continuous three-dimensional (3D) trajectory and volumetric random-access scanning modes that reaches near-cubic-millimeter scan range (up to $700 \times 700 \times 1.400 \mu\text{m}^3$) which allows calcium imaging of spontaneous activity from hundreds of neurons with sub-millisecond temporal resolution. We saw correlated LFP and Ca^{2+} activity in area CA1 during spontaneous rhythmic activity, and found an increased role of interneurons in network oscillations of hippocampus. Nevertheless, whether interneurons are driven by local network activity or their long-range inputs is still unclear. What's more, their connection to the local network is still to be investigated and their connectivity patterns are to be elucidated.

Larger number of pyramidal cells excites basket than axo-axonic cells in the CA3 region of mouse hippocampus

¹Orsolya Papp, ²Rita Karlócai, ¹Irén Tóth, ²Tamás Freund, ¹Norbert Hájos

¹ *Hálózat-Idegélettan Kutatócsoport, Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Agykéreg Kutatócsoport, Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary*

In the hippocampus parvalbumin-expressing axo-axonic cells (AAC) and basket cells (BC) spike differently during distinct brain states, but the cellular mechanisms underlying this difference are poorly understood. Using whole-cell patch-clamp techniques, we investigated the membrane properties and excitatory synaptic features of anatomically identified AACs and BCs in the CA3 region of mouse hippocampal slices. The analysis of the results showed that BCs had lower action potential (AP) threshold and input resistance, narrower AP and afterhyperpolarization than AACs. In addition, BCs fired with higher frequencies and with more modest accommodation compared to AACs. The kinetics and the AMPA/NMDA ratio of these currents as well as their magnitude necessary to evoke an AP were similar in both cell types. However, smaller excitatory postsynaptic potential and smaller stimulus intensity was necessary to drive the firing in BCs. Moreover, the frequency of spontaneous EPSCs in BCs was higher than in AACs. A cholinergic receptor agonist carbachol differentially increased the excitability, but comparably decreased the maximal electrically evoked EPSCs in both interneuron types. Neurolucida analysis revealed that the dendrites of BCs in strata radiatum and oriens were longer and more extensively ramified. Since the density of the excitatory synapses was estimated to be comparable in both cell types, we conclude that the more elaborated dendritic arbor of BCs ensures the larger number of proximal excitatory inputs in BCs. Thus, CA3 pyramidal cells more profoundly innervate local BCs than AACs, which could explain their distinct spiking behavior under different hippocampal network activities.

In vivo Activity of an Ascending Reticular Inhibitory Pathway

¹Viktor Plattner, ²Marco Diana, ¹Hajnalka Bokor, ¹Kristóf Giber, ²Stéphane Dieudonné, ¹László Acsády

¹ *Celluláris és Hálózatneurobiológiai Osztály, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Centre National de la Recherche Scientifique, École Normale Supérieure, Paris, France*

Ascending brainstem activating systems are known to have a crucial role in the regulation of sleep-wake cycle and sustaining conscious state. These cholinergic, noradrenergic and serotonergic pathways act via the thalamus by depolarizing thalamocortical (TC) neurons and switch their activity pattern from sleep to awake, information conveying mode. In the glycine transporter type 2 (GlyT2) – eGFP transgenic mouse line, however we discovered a massive and highly selective glycinergic inhibitory input to the intralaminar (IL) thalamic nuclei originating in the brainstem reticular pontine nucleus (RPO). In addition to glycine all IL thalamus projecting RPO cells expressed GABA. Glycinergic-GABAergic terminals innervated thick proximal dendrites of TC cells and formed synapses with multiple release sites. Selective in vitro photostimulation of the glycinergic RPO fibers resulted in non-depressing strong inhibitory postsynaptic currents in IL neurones. To examine the in vivo firing pattern of the IL thalamus projecting glycinergic-GABAergic cells we used juxtacellular recordings in the RPO in ketamine-xylazine anesthetized mice with concurrent local field potential (LFP) recording in the frontal cortex (FC), which is known to be reciprocally connected to the IL thalamus. GlyT2 positive RPO neurones fired rhythmic clusters of action potentials. The clusters were locked to different phases of the frontal cortical slow oscillation. Electrical stimulation of the FC resulted in fast and faithful response of the RPO units. Both chemical inactivation and spontaneous desynchronization of the frontal cortical activity disrupted the rhythmic firing of glycinergic cells indicating the strong cortical control over these RPO neurons. After

the injection of anterograde tracer to the FC we found labeled fibers and terminals in the RPO and cortico-RPO synapses at the electronmicroscopic level. Our results indicate that next to the ascending reticular activating systems there is a highly selective and effective inhibitory pathway originating also in the brainstem reticular core and being under frontal cortical control. This pathway can interact with the activating systems in the IL thalamus to tune the sensitivity and activity level of the thalamocortical system and regulate conscious behaviour.

P1.33.

Synaptic GABA-A receptor independent regulation of major thalamocortical rhythms

¹Zita Rovó, ¹Ferenc Mátyás, ¹Péter Barthó, ¹Andrea Slézia, ²Balázs Hangya, ³Sandro Lecci, ³Anita Lüthi, ¹László Acsády

¹ *Thalamus csoport, Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Cold Spring Harbor, Cold Spring Harbor Laboratory, Ny, United States;* ³ *Department of Fundamental Neuroscience, University of Lausanne, Lausanne, Switzerland*

GABA-A receptor-(GABA-AR) mediated inhibition is essential for the normal function of thalamocortical networks. Indeed, injection of GABA-A receptor antagonist into the thalamus elicits spike and wave discharges (SWD) in the cortex. GABA-A receptors assemble as either gamma subunit-containing synaptic GABA-A receptors (gamma-GABA-ARs), which are responsible for the fast, phasic, synaptic inhibition, or as delta subunit containing, which induce a persistently active or tonic current via extrasynaptic GABA-A receptors. To address the contribution of these two receptor populations to the diverse forms of GABA-AR-mediated inhibition in thalamocortical cells, we injected AAV1-CMV-Cre viral particles into the thalamus of mice with floxed gamma2 subunit genes, which is the only known gamma subunit expressed in the thalamus. This resulted in the deletion of the gamma2 gene, as verified by the loss of

immunoreactivity for gamma2 protein in the injected area within 10 days. Immunoreactivity for the other subunits of GABA-ARs remained unaltered. The lack of GABA-AR gamma2 subunit caused a complete loss of fast spontaneous and evoked, monophasic gabazine-sensitive IPSCs in the thalamus in acute thalamic slices but also significantly reduced tonic inhibition and increased cellular input resistance. Interestingly, however, loss of gamma2 subunit in the same thalamic region did not cause any epileptiform activity. Juxtacellular recordings of virally infected relay cells in vivo revealed no aberrant firing activity. All recorded neurons were able to produce rhythmic low-threshold bursts despite lacking gamma2-GABA-ARs. Thalamocortical spindle activity in the infected region also remained similar in many aspects to contralateral side. In order to test, what form of inhibition may be responsible to maintain the normal activity we tested the effect high-frequency burst discharge of GABAergic afferents, which prevail during sleep. Under this condition GABAergic inputs evoked slowly rising absence of gamma2-containing receptors, albeit with significantly slower kinetics. The data shows a novel form of inhibition which is phasic in a sense that it is linked to presynaptic activity but extrasynaptic in a sense that it does not require synaptically localized receptors. Together with tonic inhibition, this hybrid form of inhibition is sufficiently strong to maintain normal sleep oscillations in the absence of synaptic GABA-A receptors.

P1.34.

Comparison of tooth development in wild type and PACAP-deficient mice

¹Balázs Sándor, ²Krisztián Fintor, ³Péter Kiss, ³Adél Jungling, ³Eszter Hani, ⁴Péter Nagy, ⁴Zsuzsanna Helyes, ¹Ákos Nagy, ³Dóra Reglődi, ³Andrea Tamás

¹ Fogászati és Szájsebészeti Klinika, Pécsi Tudományegyetem Klinikai Központ, Pécs, Hungary; ² Ásványtani, Geokémiai és Kőzettani Tanszék, Szegedi Tudományegyetem TTIK, Szeged, Hungary; ³ Anatómiai Intézet,

PTE-MTA "Lendület" PACAP kutatócsoport, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary; ⁴ Farmakológiai és Farmakoterápiai Intézet; Szentágothai János Kutatóközpont, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary

Tooth buds are derived from the ectoderm of the first branchial arch and the ectomesenchyme of the neural crest, therefore, their development shows similarities with the development of the nervous system. Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide with widespread distribution. It has trophic and protective effects in the CNS and periphery and it plays a role in the development of the nervous system. PACAP-immunoreactive fibers have been found in the odontoblastic and subodontoblastic layers of the tooth pulp, but there is no data about the effect of endogenous PACAP on tooth development. Earlier we found that the dentin layer of molar teeth in PACAP deficient mice was significantly thinner, using morphometric measurements on native histological sections of 7-day-old mice. In our present study structural analysis of the dentin and the enamel of the developing teeth was performed by Thermo Scientific DXR Raman microscope in the same sections of the 7-day-old wild type and PACAP deficient mice. In adults (2 months old mice) we have done morphometric measurements on the incisors with Sky Scan Micro CT. The Raman spectra of the enamel in the wild type mice demonstrated a broader range of 1240/1270 ratio, which indicates a higher diversity in secondary structure of enamel protein in contrast to PACAP deficient mice. In the dentin of PACAP deficient mice we found higher intracrystalline disordering in the hydroxyapatite molecular structure compared to wild type mice. Differences were found with micro CT in the incisors of the adult mice: the pulp chamber was significantly smaller in PACAP deficient mice. These observations suggest that PACAP plays a role in tooth development, in the dentin and enamel formation. (Support: OTKA (K104984), TAMOP (4.2.1.B-10/2/KONV-2010-002, 4.2.2.B-10/1-2010-0029, 4.2.2.A-11/1/KONV-2012-0024), Arimura Foundation, Bolyai Scholarship, "Lendület" Program)

Distinct role of pyramidal cells and PV+ cells in sharp wave-ripple initiation, termination and ripple genesis.

¹Dániel Schlingloff, ¹Szabolcs Káli, ¹Zsolt Kohus, ¹Tamás Freund,
²Norbert Hájos, ¹Attila Gulyás

¹ *Agykéreg Kutatócsoport, MTA-KOKI, Budapest, Hungary;* ² *Hálózat-Idegélettani Kutatócsoport, MTA-KOKI, Budapest, Hungary*

Sharp wave-ripples (SWRs) occur in the hippocampus during awake immobility and NREM sleep, and have role in memory consolidation. In vivo studies revealed the firing pattern of distinct cell types during SWR activity, but several questions concerning the generation of SWRs are still open. Our goal is to reveal the mechanisms of SWR initiation, termination and ripple genesis. To understand how SWRs are initiated we examined how the activity of inhibitory and excitatory neurons changes during different phases of SWRs by recording EPSCs/IPSCs from neurons of the CA3 area in vitro. Excitatory activity increases ~60 msec before SWR peak. Inhibitory cells are activated ~20 msec later, quickly overshoot pyramidal cell (PC) activity and start to ripple-phase modulate cellular firing. The initiation of SWRs can be explained by a mixed stochastic/refractory model. To dissect LFP generators in stratum pyr. during SWRs, we used local drug applicaitons, without influencing global network activity. Blockade of inhibition in str. pyr. by local puff application of Gabazine or Agatoxin proved that SWR field generation and ripple phase locking of cellular firing is driven by parvalbumin positive perisomatic cells (PVCs). Modelling studies have shown, that interconnected network of interneurons driven with excitation, can generate high frequency oscillations. From PVCs only fast spiking basket cells (FSBCs) are interconnected and fire 4-5 action potentials (APs) during SWRs suitable for ripple generation. A small cut that selectively severs horizontally running FSBC axons and thus mutual connectivity between two subset of cells, introduced a jitter in SWR timing, and abolished ripple synchrony on the two sides of the cut. Furthermore FSBCs desynchronised and increased their firing for somatic Gabazine puff. SWR termination might be the result of the short-term synaptic depression observed in the inhibitory transmission of PVCs onto PCs. Manipulating

the firing pattern and the release probability of PVCs by bath application of Riluzole and DAMGO we checked the validity of this mechanism. Riluzole reduced the number of FSBC APs from 4-5 to 1-2 (had no effect on other cell types), shortened SWRs and decreased the number of ripple cycles from 4-5 to 2 with a curtailed period of multiunit (MU) firing. DAMGO that decreases release probability and short-term depression of PVCs eliminated ripple oscillation and phase-locking of MU firing, while prolonged SWRs along with MU.

P1.36.

Dendritic hot spots in CA1 stratum radiatum interneurons

¹Zoltán Szadai, ¹Attila Kaszás, ¹Gergely Turi, ¹Gergely Katona, ¹Miklós Madarász, ¹Balázs Rózsa

¹ *Kétfoton Képző Képző Központ, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary*

An unresolved issue regarding neuronal Ca²⁺ signaling is the phenomena of dendritic “hot spots” of backpropagating action potential evoked Ca²⁺-transients (bAP-Ca²⁺) in GABAergic interneurons. In this study, a combination of two-photon imaging and whole cell recording was used on CA1 stratum radiatum interneurons in order to analyze the regional differences considering the backpropagation of APs and the response to synaptic stimulation. In the first series of experiments, we applied a novel dual superfusion chamber, in which the physiologically relevant network activity is maintained, thus allowing the observation of spontaneous synaptic events such as excitatory postsynaptic potentials and spontaneous dendritic spikes. We observed that dendritic hot-spots of bAP-Ca²⁺ co-localize with spontaneous dendritic spikes (dSpikes) located after dendritic branching points, such that the amplitude of five bAPs was increased in regions located at the peak of dSpikes. Next, we changed to a common submerged chamber and performed the comparison of synaptic excitability of the proximal and distal dendritic regions in response to MNI-glutamate

uncaging. Here, in the second- and third-class dendrites we found a significant increase in the Ca²⁺-influx between the proximal and the distal dendritic regions. Our results suggest that, similarly to pyramidal cells, dendritic active zones can occur in CA1 interneuron dendrites.

P1.37.

State-dependent modulation of medial septal neurons by the hippocampo-septal feedback.

¹Viktor Varga, ¹Edit Papp, ¹Gábor Nyiri, ¹Tamás Freund

¹ *Celluláris és Hálózatneurobiológiai Osztály, Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary*

Mutual inhibition plays a key role in brain oscillations. Hippocampal theta is a prominent inhibition-based rhythm indispensable for the emergence of synchronously active neuronal ensembles during information acquiring behaviors. The GABAergic reciprocal connection between the medial septum and the hippocampus forms the backbone of limbic theta genesis. Septo-hippocampal GABA-releasing neurons may be the pacemakers of theta but the function of the hippocampo-septal (HS) feedback is still unknown. Here, we aimed to uncover how the selective activation of HS fibers in the medial septum modulates the activity of theta-related cells in urethane-anesthetized mice. HS projection neurons expressing somatostatin were transduced by a Channelrhodopsin2-containing construct. HS fibers were stimulated by 473 nm light and the response of MS neurons was recorded juxtacellularly followed by the labeling and identification of the neuron. Twenty-three of 32 cells responded to HS fiber stimulation. All of them showed robust inhibition at short latency and in some cases inhibition was followed by slight activation. Based on the response to trains of pulses at 5, 10, 20 and 50 Hz neurons fell into two subgroups: some of them were gradually suppressed by increasing stimulation frequency whereas unexpectedly, the majority exhibited facilitation. Purely inhibited cells were rhythmically modulated at the

stimulation frequency as opposed to facilitated neurons that followed weakly or not at all the stimulation. Importantly, activity of the latter subgroup was affected only when pulses were delivered during non-theta or spontaneous theta states. In contrast, activation of the HS input failed to alter their activity during sensory stimulation-induced theta. Our results unravel a hippocampal state-dependent modulation of the medial septal theta-pacemaking network by the hippocampo-septal feedback.

P1.38.

Balatoni algakivonatok neuronális támadáspontjai *Helix pomatia* központi idegrendszerében – acetilkolin receptorok jellemzése

¹Ágnes Vehovszky, ²János Győri, ²Attila Kovács W., ²Henriette Szabó,
²Anna Farkas

¹ Hidrobotanikai Osztály, Környezettoxikológiai munkacsoport, MTA, Ökológiai Kutatóközpont, Balatoni Limnológiai Intézet, Tihany, Hungary; ² Hidrobotanikai Osztály, Környezettoxikológiai munk, MTA, Ökológiai Kutatóközpont, Balatoni Limnológiai Intézet, Tihany, Hungary

Balatoni algavirágzások során gyűjtött és az MTA ÖK BLI algagyűjteményében fenntartott egyes kékalga (*Cylindrospermopsis raciborskii*) törzsek vizes kivonatainak neurotoxikus hatását elektrofiziológiai tesztek bizonyítják. A válaszok heterogenitása arra utal, hogy az algakivonatokban többféle neuroaktív metabolit van jelen, de nem zárhatjuk ki a bioaktív komponensekre “válaszoló” receptorok sokféleségét sem. *Helix pomatia* központi idegrendszerének azonosított neuronjait jellemeztünk extracellulárisan applikált acetilkolin (ACh) elektrofiziológiai hatásai (feszültségváltozások ill. membránáramok) alapján. A D cluster neuronok ACh válaszaik megfordulási potenciálja, kinetikai és farmakológiai tulajdonságai szerint heterogén csoportot alkotnak. A nikotinerg (dTc szenzitív) ACh receptorok elsősorban Cl⁻ csatornák nyitásával, a dTc-re kevésbé érzékeny ACh receptorok a membrán K⁺ permeabilitás növelésével hoznak létre membránáramot. Az RPas

neuronokon az ACh áram hordozói (változó arányban) Na⁺ és K⁺ lehetnek, megfordulási potenciáljuk szerint azonban nem alkotnak diszkrét csoportokat. Az RPas neuronok farmakológiailag és kinetikailag szintén homogén csoportot alkotnak, ACh receptoraik nikotinerg (nACh) és muszkarinerg (mACh) tulajdonságokat egyaránt mutatnak. Ezek a neuronok a nACh receptoron ható tiszta algatoxinra (anatoxin-a) azonban nagyságrendekkel alacsonyabb (nM) koncentrációban is érzékenyek. Az algakivonatok fizikai/kémiai tulajdonságaik (oldékonyság, polaritás) szerinti további frakcionálásával várhatóan elkülöníthetők lesznek egyes részfrakciók, melyek neurotoxikus hatásainak ismételt elektrofiziológiai jellemzését követően végül a neurotoxikus hatóanyagok kémiai azonosítását is megkísérelhetjük.

P1.39.

Effective control of principal cell firing by fast spiking and regular spiking inhibitory cells in mouse basolateral amygdala

¹Judit Veres, ¹Attila Gergő Nagy, ¹Norbert Hájos

¹ *Hálózat-Idegélettani Kutatócsoport, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary*

The amygdala plays a central role in fear and extinction memory formation, however the operation of the neuronal networks during these processes are not fully understood. Inhibitory synaptic control of principal neuronal activity has been proved to be essential for proper network function in the amygdala, however the precise role of different inhibitory cell types has yet to be determined. In this work we tested the ability of fast spiking and regular spiking interneurons to control the output properties of the principal cells (PCs) in the basolateral amygdala of mice. We recorded from synaptically connected pairs of interneurons and PCs using in vitro whole-cell patch-clamp technique. Amygdalar slices were prepared from parvalbumin-EGFP, GAD65-EGFP or CCK-DsRed transgenic mice. First, we recorded IPSCs and IPSPs in the postsynaptic PC. Next, by injecting a

sinusoidal current into PCs, regular firing was initiated. We then evoked action potentials in the interneuron ³0-40 ms before the peak of the oscillation to test their capacity to inhibit PC firing. We found that the majority of both fast spiking (n=14) and regular spiking interneurons (n=12) could veto PC firing. However, we also observed interneurons in both cell types, which were unable to completely prevent action potential generation in the postsynaptic PCs. Although fast spiking and regular spiking interneurons may play distinct roles in cortical network functions, our results suggest that under our circumstances both interneuron types can effectively control action potential generation in PCs. By regulating the output of PCs, these GABAergic cells are in the position to powerfully contribute to the formation and expression of fear memories.

P1.40.

L and D-aspartate in proliferative and non-proliferative regions of the brain of the domestic chick

¹Gergely Zachar, ²Zsolt Wagner, ²Tamás Tábi, ²Tamás Jakó, ²Éva Szökő, ¹András Csillag

¹ *Anatómiai, Szövet-és Fejlődéstani Intézet, Semmelweis Egyetem, Budapest, Hungary;* ² *Gyógyszerhatástani Intézet, Semmelweis Egyetem, Budapest, Hungary*

More and more evidence points to the fact that L-aspartic acid (L-asp) has neurotransmitter properties in the central nervous system of various vertebrates. Our earlier results suggest that L-asp together with L-glutamate is released in the striatum of young domestic chicks. Unlike glutamate, the enantiomer of L-asp, D-asp is also synthesized in the brain of mammals and birds by the asp-racemase enzyme. The function of D-asp is still disputed, however, its relatively high concentration during embryonic development raises the possibility that D-asp plays a role in the early development of the brain. A few studies support the hypothesis, that D-asp also modulates adult neuroplasticity, probably through regulation of

neurogenesis. In chicks, neurogenesis mainly takes place in the subventricular proliferation zone (SVZ) of the lateral ventricle. In the present study, the concentration of L- and D-asp was measured in brain tissue samples from the SVZ and nidopallium of young domestic chicks. Capillary electrophoresis coupled with laser induced fluorescent detection was used to detect and quantify the two enantiomers from tissue homogenates. D- but not L-asp showed higher concentration in the SVZ as compared to a non proliferative region (nidopallium). Concentration of L-glu showed no difference between the two brain regions. D-asp as percent of total asp concentration also decreased during the first 4 day of development. D-asp level seems to continue decreasing even during postembryonic development, suggesting that its developmental action still persists after hatching. Higher concentration of D-asp in an actively proliferating brain region supports a specific involvement of D-asp in the neurogenesis of young domestic chicks.

P1.41.

How the visual cortex handles stimulus noise: insights from amblyopia

¹Éva M Bankó, ^{1,2}Judit Körtvélyes, ³Béla Weiss, ^{1,3,4}Zoltán Vidnyánszky

¹*Faculty of Information Technology, Pázmány Péter Catholic University, Budapest, Hungary;* ²*Department of Ophthalmology, Semmelweis University, Budapest, Hungary;* ³*MR Research Center, Szentágothai J. Knowledge Center - Semmelweis University, Budapest, Hungary;* ⁴*Department of Cognitive Science, Budapest University of Technology and Economics, Budapest, Hungary*

Human visual object recognition is fast and efficient when viewing conditions are good. However, under low visibility conditions the visual system must recruit additional processing resources to handle the noisy and deteriorated visual images, thus object recognition becomes more effortful. Even though this is often the case, little is known about the neural mechanisms engaged in sensory processing of noisy images and their

impairments in visual disorders, such as amblyopia. Here we show in amblyopic patients that their behavioral deficit in categorizing face images with phase noise is coupled with a diminished noise-modulation of ERP response amplitudes in the case of the P1 and P2 component when stimuli are presented to the amblyopic compared to the fellow eye; the smaller the amplitude modulation, the more severe their noise-related behavioral impairment. On the other hand, the N170 ERP component, reflecting the structural processing of face images, was similarly affected by the presence of noise in the two eyes and its modulation did not predict the behavioral deficit. These results revealed that the efficient processing of noisy images depends on the engagement of additional processing resources both at the early, feature-specific as well as later, object-level stages of visual cortical processing reflected in the P1 and P2 ERP components, respectively. Our results also provide evidence for impaired visual cortical processing beyond early object recognition in amblyopes.

P1.42.

The effects of alpha7 nicotinic acetylcholine receptor agonist PHA 543613 on maintained and NMDA-evoked firing activity of CA1 hippocampal neurons *in vivo*

¹ZK Bali, ²D Budai, ¹I Hernádi

¹ *Department of Experimental Zoology and Neurobiology, University of Pécs;* ² *Kation Scientific Ltd., Minneapolis, United States*

Alpha7 nicotinic acetylcholine receptors ($\alpha 7$ nAChR) are widely investigated possible targets for cognitive enhancement strategies associated with dementia research. Previous scientific data suggest that $\alpha 7$ receptor agonists act as presynaptic neuromodulators on hippocampal pyramidal cells by enhancing glutamatergic neurotransmission, and, thus their effects result in increasing maintained firing rate. To test this hypothesis *in vivo*, we studied the effects of a known selective $\alpha 7$ nAChR agonist PHA 543613 and a selective antagonist methyllycaconitine, (MLA)

on NMDA-evoked excitation of CA1 neurons. Extracellular single-unit recording and simultaneous microiontophoresis were performed in 15 anesthetized Wistar rats. Preliminary results show that most of the neurons (60%) in the CA1 region responded to NMDA with increased excitability during simultaneous $\alpha 7$ receptor stimulation, but opposite effects (firing rate suppression) of PHA 543613 were also recorded in 25% of the neurons. Blockage of $\alpha 7$ receptors with MLA also resulted in mixed effects as NMDA-excitation was inhibited in 80% of neurons while it was facilitated in 20% of the recorded neurons. The present results suggest that simultaneous activation of $\alpha 7$ nAChRs by PHA 543613 does not necessarily result in maintained or NMDA-evoked firing rate increase, and the observed effects (incl. excitatory action induced by MLA) may rather show the involvement of $\alpha 7$ nAChRs in the fine-tuning of local cortical microcircuits. Further analysis may provide new insights in understanding how therapeutics targeting nAChRs may exert their action. The present research may also contribute to the development of new effective testing methods for chemicals that are being explored for the treatment of cognitive deficits in neurodegenerative disorders e.g., schizophrenia and Alzheimer's disease.

P1.43.

Input-output constellation of neurons at pinwheel-centers in cat primary visual cortex

¹Tahir Uddin, ²Cyril Monier, ²Yves Fregnac, ¹Zoltan Kisvarday

¹ *Anatomy, Histology & Embryology, University of Debrecen, Hungary;* ² *Unit of Neuroscience Information and Complexity, CNRS-Centre National de la Recherche Scientifique*

The purpose of the present study is to investigate the input-output constellation to and from pinwheel neurons (i) by assessing their synaptic input dynamics at the membrane potential level to complex visual stimuli, (ii) measure their output spiking activity (iii) as well as termination of the

labeled boutons on neighbouring neurons in the orientation map. In our ongoing experiments, we are using a host of functional and anatomical tools. Intrinsic signal optical imaging is applied to acquire orientation map in cat V1 that helps reveal pinwheel centers where all orientations meet as singular locations and surrounding orientation domains where neurons share similar orientation preference. Intracellular recording is used to estimate synaptic subthreshold input and its integration to generate output spikes of the individual neuron. Biocytin is used in recording solution to label recorded neurons and visualize cell morphology and output connections with the help of a neuron reconstruction system (Neurolucida). Our preliminary results demonstrate the feasibility of such a complex investigation for the same cell. We have successfully labeled a layer 2 pyramidal neuron at a near-pinwheel center location after recording subthreshold synaptic input signals. The extensive lateral projection of the axon is reconstructed in three-dimensions and correlated with the placement of axon terminals on orientation map providing information about the output constellation to laterally arranged neurons. Acknowledgements: Supported by FP7-PEOPLE-ITN-2008 (FACETS-ITN).

Session2. Molecular and cellular neurobiology, P2.1 – P2.38.

P2.1.

The immunohistochemical distribution of glutamine synthetase immunopositive cells in the rat subventricular zone during development

¹István Adorján, ¹Mihály Kálmán

¹ *Anatómiai, Szövet- és Fejlődéstani Intézet, Semmelweis Egyetem, Budapest, Hungary*

The subventricular zone (SVZ) of the lateral ventricle (LV) belongs to the few brain regions where neurogenesis takes place in adulthood. Studies published in the recent decade dealt with the cellular composition of this region, the steps of the ongoing neurogenesis and the routes of the migration. However, there is a limited information available about the development of the SVZ. Glutamine synthetase is an accepted marker of the mature astrocytes. Present study describes the appearance and migration of the glutamine synthetase immunopositive (GS-ip) cell populations along the LV from the perinatal period (E14-P20) until adulthood (P60). GS-ip cells were detectable in the medial wall of the LV and around the foramen of Monro from E18 on. These cells were located in the ventricle-contact layer and were GFAP-immunonegative. During later stages of the development (P6-P20) the GS-ip cell population was gradually confined to the adjacent layer of the ventricular zone (VZ) and to the upper corner of the lateral wall of the SVZ. The GS-ip cells were closely apposed to the GFAP-ip processes of radial glia that probably designate their migration route. In adulthood two systems of GFAP-ip processes were found along the LVs: a) a general tangential system, that was subjacent and parallel to the VZ b) a radial system that was perpendicular to the ventricular surface and was located in the lateral SVZ. The fact that in adulthood there were GS-ip cells found along the GFAP-ip processes in the lateral SVZ quite similar to the development makes it probable that GS-ip cells are produced and migrate in adulthood as well.

P2.2.

Potassium channels in the central nervous system of the snail, *Helix pomatia*

¹I. Battonyai, ¹N. Krajcs, ¹Z. Serfőző, ¹T. Kiss, ¹K. Elekes

¹ *Department of Experimental Zoology, Balaton Limnological Institute, Centre for Ecological Research, Hungarian Academy of Sciences, Tihany, Hungary*

Voltage-dependent potassium channels (K_v) are very complex and conserved proteins. They form selective pores in the neuronal membranes and play vital role in diverse cellular functions. Although potassium current was first recorded from the neuron of a mollusk, there has been very little information on K_v channels in invertebrates, including gastropods. Therefore we have studied the presence of different K_v channel subunits ($K_v2.1$, $K_v3.4$, $K_v4.3$) in the CNS of the land snail, *Helix pomatia* by immunohistochemistry, Western and dot blot as well as voltage clamp recording techniques. Cell clusters displaying immunoreactivity for the different subunits were observed in all parts of the CNS, however, their localization and number varied. A major concentration of immunolabeled neurons, including the B2 giant efferent cell, was found in the buccal ganglion which is the feeding motor center in the CNS. In the procerebral lobes of the brain, which are supposed to be the olfactory center, the three channel subtypes were expressed by small groups of globuli cells processing odor information. In the locomotory center, the pedal ganglia several groups of neurons were also immunoreactive for the three channels. $K_v4.3$ immunoreactivity occurred often in labeled fibers running in non-neural tissues such as the periganglionic connective tissue sheath and the aorta wall. Blotting analyses revealed specific signals for the $K_v2.1$, $K_v3.4$ and $K_v4.3$ channels, confirming the presence of K_v channel subunits in the *Helix* CNS. Voltage clamp recordings proved that the outward currents of $K_v4.3$ and $K_v3.4$ labeled cells contained transient components while $K_v2.1$ immunoreactive cells were characterized by delayed currents. The intracellular localization of the K_v subunits studied supports their involvement in signaling at both pre- and postsynaptic events. Neurons containing different sets of K_v subunits may help understand the dynamic modulation of neuronal networks underlying various behaviors of *Helix*. Supported by OTKA grant No. K78224 (K.E.) and P.D. ⁷5276 (Z.S.)

P2.3.

Inactivation of protein kinase D activity alters dendritic spine morphology and motility

¹Norbert Bencsik, ¹Márton Gulyás, ²Diána Hazai, ¹Krisztián Tárnok,
³Angelika Hausser, ²Bence Rácz, ¹Katalin Schlett

¹ *Élettani és Neurobiológiai Tanszék, Eötvös Lóránd Tudományegyetem, Természettudományi Kar, Budapest, Hungary;* ² *Anatómiai és Szövettani Tanszék, Szent István Egyetem, Állatorvostudomány Kar, Budapest, Hungary;* ³ *Inst. Cell Biology and Immunology, University of Stuttgart, Stuttgart, Germany*

The protein kinase D (PKD) family of serine-threonine kinases comprises 3 isoforms (PKD1, PKD2, PKD3). In model systems PKD is known to regulate proliferation, migration, autophagia and cell-growth. However, its function in neurons is obscure. Therefore in the present work, we studied the effect of genetically modified PKD on neuronal dendritic spines in vitro and in vivo using cultured hippocampal neurons and pyramidal neurons from transgenic mice. PKD inactivation was achieved by introducing a point mutation in the kinase domain leading to a dominant negative kinase inactive mutant form of PKD (kdPKD). Fluorescently tagged kdPKD (kdPKD-EGFP) was transfected into cultivated hippocampal neurons while for in vivo studies, kdPKD-EGFP expression was induced by 6 weeks of doxycycline treatment of CaMK α TaxkdPKD-EGFP mice, leading to the expression of EGFP-tagged mutant PKD in an inducible, forebrain-specific manner. Quantitative fluorescent microscopy was used to analyze the distribution and morphology of dendritic spines on transfected pyramidal neurons. Spines were classified as stubby, filamentous or mushroom, and quantitative parameters as the total length, neck width and head width were determined. Motility of the dendritic spines was followed by fluorescent live cell imaging and was analyzed by custom-written semi-automated software. In vivo, spines on kdPKD-EGFP expressing neurons were analyzed by quantitative immuno-electron microscopy in the hippocampus using single sections. Our in vitro data show that inactivation of endogenous PKD function reduces the number of mature, mushroom-shaped spines, while increases the ratio of filamentous spines which have weaker synaptic connections. This notion was supported by our in vivo ultrastructural findings, showing significant reduction in area and circularity of spine heads on kdPKD-EGFP dendrites. Based on live cell imaging, the kdPKD-expressing filamentous spines are more

motile, according to the motility analyses. Thus, loss of protein kinase D function affects the formation and/or maintenance of dendritic spines, influencing the strength of synaptic connections and synaptic efficacy. Behavioral as well as electrophysiological analyses are in progress to further support these ideas. This project was supported by OTKA K81934 and DFG PF 247/13-1 (to K. S.) and by OTKA K83830 (to B.R.) grants.

P2.4.

Pathway- and cell type-specific differences in the molecular composition of endocannabinoid signaling at neocortical and thalamocortical synapses

¹Barna Dudok, ²Hajnalka Bokor, ¹Gabriella M Urbán, ³Ken Mackie, ⁴Masahiko Watanabe, ⁵Tamás F Freund, ²László Acsády, ¹István Katona

¹ *Laboratory of Molecular Neurobiology, IEM HAS, Budapest, Hungary;* ² *Laboratory of Thalamus Research, IEM HAS, Budapest, Hungary;* ³ *Department of Psychological and Brain Sciences, Indiana University, Bloomington, United States;* ⁴ *Department of Anatomy, Hokkaido University School of Medicine, Sapporo, Japan;* ⁵ *Department of Cellular and Network Neurobiology, IEM HAS, Budapest, Hungary*

At the synaptic level, endocannabinoid signaling is a principal regulator of synaptic strength in various areas of the CNS. At the circuit level, it is indispensable for experience-dependent maturation of cortical circuits during postnatal development, and it is required for the fine-tuning of synaptic weights to ensure the acuity of the processing of sensory information in the neocortex. The fundamental importance of the endocannabinoid system in neuronal function is underlined by the notion that its malfunctioning leads to disorders of higher cognitive functions. However, the precise molecular organization of endocannabinoid signaling in cortical glutamatergic synapses has remained largely elusive. Here we describe the cellular expression as well as cell-type and synapse-specific distribution of two key molecular players of the endocannabinoid system in

the adult mouse somatosensory cortex. The synthesizing enzyme of the endocannabinoid 2-arachidonoylglycerol (2-AG), diacylglycerol lipase- α (DGL- α), is known to be required for all known forms of endocannabinoid-mediated synaptic plasticity. In situ hybridization, immunohistochemistry, and quantitative electron microscopy revealed that the enzyme is expressed by principal neurons, and is localized postsynaptically, in high quantity within dendritic spines receiving intracortical or thalamocortical excitatory synapses, whereas only a low amount of this enzyme is present in a few GABAergic synapses. 2-AG's target, the CB1 cannabinoid receptor, was expressed by a subset of GABAergic interneurons and ubiquitously by principal neurons. Contrary to DGL- α , CB1 was found presynaptically, at high levels on GABAergic boutons, and at a low amount on intracortical glutamatergic terminals, but was not detectable in thalamocortical synapses. Cell type-specific high resolution analysis of immunofluorescence, realized by diolistic labeling of pyramidal and spiny stellate neurons, revealed elevated levels of postsynaptic DGL- α specifically in afferent thalamocortical synapses on spiny stellate cells, but not on pyramidal cells. These synapse-specific mismatches in the subcellular targeting of the two key molecules of retrograde synaptic endocannabinoid signaling suggests that they play diverse functions in homo- and heterosynaptic forms of synaptic plasticity in an input-and postsynaptic cell type-specific manner. This study was supported by the European Research Council Grant 243153.

P2.5.

Competitive P2X3 receptor antagonists show dissimilar conformational state-preferences.

¹Nóra Epresi, ¹Krisztina Pesti, ¹Árpád Mike

¹ *Molekuláris Farmakológia Kutatócsoport, MTA KOKI, Budapest, Hungary*

The P2X3 receptor is an ATP-gated, cation-permeable ion channel protein expressed in the human pain perception pathway. P2X3 receptor subunits are expressed predominantly and selectively in C- and A δ -fiber primary afferent neurons. Since P2X3 receptors are responsible for mediating pain sensation, and P2X3 antagonists block the activation of these fibers by ATP, developing potent and selective P2X3 antagonist drugs is a new approach to pain management. Because P2X3 receptors are primarily expressed in pain sensing neurons, adverse effects are less likely. Therefore P2X3 receptor antagonist agents offer a new alternative approach to the management of pain and persistent discomfort. Only few P2X3-selective antagonists have been reported to date, and a detailed analysis of their mode of action is still lacking. We conducted whole-cell patch clamp experiments on HEK-293 cells expressing the human P2X3 receptor. We investigated two competitive antagonists, the non-selective suramin, and the selective P2X3 antagonist A-317491. We found that both drugs showed a definite conformational state preference, however, their mode of action were radically different. Suramin preferred (and therefore energetically stabilized) the desensitized state – which is somewhat unusual from an antagonist. A-317491, on the other hand stabilized resting state. This latter mechanism of action resulted paradoxically in an enhancement of agonist-evoked currents under certain experimental conditions. In kinetic simulations introduction of conformational state-preference was enough to reproduce the major qualitative properties of inhibition by both antagonists. We suggest that during drug development, identification of the mode of action may help to predict the therapeutic potential of drug candidates.

P2.6.

PACAP influences apoptotic and angiogenic pathways in human retinal pigment epithelial cells

¹Eszter Fábíán, ²László Mester, ²Alíz Szabó, ¹Dóra Reglődi, ¹Péter Kiss, ³Krisztina Szabadfi, ⁴Tamás Atlasz, ³Róbert Gábel, ¹Andrea Tamás, ²Krisztina Kovács

¹ *Anatómia Intézet, Pécsi Tudományegyetem - Orvosi kar, Pécs, Hungary;* ² *Biokémiai Intézet, PTE ÁOK, Pécs, Hungary;* ³ *Zoológiai és Neurobiológiai Intézet, PTE- TTK, Pécs, Hungary;* ⁴ *Sportbiológiai Intézet, PTE- TTK, Pécs, Hungary*

In the retina, the integrity of the pigment epithelial cells is critical for the photoreceptor survival and vision. PACAP is known to exert retinoprotective effects, against several types of retinal injuries in vivo, including optic nerve transection, retinal ischemia, excitotoxic injuries, UV-A-induced lesion and diabetic retinopathy. We have shown that PACAP activates antiapoptotic pathways and inhibits proapoptotic signaling in retinal lesions in vivo. In a recent study we have proven that PACAP is also protective in oxidative stress-induced injury in human pigment epithelial cells (ARPE cells). The aim of the present study was to investigate the possible mechanisms of the protective effect of PACAP in ARPE cells. Cells were exposed to 24 h hydrogen peroxide treatment. Expression of apoptotic and angiogenetic markers was investigated by specific arrays, while the expression of MAP kinases and Akt was studied by Western blot analysis. Our results showed that oxidative stress increased the activation of bad, bax, HIF-1 α , several heat shock proteins, while PACAP treatment decreased them. Among the MAPKs, we found that PACAP activated the protective ERK $\frac{1}{2}$, and decreased the activation of the proapoptotic p38MAPK and JNK. With the angiogenesis array we showed that oxidative stress induced the activation of pro-angiogenic factors like thrombospondin and endothelin, while PACAP treatment could decrease most of them. In summary, our results show that PACAP influences numerous apoptotic markers in direction of an overall antiapoptotic effect and also regulates angiogenetic processes in pigment epithelial cells. These mechanisms may have clinical importance in several retinopathies.

P2.7.

Morphological, gene expression and behavioral changes in PV/GAD67- mouse with a conditional deletion of GAD67 in the PV+ neurons

¹Anikó Fülöp, ¹Zoltán Máté, ²Zsuzsanna Emri, ¹Zoya Katarova, ¹Gábor Szabó

¹ *Orvosi Géntechnológiai Részleg, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Allattani Tanszék, Eszterházy Károly Főiskola, Eger, Hungary*

Decreased expression of gad1 gene coding the GAD67 form, one of two known molecular forms of glutamic acid decarboxylase (GAD) in the parvalbumin (PV)- expressing fast-spiking neurons of the hippocampus and medium prefrontal cortex (PFC) has been implicated in the impaired cortical inhibition, loss of synaptic connections, change in oscillatory activity of the cortical network and impaired cognitive functions of schizophrenic patients. To model these changes in the mouse, we derived a mouse mutant carrying a deletion of the both gad1 alleles in the postnatal PV+ neurons. Using in situ hybridization (ISH) and immunohistochemistry (IHC) we found that GAD67 expression levels in mice homozygous null mutants for gad1 allele in the PV+ neurons (PV+/GAD67KO) is greatly reduced in ³ weeks-old-mice compared to control, while parvalbumin expression was unaltered. GAD67 continued to decrease with age and was virtually absent from most PV+/GABAergic neurons in the cerebellum, in large cortical regions, thalamic reticular neurons (TRN) and hippocampus of two months-old (or older) mutant mice. Mutants displayed a significantly decreased number of GAD67/PV+ neurons compared to control, but no difference in PV+ neurons or total cell number. TaqMan QPCR assay revealed region-specific changes in the levels of GAD mRNAs, which are remarkably similar to the changes observed in schizophrenia. Two-months-old or older PV+/GAD67KO mice displayed a variety of behavioral impairments like progressive cerebellar ataxia, motor discoordination and decreased anxiety levels and suffer frequent epileptic seizures. Mutants also have a decreased life-span (70-90 days) and reduced body weight compared to wild-type mice. In conclusion PV+/GAD67KO mice faithfully reproduce characteristic phenotypic

features of schizophrenia, epilepsy and cerebellar ataxia and afford a valuable animal model to study the molecular mechanisms underlying these severe neurological and mental disorders and the causal role of GAD67 deficiency in their pathogenesis.

P2.8.

Endocannabinoid-mediated calcium signaling in spinal astrocytes

¹Zoltán Hegyi, ²Tamás Oláh, ¹Klaudia Docova, ¹Krisztina Holló, ²László Csernoch, ¹Miklós Antal

¹ *Anatómiai, Szövet- és Fejlődéstani Intézet, Debreceni Egyetem, Orvos- és Egészségtudományi Centrum, Debrecen, Hungary;* ² *Élettani Intézet, DE-OEC, Debrecen, Hungary*

Bidirectional communication between neurons and glial cells mediated by endocannabinoid signaling has recently been demonstrated. Endocannabinoids released by neurons can activate CB1-Rs on astrocytes, resulting in glutamate release from astrocytes and a consecutive glutamate-mediated modulation of neural functions. It has also been reported that DGL- α and NAPE-PLD, synthesizing enzymes of the two major endocannabinoids, 2-AG and anandamide, respectively, are also expressed by astrocytes. To explore the effect of astrocytic CB1-R activation on 2-AG and/or anandamide release from astrocytes, firstly, we investigated the co-expression of CB1-Rs and DGL- α as well as NAPE-PLD on astrocytes in the superficial spinal dorsal horn by using multiple immunocytochemical staining. It was found that most astrocytic (GFAP-IR) profiles showed positive immunostaining for CB1-Rs and for both enzymes. NAPE-PLD immunoreactive spots were homogeneously distributed around CB1-Rs, whereas the density of DGL- α -IR puncta showed a peak at a distance of 5-6 μ m from CB1-Rs, indicating that cytoplasmic Ca²⁺ transients evoked by the activation of CB1-Rs can reach membrane compartments expressing DGL- α and NAPE-PLD. To test the possibility whether 2-AG and/or anandamide released by astrocytes can act

on adjacent glial or neural CB1-Rs, we investigated the distribution of CB1-R-IR spots around astrocytic membrane puncta immunoreactive for DGL- α or NAPE-PLD. We found that the density of CB1-R-IR puncta showed a peak at a distance of 5-6 μm from both DGL α and NAPE-PLD immunoreactive spots on astrocytes, indicating that in case of 2-AG and/or anandamide release from astrocytes, the ligands may act on both neural and glial CB1-Rs. In order to test these possibilities in functional studies we established a primary astrocyte culture, and found that cultured spinal astrocytes also express CB1-Rs and both DGL α and NAPE-PLD. To test the functionality of CB1-Rs expressed by spinal astrocytes, whole cell calcium measurements were carried out in the presence of selective CB1-R agonists as well as the endogenous cannabinoid ligands, which induced slow, but robust calcium transients in more than 60% of the cells. Our results indicate, that spinal astrocytes can be activated by cannabinoid ligands, and the evoked calcium transients may activate the glial calcium-dependent endocannabinoid synthesizing enzymes, which may result in consecutive tonic endocannabinoid release.

P2.9.

Másodlagos glutamát neurotoxicitás kimutatása patkány idegsejt tenyészetekben

¹Gábor Bence Hámornik, ¹Erzsébet Bojti, ¹Gábor Kovács, ¹Erika Gósi, ¹Ferencné Alapi, ¹Andrea Járasi, ¹Balázs Mihalik, ¹Adrienn Pálvölgyi, ¹Ferenc A Antoni, ¹Balázs Herberth

¹ *Molekuláris Farmakológia, EGIS Preklinika, Budapest, Hungary*

A neurodegeneratív megbetegedések molekuláris és sejtszintű mechanizmusainak vizsgálatára számos esetben az idegsejtek tiszta tenyésze a legalkalmasabb. Ugyanakkor az ilyen tenyészetekben a sérült neuronokból felszabaduló másodlagos stressz-ágensek az asztrogliasejtek hiányában hatványozottan érvényesülhetnek és torzíthatják a kísérletek eredményeit. A legkézenfekvőbb másodlagos sejt stresszor a glutamin sav.

Kísérleteinkben a másodlagosan kialakuló glutamát hatását vizsgáltuk oxidatív stressz illetve b-Amyloid oligomerekenek (bAO) kitett érett (15-18 napos) embrionális patkány idegsejt tenyészeteken. A tenyészetek életképességét és a tápfolyadék glutamát tartalmát mértük. Az oxidatív stressz okozta sejtpusztulást az MK-801 NMDA receptor antagonistá koncentráció-függően - a tenyészetek felülúszóinak glutamátszint-növekedésével arányosan - részlegesen kivédte. A másodlagos excitotoxicitás a H₂O₂ kezelés megkezdését követően 1 órával jelent meg. A bAO okozta koncentráció-függő toxicitást az MK-801 a tenyészetek glutamátszint emelkedése előtt is teljesen kivédte, alátámasztva azokat az eredményeket, amelyek azt mutatták, hogy a bAO toxikus hatásának kialakulásában az NMDA receptoroknak döntő szerepe van. A tenyészetek glutamátszintjének nyomon követésével beállítottuk azokat a kísérleti körülményeket, amelyek mellett a glutamát receptorokon keresztül érvényesülő toxikus hatások az endogen glutamát hatásaitól elkülönítve vizsgálhatók.

P2.10.

IL-1 beta production by cultured spinal cord astrocytes upon LPS treatment is influenced by ATP and glutamate

¹Krisztina Holló, ¹Andrea Gajtkó, ¹Krisztina Hegedűs, ¹Erzsébet Bakk, ¹Zoltán Hegyi, ¹Miklós Antal

¹ *Anatómiai, Szövet- és Fejlődéstani Intézet, Debreceni Egyetem, Orvos- és Egészségtudományi Centrum, Debrecen, Hungary*

It is now clear that spinal glia are involved in the maintenance of chronic pain which occurs in response to peripheral inflammation. Glia are activated due to neuronal excitation that occurs with inflammation, and in turn release cytokines (eg. IL-1 β) that modulate neuronal excitability by influencing ion channel function. The interactions between neurons and glia facilitate the establishment of peripheral inflammation induced chronic pain. However, the neuron–glia signaling mechanisms involved in the

initiation and maintenance of inflammatory pain are just beginning to emerge. Our earlier data shows that the spinal astrocytes (and not microglia) are involved in the maintenance of chronic inflammatory pain, partly by their proinflammatory cytokine production. The major goal of our current experiments was to generate spinal astrocyte primary cultures to further study their IL-1 β production upon different stimuli. Activation of cultured astrocytes was achieved by stimulation of Toll-like receptor-4 upon lipopolysaccharide (LPS) treatment. LPS also enhance the activation of caspase-1 enzyme which is involved in the maturation of IL-1 β into its active form. Among other factors the stimulation of purinergic and glutamate receptors may contribute to the increased production of proinflammatory cytokines by the activated astrocytes. So we studied the modulation of the IL-1 β release by ATP and glutamate treatment and we found that both influence the IL-1 β production. As in inflammatory situations astrocytes are exposed to increased levels of ATP and glutamate our results suggest that both astrocytic purinergic and glutamate receptors can have a role through the enhanced and prolonged release of IL-1 β in the maintenance of chronic inflammatory pain.

P2.11.

Metabolic changes during differentiation of neural stem cells

¹Attila Jády, ²Tünde Kovács, ³Susan Vanwert, ²László Tretter, ⁴Emília Madarász

¹ *Doktori Iskola, Pázmány Péter Katolikus Egyetem, Információs Technológiai Kar, Budapest, Hungary;* ² *Neurobiochemistry Research Division, Department of Medical Biochemistry, Semmelweis University, Budapest, Hungary;* ³ *János Szentágothai Doctoral School, Semmelweis University, School of Ph. D. Studies, Budapest, Hungary;* ⁴ *Laboratory of Cellular and Developmental Neurobiology, Institute of Experimental Medicine of the Hungarian Academy of Sciences, Budapest, Hungary*

According to previous results[1], neural stem cells survive at much lower oxygen supply than neurons, both in vivo and in vitro. In order to understand the diverse O₂-demand, metabolic analyses were carried out on one-cell derived populations of neural stem cells representing progenitors of the neural plate/early neural tube (NE-4C[2]) and the adult neurogenic zones (HC_A and SVZ_M)[3]. The embryonic (E9) neuroectoderm derived NE-4C neural stem cells displayed very low O₂ consumption, and it was further decreased by starvation. Differentiating NE-4C progenies on the other hand, increased O₂ consumption in response to starvation indicating that neural precursors gain energy from catabolizing own cellular material. Depending on origin and developmental stages, different stem cells displayed different responses in response to supplementing the “starvation” medium with single metabolites (glucose, lactate, β -OH-butyrate, amino acids). Non-differentiated NE-4C cells increased O₂-consumption in response to any of the metabolites. In contrast, neuronal derivatives of NE-4C cells decreased O₂-consumption and increased H⁺ production in response to glucose, indicating that glucose is not used for mitochondrial energy production by these cells. Adult-derived non-differentiated neuronal stem cells also decreased O₂-consumption in response to glucose. In these cells, addition of amino acids resulted in a sudden but transient increase, while ketone bodies caused a slow but permanent increase of oxygen consumption. The data indicate that the basic metabolism shifts with the advancement of neural differentiation, and the metabolic profile reflects the origin and stage of differentiation of distinct neural stem/progenitor populations. References [1] Anita Zádori, Viktor Antal Ágoston, Kornél Demeter, Nóra Hádinger, Linda Várady, Tímea Kőhídi, Anna Göbl, Zoltán Nagy, Emília Madarász, 2011. Survival and differentiation of neuroectodermal cells with stem cell properties at different oxygen levels; *Experimental Neurology* (227)136–148 [2] Katalin Schlett and Emília Madarász, 1997. Retinoic Acid Induced Neural Differentiation in a Neuroectodermal Cell Line Immortalized by p53 Deficiency; *Journal of Neuroscience Research* (47)405–415 [3] Károly Markó, Tímea Kőhídi, Nóra Hádinger, Márta Jelítai, Gábor Mező, Emília Madarász, 2011. Isolation of radial glia-like neural stem cells from fetal and adult mouse forebrain via selective adhesion to a novel adhesive peptide-conjugate; *PLoS One* (6)e28538

P2.12.

Astrocytes compensate neuronal and glial overexcitation

¹Orsolya Kékesi, ¹Gabriella Nyitrai, ²Pál Szabó, ³Richárd Fiáth, ³István Ulbert, ¹Julianna Kardos, ¹László Héja

¹ *Funkcionális Farmakológiai Osztály, MTA TTK, Molekuláris Farmakológiai Intézet, Budapest, Hungary;* ² *Biokémiai Farmakológiai Osztály, MTA TTK, Molekuláris Farmakológiai Intézet, Budapest, Hungary;* ³ *Összehasonlító Pszichofiziológiai Osztály, MTA TTK, KPI, Budapest, Hungary*

Increasing evidence suggest that glial cells modulates neuronal activity by regulating the concentration of excitatory and inhibitory neurotransmitters in the extracellular space. In the current work we show that uptake of glutamate by astrocytes induces the reverse mode of glial GAT-2/3 transporters and the released GABA modulates neuronal activity both in vitro and in vivo. In in vitro hippocampus blockade of the GAT-2/3 transporters during enhanced neuronal activity reduces the level of tonic inhibition on CA1 pyramidal cells demonstrating that glial GABA significantly contributes to tonic inhibition. We also prove that the released GABA originates from an alternative polyamine synthetic pathway. We show that inhibition of this negative feedback mechanism in the low-[Mg²⁺] epilepsy model increases the duration of seizure like events (SLE), confirming its neuroprotective role. Furthermore, glial Ca²⁺ and Na⁺ imaging demonstrate that the Glu/GABA exchange mechanism counteracts overactivation not only of neurons, but also of astrocytes. Under in vivo conditions in the hippocampus the released GABA modulates the power of gamma range oscillations. The results suggest the existence of a novel mechanism by which astrocytes transform glutamatergic excitation into GABAergic inhibition providing an adjustable, in situ negative feedback on the activity of both neurons and astrocytes. This work was supported by grants ERA-Chemistry OTKA 102166, OTKA K⁸1357, TÉT

P2.13.

Protein differences between somatic and synaptic mitochondria: possible functional consequences

¹Blanka Kellermayer, ¹Péter Gulyácssy, ¹Katalin Adrienna Kékesi, ¹Gábor Juhász, ¹Katalin Völgyi

¹ *Laboratory of Proteomics, Eötvös Loránd University, Budapest, Hungary*

P2.14.

Blood-brain barrier changes in pancreatitis: an electron microscopic study

¹Ágnes Kittel, ²Fruzsina R. Walter, ²Szilvia Veszélka, ¹Lilla Otrókocsi,
³Péter Hegyi, ³Tamás Takács, ³Zoltán Rakonczay, ²Mária A. Deli

¹ *Farmakológia, Kísérleti Orvostudományi Kutatóintézet, MTA, Budapest, Hungary;* ² *Molekuláris Neurobiológiai Laboratórium, SZBK, MTA, Szeged, Hungary;* ³ *I. ÁOK, Szegedi Tudományegyetem, Szeged, Hungary*

The blood-brain barrier (BBB) composed by capillary endothelial cells (EC), pericytes and astrocytes is crucial for the proper working of the central nervous system. The BBB regulates the brain microenvironment, supplies brain cells with nutrients and protects them from toxic materials in the blood. Several diseases, including bacterial and virus infections, neurodegenerative diseases and inflammations of different origin can result in lesion of BBB and relatively small changes in its functions may lead to serious neuronal dysfunctions. Acute necrotising pancreatitis may lead to

pancreatic encephalopathy. Previous work from our teams demonstrated an elevated BBB permeability in parallel with increased blood cytokine levels in taurocholate induced pancreatitis in rats. A new non-invasive rat pancreatitis model was recently developed and characterised by intraperitoneal injection of the cationic amino acid L-ornithine at the dose of 3 g/kg bw. Using this in vivo model and our triple co-culture model of the BBB functional and morphological changes were examined. Electron microscopy was used to monitor ultrastructural changes in the cells forming the BBB following L-ornithine treatment. In the permeability experiments an elevated extravasation of the marker molecules to the brain was determined in rats with pancreatitis. Fragmented glycocalyx, plasma and basal membrane, serious oedema of ECs and glial endfeet, mitochondrial damage and increased number of vesicular elements were observed. Interendothelial tight junctions (TJ) were loosened. Alcian blue used for indication of intact EC function and staining of glycocalyx were found in transcellular vesicles and on /in TJs after 24 h in ornithine-induced pancreatitis, and the dye was also observed in the brain parenchyma. In case of BBB culture model, the effect of L-ornithine also manifested in increased permeability, decreased resistance and in similar morphological changes as observed in vivo. Deformities of ECs were more profound, including the fragmentation of glycocalyx, spectacularly increased number of caveolae and caveolae-like structures, irregular and open interendothelial junctions and high number of apoptotic bodies. Conclusion: increased BBB permeability could be observed by both functional and morphological methods in L-ornithine induced pancreatitis in rats, at least partially mediated by a direct effect of L-ornithine on brain ECs demonstrated by the results obtained on the culture model.

P2.15.

In vitro models of radial glia like neural stem cells- isolated via selective adhesion from different brain regions

¹Tímea Kőhidi, ¹Károly Markó, ¹Nóra Háding, ²Gábor Mező, ¹Emília Madarász

¹ *Idegi Sejt- és Fejlődésbiológiai Laboratórium, MTA-KOKI, Budapest, Hungary;* ² *Peptidkémiai Kutatócsoport, ELTE-MTA, Budapest, Hungary*

Preferential adhesion of neural stem cells to surfaces covered with a novel synthetic adhesive polypeptide (AK-cyclo[RGDfC]) provided a unique, rapid procedure for isolating radial glia-like cells from both fetal, perinatal or adult rodent brain. Radial glia-like (RGl) neural stem/progenitor cells grew readily on the peptide-covered surfaces under serum-free culture conditions in the presence of EGF as the only growth factor supplement. Proliferating cells derived either from fetal (E 14.5) forebrain or from different regions of perinatal or adult brain maintained several radial glia-specific features including nestin, and RC2 immunoreactivity as well as Pax6, Sox2, Blbp, Glast gene expression. Proliferating RGl cells were obtained also from non-neurogenic zones including the parenchyma of the adult cerebral cortex and dorsal midbrain. Many of the investigated positional genes (Pax6, Olig2, Dlx2, Emx2) characterizing regional determination in the developing forebrain, were expressed by all RGl populations, regardless of their origin (either spatial or age). Cell populations, derived from different areas of either fetal or adult brains, could be differentiated to neurons of different phenotypes, astrocytes and oligodendrocytes under appropriate inducing conditions. Upon induction, all RGl populations gave rise to GABAergic neurons. Significant differences among the cell populations however, were found in the generation of glutamatergic and catecholamine-synthesizing neurons and in the production of oligodendrocytes. Despite of uniform expression of positional genes, the mature derivatives of RGl cells displayed characteristics predicted by their regional origin. In the future we plan to establish an in vitro optogenetic model, based on RGl cells derived from distinct brain regions, to further investigate the cell biological responses upon light- triggered bioelectric stimuli.

P2.16.

Developmental changes in the gap-junction forming connexin 36 expression levels in rat retina

Purpose: Connexin36 (Cx36) is one of the major gap junction forming protein subunits in the brain and the retina. Many structural changes (proliferation, migration, synapse formation and reorganization) occur in the early postnatal retina thus we examined how Cx36 mRNA and protein levels are changing in the developing rat retina.

Methods: Wistar rats (P0, 1, 3, 5, 10, 15 and 20-days-old adults) were sacrificed. Eyes were processed for quantitative real-time polymerase chain reaction (qPCR), western blot (WB) and immunocytochemistry (IC). cDNAs from total RNA extracts were used for SYBR Green based qPCR with primers detecting the Cx36 mRNA transcript. RPL13 was used as endogenous control. In the WB analyses proteins were separated, blotted and incubated with α -Cx36 and HRP-conjugated antibodies. The reaction was visualized by chemiluminescence and quantified by ImageJ. Cx36 protein levels were normalized to those of β -Tubuline. Eyes were also hemisected and fixed in 4% paraformaldehyde for IC. 12 μ m thick cryostat sections were collected on slides, treated with the primary and the secondary antibodies and photographed.

Results: We found no significant difference in the Cx36 mRNA levels between P0 and P1. In contrast, a continuous increment was observed in the relative Cx36 transcript level between P1 and P15. The Cx36 mRNA expression reached its peak at P15 where it showed a 68-fold increment relative to P0. Following P15, however a significant (~31%) drop of the Cx36 mRNA level was detected. The WB analysis showed a corresponding increase in Cx36 protein expression with a ~60% elevation at P20 relative to P0. Unlike mRNA transcripts, Cx36 protein levels displayed a local plateau between P3 and P10. In addition, the observed prevalent drop of Cx36 mRNA level at P20 was not mimicked by the protein product. These latter findings were reinforced by IC analyses as

retinal Cx36 immunostains gradually increased throughout postnatal development.

Conclusion: The data shows a continuous increase in both Cx36 mRNA and protein levels between P0–P15. Interestingly, the relative Cx36 mRNA level dropped significantly in the adult, that reflect changes related to early postnatal synaptic reorganization around eye opening. It is likely that the observed discrepancies between Cx36 mRNA and protein levels are due to post-transcriptional mechanisms (RNAi) that fine-tune the ultimate Cx36 protein levels throughout retinal development.

P2.17.

Antihyperalgesic effects of CART peptide fragments in carrageenan-induced hyperalgesia model

¹Kornél Király, ²Márk Kozsurek, ²Erika Lukácsi, ²Zita Puskár

¹ *Farmakológiai és Farmakoterápiás Intézet, Semmelweis Egyetem, Budapest, Hungary;* ² *Anatómiai, Szövet- és Fejlődéstan Intézet, Semmelweis Egyetem, Budapest, Hungary*

Cocaine- and amphetamine regulated transcript (CART) peptides of different sizes were identified in diverse tissue types. The biologically active forms were implicated in several physiological functions, but the supposed CART receptor(s) has (have) not been cloned and the signal transduction mechanisms have not been characterized. In this study the dose-response and time-response properties of CART(55-102), CART(55-76) and CART(62-76) peptides and their relationship to the opioid system have been investigated in a rat inflammatory hyperalgesic model. Hyperalgesia was induced by intraplantar injection of 1% carrageenan and nociceptive threshold to pressure was measured by the Randall-Selitto test. Antihyperalgesic ED₅₀ values of intrathecally injected CART(55-102), CART(55-76) and CART(62-76) were determined as 0.78 (0.44-1.37),

0.28 (0.23-0.35) and 2.76 (2.31-3.30), respectively (all values are given in nM, mean and range). All CART fragments had obvious antihyperalgesic effect, but application of CART(55-102) in 0.3 nM or above resulted in a substantial tremor, which made the recordings unreliable. CART(62-76) in 10 nM showed not only antihyperalgesic but also analgesic effect. The antihyperalgesic effect of CART(55-76) was completely antagonized by naltrexon or Endomorphin-2 (E2) antiserum. Using multiple immunofluorescent labelling the co-localization of E2 and CART in the rat dorsal horn was also examined. Quantitative analysis showed that the majority (~66%) of E2 immunoreactive axons also expressed CART peptide. Our data suggest that different regions or fragments of CART peptides may mediate different effects in a dose dependent manner. CART(55-102) and CART(55-76) peptides in low doses might be involved in mu opioid receptor activation by induction of E2 generation or inhibition of E2 degradation while high dose of these peptides and CART(62-76) activate specific receptors or other signal transduction pathways.

P2.18.

Genes affected by intravitreal administration of PACAP 1-38 and PACAP 6-38 in newborn rat retina

¹Mónika Lakk, ¹Bence Szabó, ¹Zoltán Gödri, ¹Róbert Gábel, ¹Viktória Dénes

¹ *Kísérletes Állattani és Neurobiológiai Tanszék, Pécsi Tudományegyetem, Természettudományi Kar, Pécs, Hungary*

Pituitary adenylate cyclase-activating polypeptide (PACAP), a pleiotropic hormone/neurotransmitter, is responsible for a variety of physiological actions in the developing nervous systems (i.e. cell proliferation, differentiation, neurite outgrowth). The presence of both PACAP and its receptors in the premature mammalian retina indicates regulatory potential for PACAP. In the newborn rat retina VPAC1, VPAC2 and four PAC1 isoforms, namely Null, Hip, Hop1 and HipHop1 have been identified.

However, the paucity of detailed information on the PAC1 receptor expressing elements and function of PACAP in the retinal development is striking. Thus, we aimed to show the PAC1-R immunoreactive elements and identify the genes regulated by PACAP in the rat retina at postnatal day 1 (P1). For immunocytochemistry, native eyecups of P1 Wistar rats were used. PAC1-R expressing structures were detected by anti-PAC1 antibody. To identify genes regulated by PACAP, one group of P1 pups were injected intravitreally (i.v) with 100 pmol PACAP 1-38. To analyze the effect of endogenous PACAP too, the second group received i.v 2.5 nmol PACAP 6-38, a potential PAC1 receptor antagonist. The control group was injected with saline in both cases. Gene expression differences were examined 6 hours after treatments. Changes were determined by quantitative real-time PCR. In the newborn rat retina, PAC1 immunoreactivity was detected in the ganglion cell layer (GCL), inner plexiform layer, neuroblast layer (NBL) and pigment epithelium. Administration of PACAP 1-38 affected critical regulatory pathways that direct neural development: Hedgehogs, Gdf/Bmp, Fgf and Wnt. Genes responsible for pluripotency and self-renewal (Nanog, T) were downregulated whereas genes involved in cell communication (Gjb1), retinal neuronal subtype specification (Dlx1, Math5, Otx2) were upregulated. The PACAP 6-38 treatment affected Fgf pathway only, furthermore, decreased the expression of genes responsible for stem cell maintenance (T, Nanog, Pax6). Among transcriptional factors required for retinal cell fate commitment NeuroD1, Neurog1, Otx2 were repressed whereas Mash1 was activated. Our results provide evidence that (i) PACAP promotes specific neuronal differentiation, (ii) ganglion cells, a specific cell population of the NBL and pigment epithelial cells can respond to PACAP in the newborn rat retina, (iii) exogenously applied PACAP and endogenous PACAP can exert different effects on retinal development.

P2.19.

The distribution of alpha/beta hydrolase 4 (Abhd4) and its potential role during the development of the cerebral cortex

¹Zsófia László, ¹Zsolt Lele, ¹Ashley Dorning, ¹István Katona

¹ *Molekuláris Neurobiológia Kutatócsoport, MTA KOKI, Budapest, Hungary*

Anandamide is an endogenous ligand of cannabinoid and vanilloid receptors. Although its physiological role in the brain have been studied extensively, its biosynthesis, particularly in the embryonic brain remains poorly characterized. Here we report that a recently identified serine hydrolase involved in anandamide biogenesis and called alpha/beta hydrolase ⁴ (Abhd4) is highly expressed in the ventricular zone of the lateral and third ventricles in the E14 mouse brain. After birth, Abhd4 expression is limited to a small number of cells in the dentate gyrus and in the lateral subventricular zone of the telencephalon. Gain-of-function experiments by in utero electroporation revealed that ectopic Abhd4 expression causes neuroblast migration defect, in which cells are arrested in the subventricular and intermediate zones. Long-term survival experiments indicated the loss of Abhd-GFP expression from the postnatal cortex. Finally, TUNEL tests revealed increased cell death in the Abhd4-electroporated areas of cortex suggesting that Abhd4 overexpression results in cell death. These results imply that Abhd4, being exclusively expressed by cells in the germinative zones, functions as a „stand-by” enzyme. Its activity may be triggered by appropriate signals, which induce cell death, thereby its expression must be switched off prior to neuroblast migration, when migrating neurons need to escape from apoptosis triggered by loss of connections to the extracellular matrix.

P2.20.

Regulation of presynaptic [Ca²⁺] and GABA release by CB1 cannabinoid receptors at individual boutons

¹Nora Lenkey, ¹Gergely G Szabo, ¹Noemi Holderith, ¹Norbert Hajos,
¹Zoltan Nusser

A diverse population of CCK-expressing GABAergic interneurons (INs) controls the function of hippocampal pyramidal cells. It has been demonstrated that the release of GABA from these INs is under the control of CB1 cannabinoid receptors (CB1) that inhibit presynaptic Ca^{2+} channels. The release of GABA from distinct IN types is differentially regulated by CB1 activity. However, the way in which CB1 receptors regulate presynaptic $[\text{Ca}^{2+}]$ and consequently GABA release at individual boutons is unknown. Here we performed *in vitro* two-photon fluorescent $[\text{Ca}^{2+}]$ measurements in individual boutons in combination with CB1 pharmacology and *post hoc* immunolabeling for CB1 receptors (CB1-IR) to address how the amplitude of $[\text{Ca}^{2+}]$ transients and the effect of a CB1 antagonist (AM251) depend on the amount of presynaptic CB1s. First we measured the CB1 content of IN boutons in mouse hippocampal CA3 area and found large variability among boutons. This was the result of a similar intra-cell bouton-to-bouton ($\text{CV}=0.38$) and an inter-cell ($\text{CV}=0.44$) variability. We also demonstrate that the CB1-IR of axon terminals does not differ significantly between different cell types (basket vs. dendritic layer innervating cells; DLI). There is a moderate intra-cell, inter-bouton variability ($\text{CV}=0.32$) in the peak $[\text{Ca}^{2+}]$ and this variability is cell-type independent. The mean $[\text{Ca}^{2+}]$ transient is significantly smaller in basket ($0.15 \pm 0.08 \text{ G/G}_{\text{max}}$) than in DLI ($0.24 \pm 0.08 \text{ G/G}_{\text{max}}$) cells. No significant correlation was found between the peak $[\text{Ca}^{2+}]$ and CB1-IR of different cells. Our results also demonstrate that AM251 increases the amplitude (70%) and decreases the variability of $[\text{Ca}^{2+}]$ transients in basket, but not in DLI cells, and the effect of AM251 negatively correlates with the amplitude of the $[\text{Ca}^{2+}]$ transient. To understand how the variability in action potential-evoked axonal $[\text{Ca}^{2+}]$ relates to GABA release, we generated a $[\text{Ca}^{2+}]$ – uIPSC relationship curve by measuring $[\text{Ca}^{2+}]$ transients and uIPSCs in the presence of different concentrations of ω -conotoxin-GVIA. Our results show that ω -conotoxin-GVIA completely blocks the postsynaptic responses as well as the presynaptic $[\text{Ca}^{2+}]$ transients in these IN boutons. Furthermore, due to the power relationship ($m=2.2$) a slight reduction in peak $[\text{Ca}^{2+}]$ causes a strong decrease in

postsynaptic responses, demonstrating that the variability in presynaptic $[Ca^{2+}]$ strongly affects inhibitory synaptic transmission.

P2.21.

The role of protein kinase D activation in response to oxidative stress-induced neuronal cell death

¹Hanna Laura Liliom, ¹Krisztián Tárnok, ²Attila Varga, ²György Kéri,
²Tibor Vántus, ¹Schlett Katalin

¹ *Élettani és Neurobiológiai Tanszék, Eötvös Loránd Tudományegyetem, Természettudományi Kar, Budapest, Hungary;* ² *Patobiokémiai Kutatócsoport, MTA TKI Semmelweis Egyetem, Budapest, Hungary*

Protein kinase D (PKD), a serine/threonine kinase is highly expressed in the mammalian brain and fulfills many roles in cellular physiology, including the modulation of signaling pathways activated in response to cellular stress. According to studies on non-neuronal cells, reactive oxygen species originating from the mitochondria during oxidative stress activate PKD's downstream signaling. Our knowledge on the relation between oxidative stress and PKD-mediated changes in neurons, however, is still limited. During the present work we have shown that PKD undergoes a rapid and transient activation upon H₂O₂ treatment in mouse primary cortical neuronal cultures. Activation of PKD was verified by the increased autophosphorylation level of PKD as well as by PKD FRET reporters. Using MTT assays, we have demonstrated that treatment with PKD-specific inhibitors significantly attenuated cell death induced by 24 hours of oxidative stress. The neuroprotective effect of the inhibitors was the most profound when neurons were preincubated for 2 hours before the onset of the H₂O₂ treatment. This can be explained by the finding that PKD inhibitors activate NFκB, which might help neurons withstand oxidative stress. This work was supported by OTKA K81934 to K.S.

Difference between fast-spiking PV- and non-fast-spiking CCK-containing GABAergic interneurons in Ca²⁺ transients recorded by two-photon laser scanning microscopy

¹Tibor Lőrincz, ²Máté Kisfali, ²Katalin Eszter Sós, ²E. Sylvester Vizi

¹ *Gyógyszerkutató Kutatócsoport, MTA-Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Department of Pharmacology, Institute of Experimental Medicine-HAS, Budapest, Hungary*

Despite the fundamental role of GABA as inhibitory transmitter in brain function, the mechanisms of its release e.g. the presynaptic events including receptor-mediated modulation of the release and Ca²⁺ transients in the presynaptic terminal are poorly understood. To address these questions we used two-photon laser scanning microscopy and acute hippocampal slice preparations from rats and compared the properties of Ca²⁺ transients in fast-spiking and non-fast-spiking GABAergic interneurons. Using somatic patch electrode to deliver anatomical tracer (Alexa 594) and Ca²⁺ indicator dye (OGB-1) and to stimulate electrically (2 ms; 1000-1300 pA) the interneurons we observed that Ca²⁺ transients and their characteristic features could be easily studied. In order to have a fast accurate determination of the time course (τ) and amplitude of the calcium transients, line scans were used. The amplitude of the calcium transients was expressed as the fractional change in basal fluorescence ($\Delta F/F$), which is proportional to the changes in [Ca²⁺]_i. The application of different equations we calculated [Ca²⁺]_i at rest and Δ [Ca²⁺]_i in response to single stimulation: the resting level of [Ca²⁺]_i proved to be ~50 and ~35 nM, the stimulation-evoked [Ca²⁺]_i was ~320 nM and ~90 nM measured on non-fast-spiking and fast-spiking interneurons, respectively. The decay of Ca²⁺ transients measured on boutons was longer lasting on non-fast-spiking interneuron than those recorded on fast-spiking interneurons. The endogenous buffer capacities of fast- and non-fast-spiking interneurons were also significantly different. Ca²⁺ is an ubiquitous intracellular messenger that influences different cellular function including transmitter release. The temporal and spatial characteristics of Ca²⁺ signals evoked by

APs arriving at the terminals play important decisive role in the release of GABA heavily involved in controlling pyramidal cell activity.

P2.23.

Immunodetection of phosphorylated and nitrosylated proteins the potential targets of nitric oxide signaling in the olfactory center of the snail (*Helix pomatia*)

¹Kálmán Nacsá, ¹Károly Elekes, ¹Zoltán Serfőző

¹ *Kísérletes Állattani Osztály, MTA Ökológiai Kutatóközpont Balatoni Limnológiai Intézet, Tihany, Hungary*

The olfactory center (procerebral lobe) of stylommatophoran terrestrial snails is known to produce nitric oxide (NO)-modulated local field potential oscillatory waves when the animal receives odor stimulus. This electrical wave propagation forms the basis of olfactory information processing and learning. Up to date little is known on the distribution and activity of signaling pathways mediated by NO in the procerebral lobe. Therefore our aim was to find the potential targets of NO by using phospho- and nitroso-specific antibodies in immunohistochemical and blotting experiments. Diffuse immunoreaction indicating protein nitrosylation was detected in the cell body layer and internal neuropil of the procerebral lobe, and in the adjacent intermediate neuropil region of the metacerebrum, all displaying intensive nitric oxide synthase (NOS) reaction demonstrated previously by us. The activated substrates of the members of the AGC family protein kinases (PKA, Akt) could be localized in all procerebral areas, but the labeling was the most prominent in labeled trajectories in the globuli cell layer and in the internal and intermediate neuropils. Opposite to PKA and Akt, the anti-protein kinase G(-1) antibody labeled exclusively the lateral neuropil and the efferent pathway along the medial edge of the terminal neuropil. Within the intermediate neuropil fine neural varicosities were labeled by PKG. In Western blot experiments there were no differences in the amount and intensity of possible S-

nitrosylated proteins, compared control and NO-donor (10^{-4} M SNP) stimulated procerebral homogenates. Probing the PKG-1 antibody a ~140 kDa protein band was detected which is considered the active (dimer) form of the enzyme. The PKG activated substrate antibody labeled two, while the PKA activated substrate antibody detected four bands, without differences between the control and SNP stimulated preparations. Two protein bands demonstrated by the Akt activated substrate antibody were more intensively labeled in the SNP treated preparation. The present findings provide a basis for the detailed description of the molecular targets of NO signaling in the snail olfactory center and open the way for further investigation of these molecules in odor elicited behavioral experiments. Support: OTKA grants, Nos. 78228 (K.E.), PD75276 (Z.S.). Z.S. also Support: the János Bolyai Research Scholarship of HAS.

P2.24.

A PAC1 receptor isoform shift affects postnatal retinal development: molecular and morphological approaches

¹Viktória Dénes, ¹Nikoletta Czotter, ¹Zsolt Nyisztor, ²Gergely Berta, ¹Róbert Gábel

¹ *Kísérletes Állattani és Neurobiológiai Tanszék, Pécsi Tudományegyetem, Természettudományi Kar, Pécs, Hungary;* ² *Orvosi Biológiai Intézet, Pécsi Tudományegyetem, Orvostudományi Kar, Pécs, Hungary*

The ubiquitous pituitary adenylate cyclase-activating polypeptide (PACAP), the member of secretin/glucagon/VIP family, exerts versatile effects on neuronal development (e.g. cell proliferation, differentiation, apoptosis and neurite outgrowth) due to differential expression of PAC1 receptor (PAC1-R) isoforms. The expression as well as a dynamic splicing regulation of PAC1 isoforms (Null, Hip, Hop1 and Hiphop1) was previously reported in correlation with postnatal retinal development. The transcript levels of Hip splice variant showed a decline while expression of Hop1 variant was elevated from postnatal day 1 (P1) to P10. We aimed in

the present study (i) to determine the period of Hip/Hop1 isoform shift in the postnatal rat retina, (ii) describe the PAC1-R immunoreactive structures from P5 to P10, (iii) examine the effects of PACAP administration on retina morphology. Native retinas from P5 to P10 were used to assess the ratio of Hip and Hop1 receptors and follow their expression changes by Taqman and SybrGreen real-time PCR methods. To detect PAC1-R expressing structures, we used anti-PAC1 antibody on cryostat sections. To examine the effects of PACAP, one eye of P5 rats were injected intravitreally (i.v) with 100 pmol PACAP 1-38, the other eye was injected with equal volume of saline. Pups were treated either at P7 and P9 or at P5, P7, P9 prior dissection. After fixation and embedding, semithin sections were cut and visualized with toluidine staining. Confocal images show a definite PAC1-R immunoreactivity in ganglion cells, in the fibers of the inner plexiform layer, amacrine cells and calbindin containing horizontal cells between P5 and P10. However, a marked decrease was measured in both Hop1 and Hip message levels. In fact, transcription of the Hip isoform seemed to cease at P8 and P9. Hip/Hop1 isoform shift occurred between P6 and P7. Toluidine staining of the PACAP treated retina revealed that PACAP affects ganglion cell number and the maturation of amacrine cells during this period. Based on our findings, one can conclude that (i) the PAC1-R expressing elements of rat retina are not changing between P5 and P10 (ii) thus the isoform shift may take place within the same structures, (iii) Hop1 isoform predominantly mediate effects of PACAP from P7, (iv) in spite of the reduced receptor expression, exogenously applied PACAP does have effect on the differentiation of amacrine as well as the ganglion cells in the postnatal rat retina.

P2.25.

Cannabinoid type 1 receptor stimulation exerts compound and age-dependent actions in the pedunculopontine nucleus of mice

¹Áron Kőszeghy, ¹Adrienn Kovács, ¹Csilla Bordás, ¹Tamás Bíró, ¹Balázs Pál

The pedunculo pontine nucleus (PPN) is part of the reticular activating system (RAS) and one of the main sources of the cholinergic fibers in the midbrain. This nucleus is thought to have an important role in REM sleep and wakefulness. Several neuromodulatory mechanisms were described in the PPN, but the cannabinoid neuromodulation has not been characterized on a cellular level. In our study we observed that neurons from the PPN respond to arachidonyl – 2'- chloroethylamide (ACEA) and WIN55,212-2, cannabinoid type 1 (CB1) receptor agonists. Responding neurons can be depolarized or hyperpolarized by this drug. The depolarization was reverted by AM251, a CB1 receptor antagonist, while the hyperpolarization did not show any change with this drug. This effect could not be prevented by antagonists of AMPA-, NMDA- type glutamate receptors, and glycine- or GABAA-receptors. Age-dependence of the observed effect was also revealed: the dominating depolarization was replaced by hyperpolarization after the 12th postnatal day. Carbachol and ACEA elicited a similar depolarization on the same cell, but no correlation between the effects of these drugs could be observed on cells hyperpolarized by carbachol. Summarizing our data, we found that cells respond to cannabinoid stimulation in the PPN. The neurons form distinct populations according to the cannabinoid effect: a likely CB1 receptor-dependent depolarization and a CB1 receptor-independent hyperpolarization can be observed. The observed effect does not seem to depend on ionotropic glutamate-, GABAA-, or glycine-receptors. The developmental shift from the depolarizing effect to hyperpolarizing indicates that endocannabinoids might play an important role in the maturation of the reticular activating system.

P2.26.

Time course, distribution and cell types of induction of transforming growth factor betas following middle cerebral artery occlusion in the rat brain

¹Gabriella Pál, ^{1,2}Csilla Vincze, ¹Éva Renner, ^{3,4}Edina Wappler, ³Zoltán Nagy, ^{2,5}Gábor Lovas, ¹Árpád Dobolyi

¹*Neuromorphological and Neuroendocrine Research Laboratory, Department of Anatomy, Histology and Embryology, Semmelweis University and the Hungarian Academy of Sciences, Budapest;*
²*Department of Neurology, Semmelweis University;* ³*Cardiovascular Center, Department Section of Vascular Neurology, Semmelweis University;* ⁴*Department of Anesthesiology and Intensive Therapy, Semmelweis University;* ⁵*Department of Neurology, Jahn Ferenc Teaching Hospital*

Transforming growth factor- β s (TGF- β 1-3) are cytokines that regulate the proliferation, differentiation, and survival of various cell types. TGF- β was suggested to be neuroprotective because injection of TGF- β decreased, while injection of an antagonist increased the infarct size following middle cerebral artery occlusion (MCAO) in rats. The present study describes the induction of TGF- β 1-3 at 3h, 24h, 72h and 1 month after transient (1h) or permanent (24h) MCAO using in situ hybridization histochemistry and quantitative analysis. Double labeling with different markers was used to identify the localization of TGF- β mRNA relative to the penumbra and glial scar, and the types of cells expressing TGF- β s. TGF- β 1 expression increased 3h after MCAO in the penumbra. At 24h following MCAO, TGF- β 1 showed elevated expression level around the lesion, and the most of TGF- β 1-expressing cells co-localized with IBA-1, a microglial marker, while a few TGF- β 1-expressing cells co-localized with GFAP, an astroglial marker. By 72h and 1 month after the occlusion, TGF- β 1 also appeared in microglia within the ischemic core and in the glial scar. In contrast, TGF- β 2 mRNA level was increased in neurons in layers II, III, and V of the ipsilateral cerebral cortex 24h after MCAO. None of the TGF- β 2-expressing cells contained GFAP or IBA-1 immunoreactivity. TGF- β 3 was not induced in cells around the penumbra. Its expression increased in only a few cells in layer II of the cerebral cortex 24h after MCAO. The levels of TGF- β 2 and - β 3 decreased at subsequent time points. Permanent MCAO further elevated the levels of all 3 subtypes of TGF- β s, it is likely that ischemia itself rather than reperfusion evokes the induction of TGF- β s. The area of TGF- β 1 expressing cells overlapped with Hsp70 a marker of the

penumbra, but they were not present in the same cells. In contrast, TGF- β 2 expressing cells contained Hsp70 in the penumbra. TGF- β 1 did not co-localize with either Fos or ATF-3, while the co-localization of TGF- β 2 with Fos but not with ATF-3 suggests that cortical spreading depolarization, but not damage to neural processes, might be the mechanism of induction for TGF- β 2. These data suggest that TGF- β s are produced in different cell types at different time points after an ischemic attack, which may contribute to spatially and timely regulated neuroprotective and inflammatory processes. Support was provided by OTKA NNF2 85612 and OTKA K100319, as well as by the Bolyai Fellowship of the HAS

P2.27.

Neuronal and glial correlates underlying label-free intrinsic optical signal

¹Ildikó Pál, ¹László Héja, ¹Julianna Kardos, ¹Gabriella Nyitrai

¹ *Department of Functional Pharmacology, Institute of Molecular Pharmacology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary*

The intrinsic optical signal (IOS) is widely used for mapping neuronal activity in afferent activated brain areas. IOS evoked by afferent stimulation *in vitro* is generally attributed to glial cell swelling via activation of Na⁺/K⁺/Cl⁻ cotransporter that follows postsynaptic activation. Despite the phenomenon is known for decades, the underlying mechanisms have not been disclosed in detail. Our goal was to explore the molecular mechanisms underlying *in vitro* IOS genesis. We characterized IOS initiated by Schaffer collateral stimulation in the rat hippocampal slice using simultaneous high-frequency imaging and field potential recordings. We used a 464-element photodiode-array device (PDA) that enables IOS detection with 0.6 ms time resolution, making it achievable to align optical and electrophysiological signals. IOS was primarily observed in the

stratum pyramidale and proximal region of the *stratum radiatum* of the hippocampus. IOS was decreased by blockade of neuronal activity by voltage-gated Na⁺ channel inhibitor tetrodotoxin and was significantly enhanced by suppressing inhibitory signaling with the GABA_A antagonist picrotoxin. We found that IOS was predominantly initiated by postsynaptic glutamate receptor activation and progressed by the activation of astroglial glutamate transporters and Mg²⁺-independent astroglial NMDA receptors. In agreement with previous studies, furosemide, the blocker of both, the neuronal K⁺/Cl⁻ cotransporter KCC2 and the glial Na⁺/K⁺/Cl⁻ cotransporter NKCC1 decreased the IOS amplitude. To decide which cotransporter is responsible for this effect of furosemide, we applied selective blockers of NKCC1 and KCC2, bumetanide and (+)[R]-DIOA, respectively. We evidenced, that NKCC1 did not contribute to the IOS generation, in contrast to previous suggestions. Enhancement and slight inhibition of IOS through anion and volume-regulated anion channels, respectively, were also depicted. Major players of IOS mechanisms disclosed by high-frequency IOS imaging imply that spatiotemporal IOS reflects glutamatergic neuronal activation and astroglial response, as observed within the hippocampus. Our model may help to better interpret in vivo IOS and support diagnosis in the future. This work was supported by grants ERA-Chemistry OTKA 102166 and TECH-09-AI-2009-0117 NKFP NANOSEN9.

P2.28.

The mechanism of positive modulation by PNU 120596 on alpha7 nicotinic receptors

¹Krisztina Pesti, ¹Anett Szabó, ²E. Szilveszter Vizi, ¹Árpád Mike

¹ *Molekuláris Farmakológia Kutatócsoport, MTA KOKI, Budapest, Hungary;* ² *Farmakológiai Osztály, MTA KOKI, Budapest, Hungary*

PNU 120596 is a “Type II” positive modulator of alpha7 nicotinic acetylcholine receptors, i.e., it does not only augment the amplitude of

agonist-evoked currents, but also causes a drastic prolongation of mean channel open time. It has been observed that PNU-120596 causes an apparent increase in the affinity of agonists; however, the mechanism of this apparent cooperativity between the orthosteric and allosteric binding sites is currently unknown. We aimed to better understand the mechanisms of PNU-120596-modulation. We performed patch-clamp experiments in whole-cell and outside-out configurations on $\alpha 7$ nicotinic acetylcholine receptor-expressing GH4C1 cells (obtained from Siena Biotech SpA), using a theta-tube system for rapid solution exchange. We found that PNU 120596 was virtually ineffective at both the resting and the slow desensitized states, while it readily bound to the desensitized state and re-activated the receptors. In addition, we observed that in the presence of PNU-120596, dissociation of choline was slowed down radically. In order to test hypotheses for possible underlying mechanisms, kinetic modeling was used. We concluded that the experimentally observed behavior does not require a genuine increase in agonist affinity, but it does require a radically decreased accessibility of the agonist binding site upon modulator binding. We propose a “trapped agonist cycle” mechanism, in which (1) the agonist always associates to the easily accessible modulator-unbound receptor, (2) it causes the receptor to desensitize, thereby providing easy access to the modulator and (3) it dissociates from the reduced-accessibility modulator-bound conformation. The trapped agonist cycle mechanism might be the essence of Type II PAM action.

P2.29.

Cirkumventrikuláris szervek gliaszerkezetének vizsgálata - Hol a barrier?

¹Károly Pócsai, ¹Mihály Kálmán

¹ *Anatómiai, Szövet- és Fejlődéstani Intézet, Semmelweis Egyetem, Általános Orvostudományi Kar, Budapest, Hungary*

A cirkumventrikuláris szervek gliaszerkezetét illetve az erek tulajdonságait immunhisztokémiai markerekkel eddig elsősorban az eminentia medianában (EM) és a subcommissuralis szervben (SCO) vizsgálták. A jelen vizsgálat a fentieken kívül az area postrema (AP), az organon vasculosum laminae terminalis (OVL) és a subfornicalis szerv (SFO) szerkezetét is vizsgálta glia markerekkel (GFAP, vimentin - Vim, S100, glutamin szintetáz - GS). Vizsgáltunk továbbá, a bazális lamina markereként laminint és a bazális lamina receptoraként szolgáló disztroglikánt. Különösen érdekes kérdés, mi zárja le a vér-agy gát mentes cirkumventrikuláris szervek szövetterét a szomszédos agy állomány felé. A vér-agy gát átjárhatóságát rhodamine (Rh) feltöltéssel, valamint fibronektin illetve immunglobulinok kimutatásával vizsgáltuk. A fenti markerek mellett használtunk Wisteria floribunda agglutininint (Wfa) az extracelluláris mátrix (ECM) proteoglikánjainak kimutatására. A vizsgálatokat immerziós vagy perfúziós fixálás után lebegő vibratom metszeteken végeztük. Az érfeltöltés vizsgálatok során Rh a szervek anatómiai határainak megfelelően oszlott el. Az állat saját immunglobulinjai azonban változatos mértékű intraparenchymális penetrációt mutattak az AP, EM és SFO melletti agyterületeken. Fibronektin immunreaktivitás viszont inkább az erek környezetében volt megfigyelhető. Immunhisztokémiai vizsgálatok alapján a szervek gliaszerkezete különböző. A GS-, S100-, Vim- és GFAP immunreaktivitás általában nem kolokalizáltan fordul elő a gliasejtekben. Az SFO széli zónájában erőteljes S100 immunreaktivitás látható. AP esetében Vim immunreaktivitás figyelhető meg a széli részekben. A vizsgált szervek állománya Wfa-negatívnak bizonyult. A szomszédos agyterületek OVL és SFO esetében kiterjedt Wfa pozitivitást mutatnak, míg EM és AP szélei mentén keskenyebb Wfa-pozitív zóna figyelhető meg. Éles határképző struktúrát nem találtunk ezért a továbbiakban elektronmikroszkópos vizsgálatok elvégzését tervezzük. A Wfa eloszlása arra utal, hogy a diffúzió gátlásában az ECM szerkezetének is szerepe lehet.

Szinaptikus változások transzgenikus szkizofrénia-modell állat hippokampuszában

¹Balázs Pósfai, ²Csaba Cserép, ²Eszter Szabadits, ²Tamás F. Freund, ²Gábor Nyiri

¹ *Celluláris és Hálózat Neurobiológia Osztály/Agykéreg Kutatócsoport, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Celluláris és Hálózat Neurobiológia Osztály/Agykér, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary*

A szkizofrénia egy mentális zavar, amely az emberiség körülbelül 1%-át érinti. A betegséggel kapcsolatos genetikai okok közül az egyik legerősebb összefüggést a G72 gén mutatja. Ezt a humán gént egérbe juttatva az állat viselkedése megfelel a szkizofrénia-modell állattól elvártaknak. A G72 génről átíródó LG72 fehérje a mitokondriumban lokalizálódik, mely bizonyítottan fontos szerepet játszik a szinapszisok morfológiájának kialakulásában. Emellett a fehérje módosíthatja az agyban lévő D-szerin mennyiségét, ami az NMDA-receptorok egyik fő koagonistája, így közvetetten változásokat okozhat a szinaptikus jelátvitelben is. Továbbá, fiziológiai mérések eltéréseket tártak fel a G72-transzgenikus egerek gyrus dentatus szemcsesejtjeire érkező perforáns pályák által adott szinapszisok tulajdonságaiban, melyek mind pre- mind posztszinaptikusan kifejezik az LG72-fehérjét. Azonban, e változások anatómiai háttere ismeretlen. Kérdéseink a következők voltak: megváltozik-e az LG72-t kifejező egerekben a perforáns pálya által létesített szinapszisok mérete, aktív zónájuk morfológiája, és a bennük lévő NMDA-receptorok mennyisége? A kérdések megválaszolásához elektronmikroszkópos technikákkal vizsgáltuk a G72 génnel rendelkező egerek és vad típusú testvéreik szinapszisait. Azt találtuk, hogy a G72-transzgenikus egerek szinapszisai mintegy ³3%-kal nagyobbak, mint vad típusú alomtársaiké (n=2x5 állat). Az NMDA-receptorok kvantifikálására beágyazás utáni immunarany technikát használtunk a receptor GluN1-alegységére, amely minden NMDA-receptorban jelen van. Megállapítottuk, hogy a G72-transzgenikus állatok szinapszisai nem csak nagyobbak, de több NMDA-receptort is tartalmaznak, amely módosult szinaptikus hatékonyságra utalhat. A posztszinaptikus denzitás vastagságát elektronmikroszkópban készült

képeken vizsgálva nem találtunk különbséget az állatpárok között. A G72-transzgenikus egerek gyrus dentatus szemcsesejtjeire érkező szinapszisok tehát nagyobbak és több NMDA-receptort tartalmaznak, mint vad típusú alomtársaik szinapszisa. Ezen változások oka lehet az LG72 hatására bekövetkező mitokondriális módosulás, vagy a fehérje indukálta megváltozott NMDA-koagonista D-szerin koncentráció. Munkánk jó alapot szolgáltathat annak megértéséhez, hogy milyen kapcsolat áll fenn a G72 gén kifejeződése és a betegségben jól ismert sérült glutamaterg transzmisszió között.

P2.32.

Short-term caloric restriction induced ultrastructural changes in hippocampus

¹Réka Blanka Babics, ¹Diána Hazai, ¹Balázs Szőke, ¹Péter Sótónyi, ¹Bence Rác

¹ *Anatómiai és Szöveti Tanszék, Szent István Egyetem, Állatorvostudományi Kar, Budapest, Hungary*

The recent epidemic of obesity has stimulated extensive research to identify brain regions where metabolic and hormonal signals modify food intake. Accumulating evidence suggest that a major component of this problem involves learned behavior; thus, the role of learning and memory in the control of eating, appetitive- and food searching behavior must be considered in order to understand underlying neuronal mechanisms. Recent work implicates the hippocampus (a brain structure critical to learning and memory) in energy regulation. Beyond coding spatiotemporal context, the hippocampus plays an important role in encoding relationships between internal states (especially thirst or hunger) and action, thus providing a mechanism by which motivation and memory are coordinated to guide behavior. Besides inducing physiological changes in hippocampal activity, appetitive drives likely trigger morphological changes in this plastic network. To date we have very little knowledge about possible

morphological changes in the synaptic neuropil under specific defined metabolic conditions. Here we used quantitative electron microscopy and a caloric restriction paradigm - which is a reduction of total caloric intake without a decrease in nutrients or any one dietary component - to study the neuropil of the CA1 hippocampus . Our aim was to elucidate possible changes in the architecture and dimensions of synapses during fasting. We analyzed synapse number and size as well as spine surface, area PSD size, head circularity and morphology of excitatory axospinous terminals. These data provide essential information for further studies focusing on changes in the molecular composition of hippocampal excitatory synapses during caloric restriction. This project was supported by OTKA K83830 and NKB (Faculty of Veterinary Science, Budapest) grants.

P2.33.

Increased number of ectopic neurons in the neo- and archicortical white matter in human temporal lobe epilepsy

¹Zsófia Richter, ¹Chloe Reddan, ²József Janszky, ²Csilla Gyimesi, ³Zsolt Horváth, ³Tamás Dóczi, ¹László Seress, ¹Hajnalka Ábrahám

¹ *Központi Elektronmikroszkópos Laboratórium, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;* ² *Neurológiai Klinika, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;* ³ *Idegsebészeti Klinika, PTE ÁOK, Pécs, Hungary*

Temporal lobe epilepsy is the most common form of focal epilepsies and it is associated with various structural alterations. Many pathological changes were detected in the archi- and neocortex, such as neuronal cell loss, synaptic reorganization, altered protein expression etc., which can be considered to be the consequence and not the cause of hyperexcitation. These alterations may result in the formation of pathological neuronal networks, which maintain the epileptic activity. In addition to these changes, studies reported the presence of ectopic neurons in epileptic cortical tissue, such as the ectopic granule cells of the dentate gyrus. In the

present study we compared the number and distribution of white matter neurons in the archicortical subiculum and entorhinal cortex as well as in the deep temporal neocortex on neurosurgically resected tissue sections of patients with therapy-resistant temporal lobe epilepsy. Neurons were identified using immunohistochemistry with anti-NeuN antibody. Different subpopulations of neurons containing calcium binding proteins calbindin, calretinin and parvalbumin as well as neuron specific transcription factors and neurofilaments were also detected. Quantification of the neurons were performed using MicroBrightfield Neurolucida and Stereoinvestigator software. We have found that large number of neurons are present in the archi- and neocortical white matter of patients with temporal lobe epilepsy. Among them both principal cells and interneurons which contain calcium binding proteins were detected. The distribution of these neurons differed among the patients. In some patients these were evenly distributed, in others clusters formed by ectopic neurons were observed. Quantification revealed a significantly larger number of neurons in the deep archi- and neocortical white matter of patients with epilepsy than in that of controls. We suggest that these white matter neurons participate in epileptic neuronal networks, and, in addition to other factors, they are responsible for the maintenance of the epileptic activity. Furthermore, we propose that the ectopic neurons are the result of incomplete cortical migration and/or the incomplete disappearance of the fetal subplate neurons. We suggest that ectopic neurons are present in the white matter from birth and, therefore, they can be one of the causes but not the consequence of epileptic hyperexcitation.

P2.34.

Effects of pituitary adenylate-cyclase activating polypeptide analogues on cell bodies of primary sensory neurons and transfected cell lines

¹Éva Sághy, ¹Éva Szőke, ²Eszter Bánki, ¹Marietta Kelemen, ²Dóra Reglődi, ³Gábor Tóth, ⁴Alain Covineau, ¹Zsuzsanna Helyes

¹ Farmakológiai és Farmakoterápiai Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary; ² Anatómiai Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary; ³ Orvosi Vegytani Intézet, Szegedi Tudományegyetem, Szeged, Hungary; ⁴ Faculté de Médecine Xavier Bichat, Centre de Recherche Biomedicale-Bichat Beaujon, Párizs, France

Pituitary adenylate-cyclase activating polypeptide (PACAP) acts on G protein-coupled receptors: the specific PAC1 and VPAC1/VPAC2, which bind VIP and PACAP with similar affinity. PACAP6-38 was described as a potent PAC1/VPAC2 antagonist in several models. Maxadilan is a selective PAC1 agonist, while its fragment, MAXA65, is a specific antagonist. Ala11,22,28VIP is a selective VPAC1 agonist, while BAY 55-9837 is a selective VPAC2 agonist. We reported earlier that PACAP1-38 and PACAP6-38 are both able to decrease the electrical-field stimulation-induced release of the sensory neuropeptide calcitonin gene-related peptide (CGRP) from sensory nerve endings of the isolated rat trachea. Therefore, interestingly, the fragment did not behave as an antagonist in this system. In the present study we aimed at analysing the actions of peptide fragments considered as agonists and antagonists on sensory neural and cell line responses in vitro. Ratiometric technique of $[Ca^{2+}]_i$ measurement with the fluorescent indicator fura-2-AM on primary cultures of trigeminal ganglia neurons (rat and mice) and PAC1 and VPAC1 receptor-expressing cell lines were performed. On the cell bodies of rat and mouse trigeminal ganglion neurons: Slowly increasing $[Ca^{2+}]_i$ indicating Gq protein-coupled receptor activation was detected both after PACAP1-38 and PACAP6-38 administration. The PAC1 receptor agonist maxadilan, the PAC1 receptor antagonist MAXA65 and the VPAC2 receptor agonist BAY 55-9837 caused similar response. In contrast, the VPAC1 receptor agonist Ala11,22,28VIP had no significant effect on $[Ca^{2+}]_i$. On cell lines: Our data show that PACAP1-38, PACAP6-38, maxadilan and MAXA65 increased $[Ca^{2+}]_i$ on PAC1 and VPAC1 receptor-expressing cell lines. Ala11,22,28VIP activated VPAC1 receptor-expressing cell line, BAY55-9837 had no effect either on PAC1 or on VPAC1 receptor-expressing cell line. It is concluded that Ca-imaging is able to test the PACAP receptor-selective agonists and antagonists. Interestingly, some antagonist of PACAP receptors act as agonists on the sensory neurons, and cell lines.

Presently unknown receptors or splice variants linked to distinct signal transduction pathways might explain these differences. The VPAC1 receptor does not play a role in these processes. Support: SROP-4.2.2.A-10/1-2010-0029, SROP 4.1.2.B-10/2/KONV-20/0-0002, MTA Lendület program, 4.2.2.A-11/1/KONV-2012-0024, OTKA 104984

P2.35.

Ultrastructural and neurochemical differences between wild type and PACAP KO mice in adult and aging retina

¹Krisztina Szabadfi, ²Péter Kiss, ²Dóra Reglődi, ²Andrea Tamás, ³Hitoshi Hashimoto, ⁴Zsuzsanna Helyes, ⁵Seiji Shioda, ⁶Róbert Gáriel

¹ *Kísérletes Állattani és Neurobiológiai Tanszék, Pécsi Tudományegyetem, Természettudományi Kar, Pécs, Hungary;* ² *Department of Anatomy, PTE-MTA Lendület PACAP Team, University of Pécs, Pécs, Hungary;* ³ *Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan;* ⁴ *Dept. of Pharmacology and Pharmacotherapeutics, University of Pécs, Pécs, Hungary;* ⁵ *Department of Anatomy, Showa University, Tokyo, Japan;* ⁶ *Dept. of Experimental Zoology and Neurobiology, University of Pécs, Pécs, Hungary*

Pituitary adenylate cyclase activating polypeptide (PACAP) is a neurotrophic and neuroprotective peptide. PACAP and its receptors are present in the retina. We have provided evidence that PACAP is neuroprotective in retinal degenerations. The aim of this study was to examine whether histological alterations, cell type-specific differences or changes in the distribution of PAC1-R expression exist between the retinas of wild type and PACAP deficient mice in adult (3-5-months-old) and aging (1-year-old) animals. Retinas were processed for routine histology, electron microscopy and immunohistochemistry for TH, calretinin, calbindin, parvalbumin, PKC α , GFAP, PNA, Brn3a and PAC1-R. Standard histological methods revealed no major differences between the adult retinas of wild type and PACAP deficient mice. Staining for the above

markers of adult PACAP KO retinas was similar to that of wild type retinas, with no significant alterations in immunoreactivity patterns except for PAC1-R staining. We observed that fewer cells expressed PAC1-R in adult PACAP KO than in wild type retinas. Among the age-related changes, the number of cone photoreceptor terminals was reduced in both wild type and PACAP KO aging retinas compared to adult controls. Other well-known age-related differences were, however, only observed in the PACAP KO mice. These alterations included: horizontal cell processes sprouted into the photoreceptor layer; bipolar cells showed arbor-specific alterations: their dendrites sprouted but their axons remained stable; the ganglion cell number decreased and Müller glial cells showed elevated GFAP expression compared to the aged wild type retinas. In summary, while there are no major differences in the histological structure and expression of markers between adult wild type and PACAP KO mice, marked degenerative changes appear earlier in aging mice lacking endogenous PACAP. These results support the endogenous protective role of PACAP against aging processes of the nervous system. Supported by OTKA K100144, 104984, CNK78480; Bolyai Scholarship; Richter Gedeon Centenary Foundation; PTE-MTA Lendület program, SROP-4.1.2.B-10/2/KONV-20/0-0002, SROP-4.2.2.A-11/1/KONV-2012-0024.

P2.36.

Role of protein kinase D in polarized sorting of neuronal membrane proteins

¹Zsófia Szíber, ¹Tamás Szórádi, ¹Krisztián Tárnok, ²Angelika Hausser, ¹Katalin Schlett

¹ *Élettani és Neurobiológiai Tanszék, Eötvös Loránd Tudományegyetem, Természettudományi Kar, Budapest, Hungary;* ² *Inst. Cell Biology and Immunology, University Stuttgart, Stuttgart, Germany*

Neurons are highly polarized cells, where the somatodendritic and the axonal membrane domains possess distinguished anatomical and

functional properties. Protein kinase D (PKD) is a serine/threonine kinase known to regulate basolateral plasma membrane-directed protein sorting in non neural cells. In neurons, PKD activity controls the integrity of Golgi apparatus and the extent of dendritic arborization, however, its role in selective transmembrane receptor sorting have not been investigated in detail. To address this question, we have overexpressed PKD mutants as well as various transmembrane proteins in embryonal hippocampal neuronal cultures and analysed the distribution and the transport of the overexpressed fluorescently labeled proteins. Our data showed that in the presence of the kinase inactive mutant PKD, the intracellular distribution of transferrin receptor (TfR), a known dendritic membrane protein, and the intracellular transport of L1CAM, a known axonal membrane protein were both impaired. Interestingly, overactivation of PKD led to a resembling phenotype. We have evidence that PKD activity influences dendritic membrane turnover, as well: the endocytosis of fluorescently labeled transferrin is significantly increased upon PKD's activation. Therefore, PKD presumably controls the correct localization of membrane proteins either directly at the Golgi-network and/or at the plasmamembrane via the endosomal machinery. This work was supported by OTKA K81934 to K.S.

P2.37.

Effect of knocking out cyclophilin D on region-specific distribution of mGluR1, alfa synuclein and GFAP expression in the mouse brain

¹Viktória Vereczki, ²Ibolya Bodnar, ³Otto Pinter, ³Dora Zelena, ⁴Josef Mansour, ⁴Issa Pour-Ghaz, ⁵Lilla Kepes, ⁴Beata Torocsik, ⁵Erzsébet Oszwald, ⁴Vera Adam-Vizi, ⁴Christos Chinopoulos

¹ *Anatómiai, Szövet- és Fejlődéstani Intézet, Semmelweis Egyetem, Budapest, Hungary;* ² *Dept. of Human Morphology and Developmental Biology, Semmelweis University, Budapest, Hungary;* ³ *Laboratory of Molecular Neuroendocrinology, Ins. of Exp. Medicine of the Hung. Academy of Sci., Budapest, Hungary;* ⁴ *Department of Medical*

Genetic proof that the mitochondrial protein cyclophilin D (cypD) plays a major role in the manifestation of necrotic cell death was established by using genetically engineered cypD knock out mice. However, despite the enormous amount of work performed in isolated mitochondria and other in vitro models, the indisputable contribution of cypD to the most common neurodegenerative disease, Alzheimer's disease, has only being recently shown: in transgenic mice overexpressing mutant amyloid precursor protein and A-beta, cypD deficiency improved mitochondrial and synaptic function and learning/memory in both young and aged mice. Mindful of the recent evidence showing that genetic disruption of the cypD gene affords benefit to animals bound to develop Alzheimer's disease, we formulated the hypothesis that the same genetic manipulation may confer advantage to the aging animal population in general. We therefore examined the expression of mGluR1, alfa synuclein and GFAP, 3 aging markers of a subpopulation of neurons and astrocytes, respectively, in cypD +/+, +/- and -/- aged mice. 4 mice from each group have been perfused with paraformaldehyde plus acrolein and kept in cryoprotectant. Brains have been sectioned, and decorated with antibodies against mGluR1, alfa synuclein and GFAP. We hereby show that GFAP is upregulated in +/- mice, but downregulated in -/- mice, in agreement with the results obtained from western blotting of brain homogenates. However, mGluR1 and alfa synuclein expression exhibited a significantly different pattern of expression in brain regions among the three groups, a finding that could not have been yielded from western blotting alone. These efforts may provide valuable information in terms of pinpointing cyclophilin D as a target for an aging-combating drug.

P2.38.

The cannabinoid receptor type 1 antagonist rimonabant binds in an antagonistic fashion to mu-opioid receptors at low concentrations

¹Ferenc Zádor, ¹Ferenc Ötvös, ¹Sándor Benyhe, ¹Anna Borsodi, ¹Eszter Páldy

¹ *Biokémia Intézet, Magyar Tudományos Akadémia, Szegedi Biológiai Kutatóközpont, Szeged, Hungary*

Rimonabant, an antagonist for the G-protein coupled cannabinoid receptor type 1 (CB1), was the first CB1 receptor targeted appetite suppressant to be approved for the treatment of obesity, but due to its strong side effects (depression, anxiety) it was shortly withdrawn from the market. Since the approval and also after the withdrawn many studies highlighted the unspecific behavior of rimonabant being prone to bind to many members of the G-protein coupled receptor (GPCR) family. Our aim was to examine the binding properties of rimonabant towards the G-protein coupled mu opioid receptor (MOR), in in vitro competition binding experiments with tritiated opioid ligands in membrane preparations of Chinese hamster ovary cells stably transfected with MOR (CHO-MOR) and also in wild type and CB1 knock-out mice forebrain membrane fractions. Further on for the first time we examined the possible direct interactions between MOR and rimonabant in in silico docking calculations using the homology model of both the active and inactive states of MOR. According to the competition binding experiments rimonabant failed to displace the tritiated agonist synthetic peptide [D-Ala2, NMePhe4,Gly5-ol]enkephalin ([3H]DAMGO) in low concentrations, and it was highly unspecific on the receptor in higher concentrations in wild type, and CB1 knock-out mice forebrain, and in CHO-MOR membrane fractions. Interestingly, in the docking experiments rimonabant had a favorable binding position into the inactive state of the MOR with high binding energy, indicating that rimonabant behaves as an antagonist on MOR. This finding was confirmed by radioligand competition binding experiments in CHO-MOR cell membranes, in which rimonabant displaced the tritiated opioid antagonist naloxone in very low concentrations, specific to the “high affinity state” of the receptor, which did not occur in [3H]DAMGO displacement experiments. This study was supported by grants TÁMOP-4.2.2A-11/1KONV-2012-0024 and TÁMOP-4.2.2A-11/1KONV-2012-0052

Session 3. Methods, P3.1 – P3.17.

P3.1.

Automated on-line column-switching LC method for quantification of photostimulation-associated changes in transmitter and neuromodulator release

¹Mária Baranyi, ¹Rómeó Andó, ²Edit Papp, ¹Beáta Sperlágh

¹ *Molekuláris Farmakológia Csoport, Kísérleti Orvostudományi Kutató Intézet, Budapest, Hungary;* ² *Idegi Jelátviteli Csoport, Kísérleti Orvostudományi Kutató Intézet, Budapest, Hungary*

Changing the strength of synaptic transmission is a means to modify the communication in a neuronal network. In vitro neurochemical monitoring using micro perfusion sampling is important in neuroscience. A significant limitation of current practice is that different assays are utilized for measuring each class of neurotransmitter and neuromodulator. We present a high performance liquid chromatography (HPLC) absorbance and fluorescence method that utilizes dansyl chloride for determination of multiple neurotransmitters amino acids (aspartic acid (Asp), glutamic acid (Glu), 4-aminobutanoic acid (Gaba) and glycine (Gly)); monoamines (dopamine (DA), noradrenalin (NA) and serotonin (5-HT)) and neuromodulators (anandamide (AEA), 2-arrachidonyl glycerol (2-AG) and somatostatin (SST)) in brain slice perfusate. The procedures of cleanup, preconcentration, and separation were completed on-line in one step. To demonstrate utility of the method we have examined the dynamic changes of neurotransmitters and neuromodulators released from the prefrontal cortex (PFC) slice of transgenic mice that were microinjected with light-sensitive channelrhodopsin-2 mutant adenoviral vector (rAAV2/9-synapsin-ChR2/eGFP) in response to photostimulation. In the present study we have examined the “basal” extracellular concentrations of

transmitters and modulators using light stimulation (470 nm) with different frequencies and number of pulses (5 Hz, (3600), 10 Hz, (2400), 20 Hz, (1200)) in slice from unlabelled mice. Photostimulation elicited transient increases in the releases of Gaba; 5-HT and NA which was dependent on the stimulation frequency and the number of pulses applied. Extracellular SST changed in the opposite direction in response to light stimulation. As a control stimulation, we examined the effect of the sodium channel activator veratridine in the PFC slice. The selected samples revealed that the amount of Gaba, NA, 5-HT and SST was also elevated in response to veratridine. The findings from this study demonstrate that the increases of neurotransmitters and neuromodulators released from virus-labelled PFC slice when stimulated by light. The results show that dansylation with HPLC method allows chemically different type of small molecules to be detected at concentrations from picomolar to micromolar.

P3.2.

A novel method for quantitative molecular profiling in axon terminals using STORM super-resolution microscopy

¹László Barna, ¹Barna Dudok, ¹Eszter Szabadits, ¹Szilárd Szabó, ¹Stephen Woodhams, ¹Cristopher Henstridge, ¹Balázs Pintér, ²Masahiko Watanabe, ³Sang-Hun Lee, ³Ivan Soltesz, ¹István Katona

¹ *Laboratory of Molecular Neurobiology, Institute of Experimental Medicine HAS, Budapest, Hungary;* ² *Department of Anatomy, Hokkaido University School of Medicine, Sapporo, Japan;* ³ *Department of Anatomy and Neurobiology, University of California, Irvine, United States*

Brain circuits comprise a massive diversity of cell types at the scale of hundreds. Up-or down-regulation of given signaling pathways associated with a physiological or pathophysiological mechanism is usually restricted to a few subcellular microdomains and takes place only in some selected cell types. To date, targeted cell type- and compartment-specific molecular investigations combined with physiological and structural characterization

have been difficult to perform. Here, we present a rapid and versatile method based on STORM super-resolution microscopy to circumvent this technical obstacle at the single cell level. The CB1 cannabinoid receptor is the most abundant GPCR in the brain and endocannabinoids are among the most important presynaptic regulators of synaptic transmission. CB1 receptors mediate diverse forms of synaptic plasticity, however, the regulatory principles, which determine their precise spatial location and density in relation to the release machinery on given axon terminal types have remained elusive. To illustrate the power of this novel method, we performed electrophysiological and anatomical characterization of hippocampal GABAergic interneurons, with correlated super-resolution imaging of single axon terminals. This approach allowed determination of the abundance and spatial position of CB1 receptors at a nanoscale resolution in relation to synaptic active zones. While the distribution of CB1 receptors on single axon terminals was surprisingly homogenous, abundance of the receptors exhibited an unexpectedly large variability between individual boutons. CB1R levels showed a moderately strong correlation with the size of the axon terminal, and were correlated weakly with the size of the presynaptic active zone. However, neither the pre-, nor the postsynaptic neuron determined receptor abundance. These findings suggest that cannabinoid receptor levels are passively adjusted to the size of the bouton and the synapse. Comparison of perisomatic- and dendritic-targeting interneurons revealed that perisomatic cells have larger boutons with slightly more CB1Rs, and that on axon terminals of perisomatic interneurons, but not on those of dendritic cells, CB1R abundance is scaled with the level of Bassoon, an anchoring protein of VGCCs. These cell-type specific differences may underlie the different sensitivity of the two interneuron populations to cannabinoid agonists. This work was supported by the European Research Council Grant 243153.

P3.3.

Optimizer: A flexible software tool for fitting the parameters of neuronal models

¹ *Molekuláris Bionika szak, Pázmány Péter Katolikus Egyetem, Információs Technológiai Kar, Budapest, Hungary;* ² *Kísérleti Orvostudományi Kutatóintézet, Magyar Tudományos Akadémia, Budapest, Hungary*

Current experimental methods make it possible to create increasingly complex multicompartmental conductance-based neuron models, which have the potential to imitate the behavior of real neurons with great accuracy. However, these models have many parameters, often poorly constrained by the available data. One alternative, which is often used in network models, is to utilize much simpler (e.g., reduced compartmental or integrate-and-fire type) model neurons. These have fewer parameters than detailed biophysical models; however, the remaining parameters are often not directly related to the underlying biophysics, and need to be set such that the behavior of the model cell best approximates that of the real neuron. For both simple and detailed models, the relationship between the values of the parameters and the behavior of the model is nonlinear and complex, and the task of finding the optimal parameter values is highly non-trivial. Thus, a reliable automated parameter-constraining procedure which could handle the different scenarios relevant for model fitting and model simplification would be of great value, and the goal of our research was to develop such a flexible framework. The result of our efforts is the software called Optimizer, implemented in the Python programming language. The most important feature of the software is its flexibility: the program can run optimizations based on one or more traces; currently contains nine commonly used error functions, which may be combined arbitrarily; and implements a powerful, canonical genetic algorithm. The software can be easily modified, expanded, and customized to suit individual needs. The program currently supports the Neuron simulator, but will later support other popular modeling software as well. Optimizer has two different interfaces. One is a graphical interface, which allows the user to set the optimization parameters and observe the results in a convenient way. The other interface is command-line based, which reads the settings from a text file, and thus allows the optimization to run on platforms that do not support graphical interactions. So far, we tested the

program with three problems from different model classes, and with different complexity. As targets, we used simulated traces from the same or a more complex model class, as well as experimental data. In all tests, Optimizer showed equally good or better performance than the various methods described in the literature. Support: OTKA K83251.

P3.4.

Polymer Based Microfluidics for Biomedical Applications

¹Péter Fűrjes, ²Gabriella Eszter Holczer, ²Zoltán Fekete

¹ *Műszaki Fizikai és Anyagtudományi Intézet - MEMS LAB, MTA - Természettudományi Kutatóközpont, Budapest, Hungary;* ² *Műszaki Fizikai és Anyagtudományi Intézet - MEMS L, MTA - Természettudományi Kutatóközpont, Budapest, Hungary*

We report advances in the fabrication technology and testing of polymer microfluidics as a part of a polymer point-of-care biosensor device which is applicable to detect blood protein markers of cerebro-vascular symptoms e.g. stroke with high sensitivity. The proposed microfluidic systems are applicable for cost-effective sample transport and still can contain some simple sample preparation functions, such as mixing or dilution. The polymer based microfluidic structure was realised by fast prototyping applying SU-8 epoxy based negative photoresist as moulding replica for PDMS moulding. An improved ³D multilayer formation process was developed in order to achieve reliable SU-8 structuring for the formation of advanced functional elements (e.g. chaotic mixers) of the microfluidic system. The practical performance of the microfluidic system was verified by biological analyte. The key issue of the research from the point of the integrability and final applicability of the device is the appropriate polymer material selection, which has a natural impact on the leakage-free bonding between the sensing and the fluidic part of the device. To obtain stable and high adhesion between the fluidic (PDMS) and other functional materials (polyimide, poly(methyl-metacrylate), SU-8) various bonding strategies

were qualified. Adequate adhesion were achieved by subsequent silanisation (applying organofunctional alkoxysilanes as (3-aminopropyl)-triethoxysilane – APTES) processes and oxygen plasma treatments applied for the polymer surfaces. Notable drawback of the application of PDMS in biosensors is the hydrophobic surface characteristics, significantly affecting the maximal flow rate in the channel system. Moreover, the non-specific binding of proteins or ligands on the channel surfaces is also a critical issue due to the possible depletion of the target molecules during the transport to the active sensing area of the device. Our work is also intended to define a modified material composition, which is appropriate to significantly improve both the wettability and the non-specific binding characteristics of PDMS. TX-100 and PDMS-PEO surfactant was added to the raw PDMS before polymerisation and the influence of the tensides was studied considering the polymerisation reaction and the surface characteristics. For testing the hydrodynamic behaviour and non-specific protein adsorption on the surfaces fluorescently labeled human serum albumin was applied.

P3.5.

In vitro characterization of convention enhanced drug delivery capabilities of silicon-based multielectrode arrays with fluidic channels

¹Áron Horváth, ²Gergely Márton, ²Zoltán Fekete, ³István Ulbert, ²Anita Pongrácz

¹ *Elektrofizioógia, Pázmány Péter Katolikus Egyetem Információs Technológiai Kar, Budakeszi, Hungary;* ² *Mikrotechnológia Osztály, MTA-TTK, Műszaki Fizikai és Anyagtudományi Intézet, Budapest, Hungary;* ³ *Összehasonlító Pszichofiziológia Csoport, MTA-TTK Kognitív Idegtudományi és Pszichológiai Intézet, Budapest, Hungary*

Implanted neural sensors, such as silicon-based multielectrode arrays (MEAs), manufactured with the technology of microelectromechanical systems (MEMS) are frequently applied for recording extracellular

activities (local field potential – LFP, single and multiunit activities (SUA, MUA)). Our research group has successfully integrated longitudinal channels into the shafts of such microelectrodes, which can therefore be used for controlled material release, such as convention enhanced delivery. The benefits of precise injection of materials in the very proximity of the electrodes are numerous. The microchannels can be used for storing and slowly emitting drugs or labeling molecules. A system like this can be employed for the treatment of tumors, Parkinson's disease or epilepsy effectively. Local injection of anti-inflammatory drugs and bioactive molecules might reduce or delay inflammation and enhance proliferation and survival of neurons around the implant. The distribution of the injected solution is a crucial matter. It depends on several factors, such as the geometry and material of the implanted shafts, flow rate and injected dosage. One of the greatest concerns regarding convention enhanced delivery systems is that above a certain injection speed the liquid flows back through the track of the needle. In this work, an in vitro testing system is presented, which is suitable for modeling the distribution of the injected material in vivo. Agarose (0.6%) was applied as gel material and blue dye was injected into the gel with flow rates of 0.2-2 $\mu\text{l}/\text{min}$. The Matlab Image Processing Toolbox was used to analyze microscopic images, unrevealing spatial distribution of the solution around the implant.

P3.6.

Flexible MEMS-based microelectrocorticograms (uECoG)

¹Marcell Kiss, ¹Gergely Márton, ¹Anita Pongrácz

¹ *Mikrotechnológia Osztály, MTA TTK MFA, Budapest, Hungary*

Electrocorticography (ECoG) or intracranial EEG can be applied to measure the local field potential (LFP). Significant modulations in LFP are related to sensory processing, motor planning, visuomotor interactions and higher cognitive functions such as attention, memory and decision-making. Electrocorticography is also used to locate epilepsy seizure focus areas,

before surgery. EcoGs used in clinical practice have an electrode diameter of 1 mm, which provide insufficient spatial resolution. In this work, micro-electrocorticograms (μ ECoG) are realised using MEMS technology. The achieved dimension of electrode sites is in a hundred micron range. Devices consist of a polyimide structural and SU-8 passivation layer, while gold is used as electrode material. This multilayer structure allows chronic implantation due to high flexibility. The presented technology includes several improvements in polyimide-based device fabrication. μ ECoGs were designed specifically for chronic implantation into rat and feline cortices, since micromachined holes are integrated in order to help circulation and avoid traumatic impact. The electrical connections were realised in a compact way, with both solderable precuDIP-24 and mechanical contact ZIF connectors. The manufactured μ ECoGs were characterized using impedance spectroscopy.

P3.7.

An approach to increase the yield of physiologically characterized and intracellularly labeled neurons in cortical areas exposed for intrinsic signal optical imaging in the primary visual cortex of the cat.

¹Zoltan Kisvárdy, ¹Tahir Uddin

¹ *Anatómiai, Szövet- és Fejlődéstani Intézet, Debreceni Egyetem, Hevesvezekény, Hungary*

In primary visual cortex (V1) orientation preference is arranged in iso-orientation columns or domains around pinwheel centers where all orientations meet in a point like fashion. Recent data (i) provided controversial results on the orientation tuning properties of pinwheel center neurons as well as showed their differing adaptational properties compared to those of orientation domains, (ii) anatomical data also indicated marked differences in the connectivity pattern between the two populations of cell. Hence investigation of the response characteristics and connectivity of single pinwheel neurons would unravel further details of the organization

of V1. Here we developed a technique whereby single pinwheel neurons can be studied using intrinsic signal optical imaging, electrophysiological characterization and labeling axonal connections at utmost detail. The limiting factor of this combined approach that hinders all three types of measurements is brain pulsation resulting from heart beat and ventilation related movement. Since intrinsic signal optical imaging requires large surface area to be exposed topical application of agar and wax alone are insufficient for dampening brain movement. In order to attenuate brain pulsation we have developed a light weight chamber that is covered by a silk mesh at its bottom. The chamber is gently lowered on the exposed cortical surface until a physical contact is reached. Surface blood vessels, which are used for guiding the glass electrode for penetrating the cortex at pinwheel centers of the orientation map, are readily seen through the openings of the mesh. Once the electrode tip is in place, i.e. touching the cortex at the aimed pinwheel center position, agar and wax can be topically applied in addition to conventional methods (pneumothorax, cysternal puncture) for attenuating brain pulsation. In this way, the exposed cortex is sandwiched between the relatively elastic silk-mesh and the interior wall of the skull quasi-restoring intracranial pressure. Acknowledgements: Supported by FP7-PEOPLE-ITN-2008 (FACETS-ITN) to Z.K.

P3.8.

Testing a novel 7-channel deep brain microelectrode for parallel single unit recording in the cat thalamus

¹Péter Kóbor, ¹Zoltán Petykó, ²László Papp, ¹Mary Ann Allston, ¹Péter Buzás

¹ *Élettani Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;* ² *Neuronelektród Kft., Neuronelektród Kft., Budapest, Hungary*

Multiple electrodes (e.g. tetrodes) can improve reliability of separating extracellular action potentials of concurrently recorded neurons. Our aim

was to develop an economical multitrode that is sufficiently robust to reach stereotaxically defined brain regions at depths of >1 cm without the use of a guiding tube, and that allows high quality parallel recordings. Here we present a custom-made 7-channel heptode consisting of 7 platinum-iridium wires arranged in a hexagon of 60 μm diameter. The tip of the electrode can be sharpened at various angles resulting in contact distances of about 20 μm . We confirmed in anaesthetised, paralysed and artificially ventilated cats that this construction is rigid enough to reach stereotaxically defined locations of the lateral geniculate nucleus. We recorded stimulus evoked extracellular potentials at several locations and identified single units using cluster analysis of spike waveforms. Based on testing refractory period violations, we could isolate 6.6 (± 3.8) single units per recording site which comprised 37.22% of all spike events. For comparison with single channel recordings, we analysed the sum of the multi-channel signal and found 3.1 (± 2.0) single units per recording site (19.50% of spike events). These results demonstrate the utility of our novel microelectrode for parallel recording of several neurones from deep brain structures. Supported by grants OTKA K79156 and the Bolyai Fellowship to P.B. and by TÁMOP-4.2.1./B-10/2/KONV-2010-0002.

P3.9.

In vivo durability tests on microelectrode arrays with high surface area platinized platinum sites

¹Gergely Márton, ²István Wosinski, ³István Bakos, ⁴Zoltán Fekete, ¹István Ulbert, ⁴Anita Pongrácz

¹ Kognitív Idegtudományi és Pszichológiai Intézet, Magyar Tudományos Akadémia, Természettudományi Kutatóközpont, Budapest, Hungary; ² Információs Technológiai Kar, Pázmány Péter Katolikus Egyetem, Budapest, Hungary; ³ Anyag- és Környezetkémiai Intézet, MTA Természettudományi Kutatóközpont, Budapest, Hungary; ⁴ Mikrotechnológia, MTA TTK Műszaki Fizikai és Anyagtudományi Kut., Budapest, Hungary

Implantable neural electrodes are commonly used for in vivo monitoring of potential changes within the extracellular space of the central nervous system (CNS). In order to equip these structures with electrodes, conducting materials are required, which are non-toxic and able to withstand the harsh chemical effects caused by the neural tissue. Noble metals such as gold (Au), platinum (Pt) and iridium (Ir) are commonly used, because of their corrosion resistance. The electrode surfaces must be as noiseless as possible, when functioning as recording sites and they must have the capability of injecting sufficient amount of charge into the tissue, when employed as stimulators. A key parameter, which determines whether they can meet these criteria, is their impedance. For these reasons, electrodes with large effective surface area, related to their geometric area are needed. Several porous electrode materials with huge roughness factor have been developed for this purpose, such as platinum black, carbon nanotubes and conducting polymers. In spite of the broad spectrum of the applied methods, electrode impedance reduction of microelectrode arrays (MEAs) is still an issue under active research. A key factor that has to be considered is whether these deposits are durable enough to withstand the penetration into the neural tissue. In this work, a 24-channel silicon-based MEA and a platinum deposition procedure is presented. We have developed the platinization method with the variation of parameters and applying ultrasonic treatment on the deposited layers afterwards. Parameters such as roughness factor, durability and controllability of the deposition were in our primary attention. The Pt deposits were tested with multiple acute in vivo experiments for durability and compatibility with acute stereotactic operation procedures. The probe was used for nine in vivo measurement sessions in the hippocampus of Wistar rats ($n=3$). In order to reduce the number of animals used, it was implanted three times into each rat, at three different locations. Before each triple session, electrode activation and cleaning process was performed in 0.5 M H_2SO_4 . The MEA has proven to be robust enough to withstand nine recording sessions without damage, and its electrodes have endured the procedures without significant variation of impedances at the biologically relevant frequency of 1 kHz.

P3.10.

More effective caged compounds for two photon uncaging

¹Dénes Pálfi, ²Gergely Szalay, ²Klaudia Spitzer, ²Gergely Katona, ²Attila Kaszás, ²Balázs Chiovini, ²Miklós Madarász, ³Imre Csizmadia, ²Balázs Rózsa

¹ *Két Foton Laboratórium, PPKE ITK, Budapest, Hungary;* ² *Two Photon Imaging Center, Institute of Experimental Medicine, Budapest, Hungary;* ³ *Chemistry Department, University of Toronto, Toronto, Canada*

We developed a new GABA- (γ -Aminobutyric acid) named iDMPO-DNI-GABA and a more effective glutamate uncaging material named DNI-GLU-TFA. Two-photon uncaging takes advantage of the high spatial and temporal resolution of two-photon excitation to study even the function of a single synapse. Thus, two-photon uncaging can be used to produce maps of receptor densities (e.g. GABA) even in three dimensions (Katona et al. 2011). Used in combination with two-photon imaging, two-photon uncaging provides an opportunity to study the long-term structural and functional consequences of stimulation of structures such as dendritic spikes and dendritic spines. We found that DNI-GLU-TFA has a 7.2 times higher two-photon action cross section and a faster release rate after two-photon activation as compared to MNI-Glu. We measured the relative two-photon efficiency of the compound from 700 nm to 840 nm. We found that two-photon uncaging efficiency of both materials peaked at 720-740 nm. Moreover, we developed a protective material which decreased the side effects of two-photon uncaging. The GABA receptor inhibiting side effect of DNI-Glu-TFA and MNI-Glu was also compared. DNI-Glu-TFA induced less reduction in IPSCs amplitudes due to the larger compound caging the glutamate.

P3.11.

Chronic pain threshold changes in a new complex schizophrenia model

¹Zita Petrovszki, ¹Gabriella Kekesi, ¹Gyorgy Benedek, ¹Gyongyi Horvath

¹ *Élettani Intézet, Szegedi Tudományegyetem Általános Orvostudományi Kar, Szeged, Hungary*

Introduction: The decrease in pain sensitivity is a well-known symptom of schizophrenia. We induced schizophrenia-related alterations by selective breeding and subchronic ketamine treatment with social isolation in rats. The aim of the present study was to determine the pain sensitivity in acute and chronic pain tests. **Methods:** Twelfth generation of male Wistar rats after weaning (day 21 of age) were both housed individually for 4 weeks and treated with ketamine (30 mg/kg subcutaneously, s.c.) for 3 weeks, then followed 1 week resocialization. The control group did not receive any treatment. Tail-flick latencies at 48 °C were determined at 4 and 9 weeks of age. At the age of 14 week 2 x 1 mg monosodium iodoacetate (MIA) was injected into the right ankle of animals to induce osteoarthritis leading to mechanical allodynia. The mechanical pain threshold was detected by dynamic aesthesiometer before the inflammation and one and two weeks thereafter. Additionally, the antinociceptive effect of 1mg/kg (s.c.) morphine was also assessed. The degree of edema was also measured by a digital caliper. The body weight of the animals was measured weekly. **Results:** There were no significant differences in the body weight and the tail-flick latency at the age of three weeks between the two groups. In contrast, the new substrain showed enhanced tail-flick latency at the age of 9 weeks compared to the control animals. Regarding the mechanical sensitivity before MIA injection, a significant decrease was observed in the new substrain. After injection of MIA the pain threshold significantly decreased in the inflamed side in both groups, but a higher degree of allodynia was observed in the control group. Morphine produced significant decrease in allodynia in both groups, but its antinociceptive effect appeared earlier, and was more prolonged in the treated group. Regarding the ankle cross section area, the injection of MIA caused a prolonged, significant edema, without significant difference between the groups. **Conclusion:** Our study proved that the selective breeding after a

double hit treatment led to decreased pain sensitivity in both acute and chronic pain models, suggesting that this new substrain can simulate the hypoalgesic phenomenon of schizophrenia. This work was supported by OTKA (K83810;NF72488), TÁMOP-4.2.2/B-10/1-2010-0012, TÁMOP-4.2.2.A-11/1KONV-2012-0052, grants.

P3.12.

In vivo testing of silicon based neural multielectrodes with integrated drug delivery channels

¹Anita Pongrácz, ¹Zoltán Fekete, ¹Gergely Márton, ²Zsófia Bérces, ¹Marcell Kiss, ¹Péter Fürjes, ³István Ulbert

¹ *Mikrotechnológia, MTA TTK MFA, Budapest, Hungary;* ² *ITK, PPKE, Budapest, Hungary;* ³ *Összehasonlító Pszichofiziológiai Labor, MTA TTK KPI, Budapest, Hungary*

Complex electrical and chemical interaction of neurons in the central nervous system is still in the research focus of various groups. One of the prevalent electrophysiological methods is to use penetrating multielectrodes in the extracellular space of the brain and record or stimulate neural activity. Additional local drug delivery features could strongly enhance the functionality of these multielectrodes. Simultaneous chemical and electrical interactions with the very same groups of cells of an active brain area might induce accelerated progress in numerous fields: local brain areas can be stimulated or inhibited while the neuronal activity can be recorded simultaneously; effect of pharmaceutical can be tested over the blood brain barrier on the local neurotransmitter level; long-term stability of electrophysiologic implants can be extended with the help of continuous dosage of anti-inflammatory drugs via drug-delivery channels. In this work we present the fabrication method, electrical and fluidic characterization and in-vivo testing of a silicon neural multielectrode with monolithically integrated fluidic channel. Fabrication process and schematic view of the realized probes is presented. Micromachining of the

drug delivery channels take place in the bulk substrate and leave the substrate surface available for further processing. The fluidic microchannels are geometrically characterized by cross sectional SEM imaging, cross section of the channels can easily be tuned in the 5-30 μm range. A PTFE tube connection is established between the macro-pump and the microfluidic channel and pressure-flow characteristic of the channel is measured. In-vivo testing of the novel neural probes is carried out in rat brain, where it can easily penetrate both the dura and pia mater and exhibit excellent performance in local field potential, multi-unit and single unit activity recordings. Feasibility of our integration concept is proved by locally injected bicuculline in the cortex and in the thalamical regions of rat brain in vivo, while simultaneously recording the electrical signals of the stimulated neurons on four different electrical channels.

P3.13.

Micro- Electric Imaging: Reconstruction of spatio- temporal patterns of single neuron input currents based on extracellular potential measurements

¹Zoltán Somogyvári, ²Dorottya Cserpán, ³Lucia Wittner, ⁴István Ulbert, ⁵Antal Berényi, ⁶György Buzsáki

¹ Elméleti Osztály, MTA Wigner Fizikai Kutatóközpont, Budapest, Hungary; ² Elméleti Osztály, MTA Wigner Fizikai Kutatóközpont, Budapest, University of Technology and Economics, Budapest, Hungary; ³ Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, Budapest, Hungary; ⁴ Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, Peter Pazmany Catholic University, Budapest, Hungary; ⁵ Department of Physiology, Neuroscience Institute, University of Szeged, New York University, USA, ⁶ Neuroscience Institute, School of Medicine, New York University, New York, United States

One of the main obstacle to decipher the information processing and the neural communication in the brain is the lack of any experimental technique which is able to measure the spatio-temporal distribution of synaptic currents on individual neurons in freely behaving animals. Thus, we developed a new micro electric imaging technique, which is able to determine the currents flowing on single neocortical neurons during action potentials. Our results show, that by using new mathematical source localization methods and high density, chronically implanted micro electrode arrays, fine details of initiation and spatio-temporal dynamics of neural action potentials can be revealed, which was not directly observable before. Preliminary results show, that we are able to determine the subtle effects of input currents preceding and causing the action potentials during different oscillatory states of hippocampus. The new method can provide better description of cortical microcircuits and their dynamics, which is essential for the understanding of their computation and helps to bridge the gap between the microscopic and the macroscopic neuro- electric phenomena. Furthermore, our project could lead to better understanding of the neural code, by refining our knowledge about the input-output transformation implemented by the neurons.

P3.14.

In vivo 3D measurement of neuronal network activity during visual stimulation

¹Klaudia Spitzer, ¹Gergely Szalay, ¹Gergely Katona, ²Pál Maák, ²Máté Veress, ¹Attila Kaszás, ¹Linda Sulcz-Judák, ¹Balázs Chiovini, ³Dénes Pálfi, ⁴Dániel Hiller, ⁴Botond Roska, ¹Rózsa Balázs

¹ Két Foton Képkészítő Központ, Magyar Tudományos Akadémia Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary; ² Atomfizika Tanszék, Budapesti műszaki és Gazdaságtudományi Egyetem, Budapest, Hungary; ³ Két Foton Képkészítő Laboratórium, Pázmány Péter Katolikus Egyetem, Információs Technológia Kar, Budapest, Hungary; ⁴ Neural Circuit

In order to study the memory process and neuronal network activity in a less modified environment in vivo experiments are required. Although several electrophysiological methodologies and imaging techniques have been developed for 3 dimension network measurements none of them provided the high spatial and temporal resolution that can be realized with 3D two-photon acousto optical imaging. However, the disadvantage of this technology is that current technology is too complex for miniaturization. Therefore measurement should be performed with head-fixed animals. The main problem was that 3D in vivo measurements from the V1 region of the visual cortex resulted in slow frequency synchronous activity pattern. This synchronous activity suppress the real responses from the visual stimulation. To solve this problem several methods have been tried including different type of anesthesia and habituated, awake head-fixed mice. But based on our data and other studies from the literature the only solution to solve this problem is virtual reality system, where we can examine mice in freely moving conditions. We used a high-resolution, acousto-optic two-photon random-access scanning microscope that reaches near-cubic-millimeter scan range (up to $700 \times 700 \times 1,400 \mu\text{m}^3$), with a high scanning speed, with $470 \times 490 \times 2,490 \text{ nm}^3$ resolution in the center core, and less than $1.9 \times 1.9 \times 7.9 \mu\text{m}^3$ resolution throughout the whole scanning volume, which is still sufficient to measure activity from the cell somatas in the whole scanning volume. With these tools we measured the neuronal activity of the visual cortex in 3D. Furthermore we used the combination of the two-photon imaging technique and the in vivo patch clamp technique. We show volumetric random-access scanning calcium imaging of spontaneous and visual stimulation-evoked activity in hundreds of neurons of the mouse visual cortex in vivo.

P3.15.

Optically measured propagation speed of regenerative dendritic events using fast three-dimensional (3D) random-access acousto-optical scanning

¹Gergely Szalay, ¹Gergely Katona, ²Maák Pál, ¹Kaszás Attila, ¹Máté Veress, ¹Balázs Chiovini, ¹Pálfi Dénes, ¹Klaudia Spitzer, ³Dániel Hillier, ⁴Botond Roska, ¹Balázs Rózsa

¹ Two-Photon Imaging Center, IEM HAS, Budapest, Hungary; ² Dept. of Atomic Physics, Budapest University of Technology and Economics, Budapest, Hungary; ³ Faculty of Information Technology, Pázmány Péter Catholic University, Budapest, Hungary; ⁴ Institute for Biomedical Research, FMI, Basel, Switzerland

To understand the computation functions of a single neuron requires methods to read out its activity in different time scales, which pose distinct technical challenges, since we have to resolve dendritic spines in a volume, which contains most of the branches, also have to reach sub ms temporal resolution, which is the time scale of the regenerative events in the dendrites. In spite of the advanced imaging technologies introduced in neuroscience, to meet these criteria in the same time still remains a challenge. We developed a three-dimensional random-access scanning system which have spine resolution ($\text{PSF} = 0.47 \times 0.48 \times 2.49 \mu\text{m}^3$) in the central core of the scanning volume ($290 \times 290 \times 200 \mu\text{m}^3$), and can reach 55 points/kHz scanning speed. We have validated our optical method by measuring back propagating action potentials (bAP) in large dendritic branches from several points and with large lateral ($\pm 300 \mu\text{m}$) and axial ($\pm 500 \mu\text{m}$) displacement and being able to resolve single action potentials in all cases. With the random trigger method we can distinguish onset of Ca^{2+} signals only $54 \mu\text{s}$ or in spatial regime $10 \mu\text{m}$ apart. With the combination of the random-access and the roller coaster scanning mode we can further increase the scanning speed up to 600 points/kHz. With these tools we measured propagation speed of bAPs at different temperatures, and of forward propagating subthreshold and suprathreshold spikes. Also we were able to resolve the fine details of signal propagation in the main apical branch of CA1 pyramidal cells and also in its oblique dendrites.

P3.16.

Electroporetic loading of single cells in mouse hemicochlea for functional imaging

¹Tímea Téglás, ¹Eszter Berekméri, ¹Máté Aller, ¹E. Szilveszter Vizi, ²Tibor Zelles

¹ *Celluláris Farmakológia Kutatócsoport, Magyar Tudományos Akadémia, Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Farmakológia és Farmakoterápia, Semmelweis Egyetem, Budapest, Hungary*

Hearing loss is the most common human sensory deficit. Impairments of the cochlea or the auditory nerve, i.e., sensoneuronal hearing losses (SNHLs; e.g. presbycusis or noise-induced hearing loss) are not curable by pharmacological tools at present. Invention of effective drug therapy needs deepening of our pathophysiological knowledge at the cellular level. However, investigations are set back largely by the paucity of applicable preparations from mice of mature hearing. Hemicochlea preparation from hearing mice (>P15) meets the requirements, but have not been used for functional imaging studies so far. Our aim was to set up a reliable Ca²⁺ indicator dye loading method suitable for investigating mechanism of SNHLs in different cell types of the organ of Corti by functional imaging in the hemicochlea. Cochlea of P15-27 mice were cut in half by a vibratome. The preparation was fixed in an epifluorescent microscope and perfused with oxygenated buffer solution. The selected and identified cells were touched by the fluorescent dye filled glass pipette guided by a micromanipulator. The indicator was injected into the cells by a current pulse (electroporation). We tested Oregon Green 488 BAPTA- and the ratiometric dye Fura-2. Fluorescence changes were followed by a cooled CCD camera based imaging system. We defined the optimal dye concentration in the pipette (1 mM), the amplitude (10 μ A) and width (10 ms) of the square-wave current pulse and parameters of the pipette (4-6 MOhm). We could fill up 2-6 cells in parallel in one organ of Corti,

including both sensory- and supporting cells. The background staining remained low. Although in different distribution, but all cell types of the organ of Corti have purinergic receptors (P2X and P2Y). For validation of the method, we evoked intracellular Ca²⁺ responses in the cells by ATP application. The responses were ATP concentration dependend and repeatable several times. We have set up a method of single-cell electroporation of Ca²⁺ indicators in hemicochlea preparation of hearing mice for the first time. The method supports high spatial- and temporal resolution functional imaging in both wild type and genetical modified mice. Since it is based on a cochlear preparation from mice with mature hearing, the patomechanism of adult SNHLs can be studied without contamination of developmental changes. This work was supported by the NIH-ANR Collaborative R&I Programme, TÉT_10-1-2011-0421.

P3.17.

Application of induced pluripotent stem cells (iPSCs) for disease modelling and drug screening in neurobiology

¹Gergő Vőfély, ²Zsuzsa Erdei, ²I. Tamás Orbán, ²Gabriella Nyitrai, ²Ildikó Pál, ²László Héja, ²Ágota Apáti, ²Julianna Kardos, ²Balázs Sarkadi

¹ *Doktori Iskola, Semmelweis Egyetem, Budapest, Hungary;* ² *Funkcionális Farmakológiai Osztály, MTA TTK MFI, Budapest, Hungary*

Human embryonic (HuES) and induced pluripotent (hiPS) stem cells provide new possibilities to generate in vitro developmental and disease models as well as to allow drug screening in human tissues. Recently, the reprogramming of somatic cells into pluripotent state, resulting in iPS cells became a valuable source to establish patient-specific disease models and drug screening systems. Our aims were the generation and characterization of hiPSC and comparing them to HuESC, focusing on physiological functions and drug responses. Human iPSCs were generated from human foreskin fibroblasts by introducing the reprogramming factors (Oct4, Sox2, Klf4, c-Myc, Lin28) in a single expression cassette, using the Sleeping

Beauty transposon system. We found that the hiPS and HuES cells expressed similar stem cell markers (Oct4, Nanog, PODXL) and had the ability to differentiate into all three germ layers (ecto-, meso-, and endoderm), thus representing a pluripotent state. The intracellular calcium signals induced by various ligands were akin in both cell types. The neuron-like cells differentiated from hiPS or HuES cells were characterized by immunostaining and showed comparable immunocytochemical, pharmacological and electrophysiological characteristics. Based on these results we suggest that hiPS cells generated in this work are suitable for detailed cell biology studies, and will be applicable for screening various drug effects and toxicity in neurological applications. Supported by grants OTKA NK83533 and TECH-09-AI-2009-0117 NKFP NANOSEN9.

Session 4. Neuroendocrinology, P4.1. – P4.29.

P4.1.

Protective effect of pituitary adenylate cyclase activating polypeptide in a rat diabetic nephropathy model

¹Eszter Bánki, ²Péter Degrell, ¹Adrienn Düh, ³Dániel Nagy, ¹Katalin Csanaky, ⁴Krisztina Kovács, ³Ágnes Kemény, ¹Péter Kiss, ¹Andrea Tamás, ⁵Gábor Jancsó, ⁶Dóra Reglődi

¹ *Anatómiai Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;* ² *II. sz. Belgyógyászati Klinika és Nephrológiai Centrum, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary;* ³ *Farmakológiai és Farmakoterápiai Intézet, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary;* ⁴ *Biokémiai és Orvosi Kémiai Intézet, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary;* ⁵ *Sebészeti Oktató és Kutató Intézet, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary;* ⁶

Pituitary adenylate cyclase activating polypeptide 38 (PACAP-38) is a neuropeptide, exerting diverse biological effects. One of its most frequently examined functions is the cell protection, which is achieved mainly via inhibiting apoptotic, inflammatory and oxidative processes. Our research group has already reported the renal expression of all its three receptors (PAC1, VPAC1, VPAC2) and proved its protective effect against ischemia-reperfusion induced nephropathy. Diabetic nephropathy is the leading cause of end stage renal disease. The aim of the present study was to investigate the possible ameliorative effect of PACAP in streptozotocin-induced diabetic nephropathy. Diabetes was induced by a single intravenous injection of streptozotocin (65mg/kg) in male Wistar rats. Treated animals were administered ip. 20µg PACAP every second day, while untreated animals were given vehicle. Kidneys were removed after 8-weeks survival. Besides the complex histological analysis (glomerulus diameter, glomerular PAS positive area/glomerulus area, tubular damage), expression of several cytokines was evaluated by cytokine and Luminex array. Furthermore, Western blot was performed to examine the expression of Akt and MAP kinases, while the level of oxidative stress markers (MDA, GSH, SOD) was measured by colorimetric method. Histological analysis revealed severe diabetic changes in kidneys of untreated animals (glomerular PAS-positive area expansion, tubular damage, Armanni-Ebstein phenomenon). PACAP treatment significantly diminished the damage. Diabetes resulted in mildly, while PACAP treatment in significantly higher expression of the antiapoptotic proteins (pAkt and pErk). On the other hand, significantly higher levels of the proapoptotic JNK and p38MAPK were measured in diabetic animals, the activation of which was missing in the PACAP-treated group. Diabetic kidneys showed significant cytokine activation compared to their healthy controls. PACAP was effective in downregulation of cytokines (for example selectin, TIMP-1, ICAM), and it also increased the GSH level. To conclude, PACAP is effective in ameliorating diabetic nephropathy, the polypeptide decreased the expression of the pro-, while increased the level of the antiapoptotic factors. These results raise the opportunity for the use of PACAP as a

possible therapeutic or preventive method in treating the complications of diabetes.

P4.2.

Glycinergic input to neurons of the mouse basal forebrain

¹Zsuzsanna Bardóczi, ²Barbara Vida, ³Masahiko Watanabe, ⁴László Záborszky, ¹Zsolt Liposits, ¹Imre Kalló

¹ *Endokrin Neurobiológia Kutatócsoport, Magyar Tudományos Akadémia Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Laboratory of Integrative Neuroendocrinology, Institute of Experimental Medicine, Budapest, Hungary;* ³ *Department of Anatomy, Hokkaido University School of Medicine, Sapporo, Japan;* ⁴ *Center for Molecular and Behavioral Neuroscience, Rutgers, Newark, United States*

Corticopetal cholinergic neurons in the basal forebrain occupy a space extending from the medial septum rostrally through subpallidal territories towards the centromedial amygdala caudally. Cholinergic neurons are admixed in the same region with other cell types, including gonadotropin releasing hormone (GnRH)-IR neurons. Forebrain areas are rich in glycinergic axons and we were investigating whether or not ascending glycinergic neurons participate differentially in the regulation of specific basal forebrain neurons. In this study, newly developed sera against the glycine transporter ² (GLYT2) and glycine receptor (GlyR) were used in single-label immunohistochemical studies to map the glycinergic axons and their target cells, respectively, in the mouse basal forebrain. In addition, dual-labeling was carried out to reveal, whether GLYT2-immunoreactive (IR) axons establish connection with choline acetyltransferase (ChAT), parvalbumin (PV), calbindin (CB), calretinin (CR) or gonadotropin releasing hormone (GnRH)-IR neurons. A relatively high density of GLYT2-positive axons were found in subregions of the basal forebrain including the medial septum, vertical and horizontal diagonal bands of Broca, the substantia innominata, ventral pallidum and

the extended amygdala. The strongest GlyR-immunoreactivity was observed in the lateral septum, substantia innominata and ventral pallidum. Besides overlapping areas for GLYT2-IR fibers and GlycR-IR perikarya and dendrites, areas labeled only for GLYT2 or GlycR were also observed. Analysis of double labeled samples by light and confocal microscopy identified GLYT2-IR axon varicosities in apposition to ChAT-, parvalbumin-, calbindin-, calretinin- and GnRH-IR perikarya and dendrites. The presence of synaptic contacts between GLYT2- and ChAT-, parvalbumin-, calbindin-, or calretinin-IR neurons was confirmed at the ultrastructural level. These data provide the first morphological evidence that glycinergic neurons have the capacity to directly, but non-selectively influence neurons in the mouse basal forebrain. Supported by OTKA K101326, OTKA K83710, OTKA K100722, FP7/2007-2013 Grant Agreement ²45009 and NIH grant NS023945 (LZ)

P4.3.

Mapping of brain regions activated in rat pups in response to suckling

¹János Barna, ¹Árpád Dobolyi

¹Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary

The most important activity of pups is suckling especially in rodents where pups are born blind and undeveloped. To understand the neural mechanisms related to suckling we performed a mapping study using the Fos technique. After reuniting the litters with the dams for 2 hours following a day of separation, suckling started within 5 minutes both on the 9th and 17th postnatal days. A significant increase in the number of Fos-immunoreactive (Fos-ir) neurons was found in the sensory trigeminal nucleus, the facial motor nucleus, the lateral parabrachial nucleus, the medial paralemniscal nucleus, and the central nucleus of the amygdala. Double labeling experiments demonstrated the activation of calcitonin gene-related peptide neurons in the lateral parabrachial nucleus as well as

in the motoneurons of the facial nucleus. In turn, neurons containing tuberoinfundibular peptide of 39 residues were activated in the medial paralemniscal nucleus. On the 17th postpartum day, rats were also tested by providing dry food instead of returning them to their mothers. At this age, amount of consumed dry food and milk equals. Fos activation in the central amygdaloid nucleus was decreased in pups consuming dry food as compared to suckling. In turn, Fos activated cells also appeared in the dorsomedial hypothalamic nucleus. Neurons activated by suckling may participate in sensory and motor processes as well as homeostatic regulations related to suckling while brain regions that were activated by dry food as well may play a role in general food related activities, e.g. they indicate satiation. Support was provided by the János Bolyai Fellowship of the HAS and the OTKA K100319 research grant.

P4.4.

Effects of neuropeptide SF on corticosterone release, locomotor activity and body temperature

¹Krisztina Csabafi, ¹Miklós Jászberényi, ¹Zsolt Bagosi, ¹Nándor Lipták, ¹Gyula Telegdy

¹ *Kórélettani Intézet, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary*

Neuropeptide SF, a member of the RF-amide family, is a neurotransmitter known to modulate nociception. This study investigates the effect of neuropeptide SF on the hypothalamus-pituitary-adrenal (HPA) axis, behavior and body temperature. The peptide was administered in different doses (0.5, 1, 2, 5 nmol) intracerebroventricularly to rats, the behavior of which was then observed by telemetry, open field (OF) and elevated plus maze (EPM) tests. Plasma corticosterone concentrations were measured to assess NPSF's influence on the HPA activation. In addition, the effect of NPSF on core temperature was also measured via telemetry. To identify the receptors involved in the mediation of NPSF's actions, animals were

pretreated with non-selective corticotropin-releasing factor (CRF) antagonist, selective vasopressin (AVP) V1 receptor blocker or non-selective cyclooxygenase inhibitor. Our results showed that NPSF increased the corticosterone level in the plasma, the action of which was not abolished by the CRF antagonist, rather by the vasopressin blocker. Exploratory and stereotype behavior in the OF and EPM tests was not affected, however spontaneous locomotor activity increased significantly after peptide treatment. Furthermore, NPSF caused a marked elevation in the core temperature of the animals that was not blocked by the cyclooxygenase inhibitor. These results demonstrate, that NPSF stimulates the HPA axis possibly through the activation of both CRF and AVP. In addition, NPSF causes hyperactivity and induces hyperthermia, the later of which does not seem to occur through the central production of prostaglandins.

P4.5.

A hipofízis adenilát cikláz aktiváló polipeptid (PACAP) hatásai apoptotikus jelátviteli utakra normál humán epitheliális és emlődaganatos sejtvonalakon

¹Katalin Andrea Csanaky, ²Alíz Szabó, ³András Balogh, ³Marianna Pap, ²Zita Bognár, ¹Brigitta Bede, ¹Eszter Bánki, ¹Andrea Tamás, ¹Dóra Reglódi, ³Krisztina Kovács

¹ *Anatómiai Intézet, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary;* ² *Biokémiai és Orvosi Kémiai Intézet, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary;* ³ *Orvosi Biológiai Intézet, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary*

A PACAP egy 38 aminosavból álló neuropeptid, mely az idegrendszeren kívül csaknem minden szervben előfordul. Emberi anyatejben kutatócsoportunk írta le először a PACAP felhalmozódását és vizsgáltuk változását a szoptatás előrehaladtával. A PACAP specifikus receptora, a

PAC1-receptor is fokozott expressziót mutat laktáció során az emlőmirigyben. A magas peptidkoncentráció és receptor expresszió feltételezi, hogy a peptid fontos szerepet tölt be az újszülött fejlődésében és/vagy magának az emlőmirigynek a laktáció alatti növekedésében. Ezen kérdés vizsgálatához emlőmirigy sejteken tanulmányoztuk a PACAP hatását az apoptotikus folyamatokra. A PACAP szerepét H₂O₂-indukálta oxidatív stresszben vizsgáltuk normál humán epitheliális (HMEC) és emlődaganat sejtvonalakon (MCF-7; MDA-MB 468). Ugyanezen sejtvonalakon vizsgáltuk a PAC1 receptor expresszióját konfokális mikroszkóp segítségével. A PACAP38 sejttúlélésre kifejtett hatását MTT teszttel vizsgáltuk. A mitokondriális membránpotenciál mérést JC-1 fluoreszcens festéssel tettük láthatóvá. Az apoptotikus markereket apoptózis, míg az oxidatív stresszre adott választ cell stress array kit-tel vizsgáltuk. MTT eredményeink azt mutatják, hogy a PACAP védte az MCF-7 sejteket H₂O₂-dal szemben, míg a HMEC sejteken a PACAP nem mutatott antiapoptotikus hatást. Subtoxikus dózisban azonban a PACAP kezelés kedvezően hatott az oxidatív stresszel szemben ugyanezen sejteken. A mitokondriális depolarizációs folyamatokat PACAP protektíven befolyásolta. Az apoptosis array eredmények azt mutatják, hogy az oxidatív stressz hatására bekövetkező változások közül néhányat a PACAP kezelés ellensúlyozott. Ilyen volt a XIAP, a clusterin, és a HSP70, melyek expressziója PACAP kezelés hatására megnövekedett. A konfokális mikroszkópos eredményeink azt mutatják, hogy a PAC1 receptor megtalálható mindhárom sejtvonalon. Összefoglalva azt találtuk, hogy a PACAP kezelés fokozza a sejttúlélést emlődaganat sejtekben, részben az apoptotikus útvonalak gátlásával. A laktáció során tapasztalható mirigynövekedés és az emlődaganat növekedése között számos párhuzam van, mint pl. az antiapoptotikus útvonalak szuppresszálása. Eredményeink indirekt módon azt bizonyíthatják, hogy az anyatejben lévő PACAP szerepet játszik a laktáció/involúció során az emlőben végbemenő változások szabályozásában. Támogatás: Lendület Program, Arimura Foundation, OTKA104984, TAMOP 4.2.1.B-10/2/KONV-2010-002, 4.2.2.B-10/1-2010-0029; 4.2.2.A-11/1/KONV-2012-0024

P4.6.

Maternal responses are impaired by antagonism of the receptor of tuberoinfundibular peptide of 39 residues

¹Melinda Cservenák, ¹Éva Rebeka Szabó, ²Ibolya Bodnár, ¹András Lékó, ¹Miklós Palkovits, ²György M. Nagy, ³Ted B. Usdin, ¹Árpád Dobolyi

¹Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary, ²Laboratory of Neuroendocrinology, Department of Human Morphology and Developmental Biology, Semmelweis University, Budapest, Hungary, ³Section on Fundamental Neuroscience, National Institute of Mental Health, Bethesda, Maryland, USA

Extensive maternal caring is critically important for the survival of a newborn offspring. Still, not all components of the regulatory circuits are established yet, even though such knowledge is crucial for understanding normal as well as abusive and neglectful mothering. In this work, we describe a new component involved in the regulation of maternal responses. We examined the activation of TIP39 neurons in the posterior intralaminar complex of the thalamus (PIL) in mother rats with or without close contact with their pups and showed that suckling but not olfactory, visual and auditory input from pups activated the TIP39 neurons in the PIL. Furthermore, we investigated the efferent and afferent neuronal connections of the activated TIP39 neurons by injecting anterograde and retrograde tracer into the PIL. Neurons in the PIL receive ascending information from the spinal cord and in turn project to maternal brain centers including the preoptic area and the arcuate nucleus. Indeed, the injection of retrograde tracer to these hypothalamic sites labeled TIP39 neurons in the PIL. Subsequently, a genetically modified lentivirus expressing a peptide antagonist of the parathyroid hormone 2 receptor (PTH2R) as well as only GFP-tagged virus as control was injected into the mediobasal hypothalamus of female rats, which infected a few cells around the injection site and made them release the PTH2R antagonist HYWY-TIP39. Plasma prolactin concentrations were measured in virus injected mothers on postpartum day 10. We observed a lower basal prolactin level and a significant reduction in suckling-induced prolactin levels in the

PTH2R antagonist expressing dams. The same virus was injected into the preoptic area and conditioned place preference test was performed on virus injected mothers. The results indicated that PTH2R antagonist expressing mothers showed no preference for pup-associated chamber as did control virus injected dams suggesting reduced maternal motivation following blockade of endogenous TIP39 action. This effect may be mediated by neurons expressing c-fos by pup exposure in the preoptic area, as they were demonstrated to be closely apposed by TIP39 terminals. In conclusion, TIP39 neurons are ideally positioned in the PIL to convey suckling information towards maternal brain centers to regulate different maternal responses in the postpartum period. Support was provided by OTKA K100319 and the János Bolyai Fellowship of the HAS.

P4.7.

Homeostatic alterations after IL-1 β microinjection into the cingulate cortex of the rat

¹Bettina Csetényi, ¹Edina Hormay, ¹Bernadett Nagy, ¹István Szabó, ¹Márk Bajnok Góré, ¹Barnabás Hideg, ¹Zoltán Karádi

¹ *Institute of Physiology, Pécs University, Medical School, Pécs, Hungary*

Experimental findings of our research group and data of literature prove that the cytokines have important modulatory effects in limbic forebrain structures. Interleukin-1 β (IL-1 β) is a primary cytokine which appears to be involved in several levels of the homeostatic control. In former experiments, anorexigenic, adipogenic and hyperthermic effects of this cytokine were observed. The cingulate cortex is known to play important roles in the homeostatic regulation. Extracellular single neuron recordings verified the presence of IL-1 β responsive neurons in this cortical area. It is also well established that the cingulate cortex has important interrelationships with limbic forebrain structures, therefore the present experiments were designed to examine whether local administration of IL-1 β here causes alterations in various homeostatic functions. For this

purpose, short- (2 h), medium- (12 h) and long-term (24 h) food and water intakes and 2 h body temperature were measured before and after a single bilateral microinjection of this primary cytokine (with or without paracetamol pretreatment) into the cingulate cortex of male Wistar rats. In the cytokine treated group, a somewhat decreasing tendency in medium-term food and water intakes was observed. The IL-1 β microinjection was followed by a significant increase in body temperature.

P4.8.

Subcellular localization of the components of nitric oxide system in the hypothalamic paraventricular nucleus of mice

¹Erzsébet Farkas, ²Ronald M. Lechan, ¹Csaba Fekete

¹ *Integratív Neuroendokrinológiai Kutatócsoport, Magyar Tudományos Akadémia Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Tupper Research Institute and Department of Medicine, Tufts Medical Center, Boston, Ma, USA*

Nitric oxide (NO) is a gaseous transmitter. In the hypothalamic paraventricular nucleus (PVN), it has been implicated in the regulation of energy homeostasis and neuroendocrine control. However, little information is known about the subcellular localization of the components of the NO system in the PVN and whether NO is utilized as an anterograde and/or retrograde messenger by the parvocellular neurons of this nucleus. Neuronal nitric oxide synthase (nNOS) is the enzyme responsible for the NO production of neurons, and the primary mechanism mediating the effects of NO on target neurons is soluble, guanylate cyclase-facilitated production of cGMP. Soluble guanylate cyclase is a heterodimer composed of α ($\alpha 1$ and $\alpha 2$) and β ($\beta 1$ and $\beta 2$) subunits, the most prevalent form being the $\alpha 1/\beta 1$ heterodimer. Using antisera against nNOS and the soluble guanylate cyclase $\alpha 1$ and $\beta 1$ subunits, immuno-electron microscopy was performed to determine the subcellular localization of these proteins in the parvocellular part of the PVN in mice. nNOS was abundantly present in

neuronal perikarya and dendrites and also in axon varicosities. In perikarya and dendrites, nNOS-immunoreactivity was widely distributed in the cytoplasm, primarily associated with the endoplasmatic reticulum. nNOS-immunoreactivity was also found to be associated with the perikaryal plasma membrane in close proximity to both symmetric and asymmetric synapses, as well as within axon varicosities forming both symmetric and asymmetric synapses. The soluble guanylate cyclase $\alpha 1$ subunit was found in dendrites and axon varicosities both in the pre- and postsynaptic densities of the synapses. The $\alpha 1$ subunit was associated with both symmetric and asymmetric types of synapses, whereas the $\beta 1$ subunit was primarily observed in dendrites and frequently associated with the postsynaptic density of synapses. On rare occasions when the $\beta 1$ subunit was observed in axon varicosities, the immunoreactive varicosities formed symmetric type synapses. In summary, these data indicate that nitric oxide may be utilized as both an anterograde and retrograde transmitter in the parvocellular part of the PVN.

P4.9.

Ghrelin decreases firing activity of the gonadotropin-releasing hormone (GnRH) neurons in an estrous cycle and endocannabinoid-dependent manner

¹Imre Farkas, ²Csaba Vastagh, ²Miklós Sárvári, ³Zsolt Liposits

¹ *Endokrin Neurobiológiai Csoport, Magyar Tudományos Akadémia, Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Laboratory of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary;* ³ *Department of Neuroscience, Faculty of Information Technology, Pázmány Péter Catholic University, Institute of Experimental Medicine, Hungarian Academy of Sciences, Laboratory of Endocrine Neurobiology, Budapest, Hungary*

The orexigenic peptide, ghrelin is known to influence the function of GnRH neurons, however, the direct effects of the hormone in these neurons have not been explored, yet. The present study was undertaken to reveal the putative expression of growth hormone secretagogue receptor (GHS-R) in GnRH neurons and to elucidate the mechanism of ghrelin action upon them. The expression of GHS-R mRNA was confirmed in both isolated GnRH-GFP neurons of transgenic mice and immortalized GT1-7 neurons by real-time PCR. Calcium imaging revealed the ability of ghrelin (1 μ M) to increase the intracellular concentration of Ca^{++} in GT1-7 neurons kept in a steroid-free medium. This effect was abolished by pretreatment with the GHS-R antagonist, JMV2959 (10 μ M). Estradiol (1 nM) eliminated the effect of ghrelin on the rise of intracellular Ca^{++} -content, indicating the estradiol dependency of the process. Recordings of the firing of GnRH-GFP neurons showed a significantly lower firing rate and burst frequency in metestrus (firing rate: 0.21 ± 0.04 Hz; burst frequency: 0.027 ± 0.008 Hz) than proestrus (firing rate: 0.64 ± 0.15 Hz; burst frequency: 0.12 ± 0.031 Hz) mice. Ghrelin (400 nM) administration resulted in a significantly decreased firing rate and burst frequency of GnRH neurons in metestrus mice (firing rate: 66.3 ± 9.34 %; burst frequency: 61.8 ± 14.87 %), whereas the peptide exerted no change on the firing parameters in proestrus mice. Ghrelin also decreased the firing of GnRH neurons in the male. The ghrelin-evoked alterations of the firing parameters were prevented by administration of JMV2959 (10 μ M), supporting the GHS-R-mediated action of ghrelin on GnRH neurons. The observed effects of ghrelin were also abolished by the cannabinoid receptor type-1 (CB1) antagonist AM251 (1 μ M), and the intracellularly applied DAG-lipase inhibitor THL (10 μ M), indicating the involvement of retrograde endocannabinoid signalling in the action of ghrelin. In metestrus mice, ghrelin decreased the frequency of the GABAergic mPSCs in the GnRH neurons, which was eliminated by AM251 pretreatment, implying the involvement of CB1. These findings demonstrate that ghrelin exerts direct regulatory effect on GnRH neurons via GHS-R, modulates the firing activity of GnRH neurons in an ovarian cycle-dependent manner and triggers endocannabinoid signalling which activates CB1 in attenuating the frequency of GABA-ergic mPSCs. The present work was supported by grants OTKA (K100722) and FP7/2007-2013 (No. 245009).

P4.10.

Fasting-induced alterations in the α -MSH- and AGRP innervation of thyrotropin-releasing hormone (TRH)-synthesizing neurons in the PVN

¹Andrea Kádár, ²Edith Sanchez, ¹Balázs Gereben, ³Ronald M. Lechan, ¹Csaba Fekete

¹ *Endokrin Neurobiológiai Osztály, Magyar Tudományos Akadémia, Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Dirección de Investigaciones en Neurociencias, Instituto Nacional de Psiquiatría Ramón de la Fue, Mexico;* ³ *Department of Endocrinology, Tufts Medical Center, Boston, USA*

The activation of POMC neurons in the arcuate nucleus plays important role in the development of refeeding induced satiety (Singru et al., Endocrinology 2006). These anorexigenic neurons innervate neurons in the ventral parvocellular subdivision of the hypothalamic paraventricular nucleus (PVNv) that relay satiety information to the brainstem, as well as TRH neurons in the medial and periventricular parvocellular subdivisions that regulate thyroid function. Neurons of the PVNv are activated 2h after the onset of refeeding, but hypophysiotropic TRH neurons are activated only 24h after food has been reintroduced, coincident with recovery of circulating levels of thyroid hormone. To determine whether fasting-induced changes in the neuronal input to TRH neurons contribute to melanocortin resistance of these neurons during the early phase of refeeding. For this reason, we performed triple-labeling immunofluorescence on double-transgenic TRH/Cre-Z/EG mice to study whether the number of α -MSH- and AGRP-immunoreactive axon varicosities contacting hypophysiotropic TRH neurons differ under fed and fasted conditions.. A dense network of AGRP- and α -MSH-immunoreactive axons was found in hypothalamic paraventricular nucleus surrounding TRH neurons in both fed and fasted mice. By confocal microscopic analyses, 30.9 ± 0.3 AGRP-containing boutons and 19.3 ± 0.4 α -

MSH-containing boutons were found on the surface of the perikarya and first order dendrites of the TRH neurons in the PVN of fed mice. Fasting caused a significant increase in the number of AGRP-IR varicosities on the surface of these cells (44.9 ± 0.3).

P4.11.

Effect of endocrine disruptor compound zearalenone on hypothalamus and ovarium

¹Rókus Kriszt, ¹Szilámér Ferenczi, ¹Bernadett Pintér-Kübler, ¹Ágnes Polyák, ¹Zsuzsanna Winkler, ¹Ádám Dénes, ¹Krisztina J. Kovács

¹ *Molekuláris Neuroendokrinológia Csoport, Kísérleti Orvostudományi Kutatóintézet-MTA, Budapest, Hungary*

Zearalenone (ZEA) is a nonsteroidal estrogenic mycotoxin produced by several *Fusarium* spp. on cereal grains. ZEA is a natural endocrine disruptor compound (EDC) that induces dramatic changes in uterus and gonad development and advances puberty in immature rats. However, the effects of mycotoxins on the central neuroendocrine regulation remain to be elucidated. The present study investigated the effect of zearalenone and 17-beta estradiol on hypothalamic expression of gonadotropin-releasing hormone (GnRH), kisspeptin and kisspeptin receptor (GPR54). Immature female rats (postnatal, PN 18-27 days) were treated daily per os with ZEA (10 mg/kg bw) and body weight, food intake and vaginal opening were followed and the weight of the uterus and ovaries was measured at sacrifice (PN 28). Brain samples were collected for real time PCR analysis of neuropeptide expression and for in situ hybridization histochemistry. ZEA treatment increased the uterus weight and decreased body weight gain without significant effect on food intake. ZEA administration resulted in a significant advance in vaginal opening without any reliable changes in GnRH and kisspeptin expression in the hypothalamus. Real time PCR analysis of hypothalamic samples, however, revealed 4-fold increase in kisspeptin receptor (GPR54) in response to ZEA. Using in situ

hybridization histochemistry we found site specific increase of GPR54 mRNA signal in the arcuate nuclei but not in the preoptic area of rats treated with the mycotoxin. On the other hand the ZEA treatment influenced the GPR54 expression in the ovarium but the kisspeptin remained unchanged at mRNA level. These results point to the hypothalamic kisspeptin/GPR54 mechanism as the target of endocrine disruptor mycotoxin.

P4.12.

A tranziens receptor potenciál vanilloid-1 (TRPV1) ioncsatorna protektív szerepe az életkorfüggő elhízás kialakulásában egerekben

¹Csaba Zsiborás, ¹Eszter Pákai, ¹Margit Solymár, ¹Szilvia Soós, ¹Erika Pétervári, ¹Márta Balaskó, ¹Zoltán Szelényi, ¹Miklós Székely, ¹András Garami

¹ *Kórleletani és Gerontológiai Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary*

Korábbi tanulmányainkból ismert, hogy a tranziens receptor potenciál vanilloid-1 (TRPV1) ioncsatornától genetikusan megfosztott (génkiütött, KO) egerek testtömege az életkor előrehaladtával lényegesen meghaladja vad típusú társaikét. Az egerek vizsgálata során arra is fényt derítettünk, hogy fiatal korukban a TRPV1 KO egerek anyagcseréje alacsonyabb, lokomotoros aktivitása azonban fokozottabb volt a kontroll egerekhez képest. Tisztázatlan maradt azonban az, hogy az előbbi – testtömegszabályozásban fontos szerepet játszó – effektorok, hogyan változnak az életkor függvényében. Kísérleteinkben különböző korcsoportú hím TRPV1 KO és vad típusú egereket használtunk. Heti rendszerességgel mértük az egerek testtömegét. Mozgásukban részlegesen korlátozott állatokban mértük azok nyugalmi maghőmérsékletét, emellett szabadon mozgó állatokban regisztráltuk lokomotoros aktivitásuk és maghőmérsékletük cirkadián változásait. Az egerek vizsgálata során a korábban leírtaknak megfelelően azt találtuk, hogy míg fiatal korban nem mutatkozott

szignifikáns különbség a TRPV1 KO és vad típusú egerek testtömege között, addig az életkor előrehaladtával a KO egerek testtömege szignifikánsan magasabbnak bizonyult vad típusú társaiknál. Megfigyeltük továbbá azt is, hogy a TRPV1 KO egerek nyugalmi maghőmérséklete minden korcsoportban alacsonyabb volt a kontrollokénál. A szabadon mozgó állatok vizsgálata során talált cirkadián ritmusbeli különbségek a TRPV1 KO és vad típusú egerek között tovább segítik az idősebb TRPV1 KO egerek fokozott testtömeg-növekedésének tisztázását. Eredményeink alátámasztják a TRPV1 ioncsatorna szerepét a korfüggő elhízás kialakulásának megelőzésében. Az életkor előrehaladtával TRPV1 ioncsatornák hiányában elhízás alakul ki, amely legalábbis részben az állatok csökkent nyugalmi maghőmérsékletével (anyagcseréjével) magyarázható. Következtetésül levonhatjuk, hogy a TRPV1 ioncsatorna jelenléte szükséges a nyugalmi anyagcsere – hosszabb távon pedig a testtömeg – normál értékének fenntartásához. Támogatás: OTKA PD 105532, PTE ÁOK-KA No: ³4039/11-07, MTA Bolyai János Kutatási Ösztöndíj (BO/00785/12/5).

P4.13.

A centrális projekciójú Edinger-Westphal mag idegsejtjei csökkent urocortin 1 és FosB kifejeződést mutatnak krónikus stressznek kitett PACAP heterozigóta egerekben

¹Gabriella Bodnár, ¹Tamás Gaszner, ¹László Kovács, ¹Viktória Kormos, ¹Dóra Reglődi, ¹József Farkas, ²Bálint Szalontai, ¹Balázs Gaszner

¹ *Anatómiai Intézet, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary;* ² *Kísérletes Állattani és Neurobiológiai Tanszék, Pécsi Tudományegyetem természettudományi Kar, Pécs, Hungary*

A depresszió három találat elmélete szerint a genetikai predispozíció, epigenetikai változások és stresszhatások együttesen felelnek a kórkép manifesztálódásáért. Nem ismert azonban, hogy a stresszszabályozásban szerepet játszó corticotropin releasing factor (CRF) peptidcsalád tagjai

pontosan hogyan vesznek részt az adaptációban, illetve a hibás alkalmazkodásban. A főként a centrális projekciójú Edinger-Westphal (cpEW) magban kifejeződő urocortin 1 (Ucn1) szerepét a hangulatzavarok patogenezisében mind állatkísérletes, mind humán megfigyelések alátámasztják. Ismert továbbá, hogy a a hipofízis adenilát-cikláz aktiváló polipeptid (PACAP) részleges vagy teljes hiánya depresszió jellegű viselkedési eltéréseket okoz egérben. Kísérletünk célja az volt, hogy a három találat elméletnek megfelelő egérmodellben vizsgáljuk az Ucn1 tartalmú neuronok működését. A genetikai háttér a PACAP gén részleges hiánya volt, az epigenetikai hatást az anyai megvonással modelleztük, és a stressz hatását a krónikus variábilis enyhe stressz paradigmában vizsgáltuk. Hipotézisünk szerint a krónikus aktivációs marker, a FosB segítségével az Ucn1 tartalmú neuronok aktivitásának és számának megváltozását vártuk anyai megvonás és krónikus stressz után. Eredményink szerint a PACAP heterozigóta egereink, melyek nem szenvedtek maternális deprivációt, a krónikus stresszre 30%-kal emelkedett Ucn1 immunoreaktív sejtszámmal, és 20%-kal magasabb FosB expresszióval válaszoltak. Hasonló eredményeket kaptunk a fizioológiás, 15 perces anyai depriváción átesett csoportban, ahol ugyan meglepetésünkre 34%-kal kevesebb Ucn1 tartalmú neuront láttunk a kontroll csoportban, mégis az aktiváció hasonló mértékű volt, mint a maternális depriváción át nem esett állatok esetében. A korábban napi 3 órás maternális depriváción átesett egerek Ucn1 immunoreaktív sejtszáma lényegesen nem változott a nem deprivált állatokéhoz képest, de a krónikus stressz nem volt képes a sejtszámot megemelni, és a FosB expresszió növekedése is elmaradt a maternális depriváción átesett PACAP heterozigóta állatokban. Összefoglalva az cpEW mag Ucn1 tartalmú idegsejtjeinek megváltozott aktivitását találtuk a depresszió mindhárom rizikófaktorát hordozó egerekben, így arra következtethetünk, hogy a terület szerepet játszhat a hangulatzavarok patogenezisében. Kísérletünkkel egy újabb lépést tettünk a depresszió három találat elméletének igazolása, és egy új megbízhatóbb depresszió modell kifejlesztése felé.

P4.14.

Catecholamine responsiveness of glucose-monitoring neurons in the cingulate cortex of the rat

¹Edina Hormay, ¹Bettina Csetényi, ¹István Szabó, ¹Bernadett Nagy,
¹Barnabás Hideg, ¹Márk Bajnok Góré, ¹Zoltán Karádi

¹ *Institute of Physiology, Pécs University, Medical School, Pécs, Hungary*

The cingulate cortex, as a major cortical component of the limbic system, plays important role in the central homeostatic control. It has intimate interrelationship with glucose-monitoring (GM) neuron containing limbic forebrain structures known for their involvement in the central regulation of feeding and metabolism. The GM cells in these structures were demonstrated to be influenced by catecholamines, which have already been proved to participate in a variety of regulatory processes, including feeding-associated learning and memory mechanisms. The main goal of the present experiments was to examine whether GM neurons exist in the cingulate cortex, and to determine their catecholamine and other neurochemical sensitivities. To do so, extracellular single neuron activity was recorded in the cingulate cortex of adult male anesthetized Wistar and Sprague-Dawley rats by means of tungsten wired multibarreled glass microelectrodes, during microelectrophoretic application of various chemicals, such as D-glucose, norepinephrine, dopamine, GABA, acetylcholine and glutamate. Approximately, 10 % of neurons displayed firing rate changes in response to local microelectrophoretic administration of D-glucose, and both glucose-inhibited (glucose-sensitive, GS) and glucose-excited (glucose-receptor, GR) neurons were indentified. The rate of GM neurons with activity change to microelectrophoretically applied dopamine or norepinephrine was almost two times higher compared to that of the glucose-insensitive (GIS) cells. Characteristic firing rate changes were also recorded to microelectrophoretic administration of the other neurochemicals tested. Our results provided evidence for the existence of GM neurons in the cingulate cortex of the rat. The differential catecholamine responsiveness of these GM and GIS cells is suggested to be of distinguished importance with respect to complex roles of GM neurons in the central regulation of feeding, metabolism, as well as feeding-associated learning and memory mechanisms. Supported by:

P4.15.

Diurnal variation of the melanin-concentrating hormone content of the hypothalamic melanin-concentrating hormone neurons.

¹Emese Éva Várkonyi, ¹Balázs Szőke, ¹Balázs Geric, ¹Veronika Jancsik

¹ *Anatómiai és Szövettani Tanszék, SZIE Állatorvos-tudományi Kar, Budapest, Hungary*

Melanin-concentrating hormone (MCH) expressing neurons, localized in the lateral hypothalamic area and the zona incerta, are involved in the regulation of various aspects of energy homeostasis and in the control of paradoxical (REM) sleep. The circadian variation of MCH gene expression appears to be moderate if existing at all in the mouse hypothalamus (Stütz et al., Obesity. 2007). Similarly, nearly constant MCH polypeptide levels had been observed in individual rat hypothalamic neurons in the light and dark period by multi-transcriptional profiling (Harthoorn et al., Cell Mol Neurobiol. 2005). These authors, on the other hand, observed a difference in the fasting-induced upregulation of MCH polypeptide level in relation to the day-night cycle: while upregulation was significant when measured in the early phase of the light period, it was not significant in the early phase of the dark period. To get deeper insight into a putative diurnal variation of the MCH polypeptide level, we performed comparative MCH immunohistochemistry by evaluating the density of DAB precipitate in the zona incerta and the lateral hypothalamic area separately in mouse hypothalamic coronal sections. Two sets of experiments have been performed, involving 6 mice each. Mice were divided into two groups, one group being sacrificed immediately after the night cycle (lights off, from 8 PM to 8 AM), the second one after the day cycle (lights on, from 8 AM to 8 PM), respectively. After fixation, brain slices of both groups were parallel processed with anti-MCH (Sigma-Aldrich) followed by the use of

the ABC technique and DAB as chromophore. Digitally photographed sections were evaluated with computer-assisted densitometry. Image analysis was performed by Scion Image programme version v4.02 β for Windows with thresholding to the optical density level of the background staining. In the lateral hypothalamic area, MCH level appeared to be significantly higher ($P < 0.05$) at the end of the light period, as compared to data at the end of the dark period, whereas no significant differences were observed ($P > 0.1$) in the zona incerta. These observations put forward the possibility of MCH neuron subpopulations of distinct diurnal fluctuation patterns. This work was supported by the Hungarian Scientific Research Fund (OTKA, K 81419) for V.J.

P4.16.

Nesfatin-1: a new agent in the control of the energy balance

¹Katalin Könczöl, ²Dóra Zelena, ²Ottó Pintér, ²János Varga, ³Máté Durst, ³Miklós Palkovits, ³Zsuzsanna E. Tóth

¹ *Neuromorfológiai Laboratórium, MTA-SE, Anatómiai, Szövet- és Fejlődéstani Intézet, Budapest, Hungary;* ² *Magatartásélettan és Stressz Kutatócsoport, Kísérleti Orvostudomány Kutatóintézet - MTA, Budapest, Hungary;* ³ *Neuromorfológiai és Neuroendokrin Kutatócsoport, MTA-SE, Anatómiai, Szövet- és Fejlődéstani Intézet, Budapest, Hungary*

Nesfatin-1, a fragment of the nucleobinding 2 (NUCB2) protein has been recently discovered as a new agent reducing food intake. However, its wide distribution in the brain autonomic centers suggests some additional functional relevances. To investigate the possible role of nesfatin-1 in regulating the energy expenditure, we performed telemetric experiments and measured core body temperature and locomotor activity of rats for 48h, after icv administration of 25 or 100 pmol nesfatin-1, at the beginning of the light phase. Lower dose of nesfatin-1 raised body temperature slightly 1,5h after administration. There was a bigger difference in body temperature of treated and control rats during the next light phase, since

the circadian curve of temperature in the nesfatin-1 injected rats was flattened. The locomotor activity was not affected. Higher dose of nesfatin-1 elicited a bigger initial effect, otherwise the tendency was the same as observed when lower dose was applied. After cold exposure, many activated (Fos-positive) nesfatin neurons were detected in brain nuclei showing that they were involved in cold adaptation. Nesfatin colocalised with prepro-thyrotropin-releasing hormone (pTRH) expressing cold activated neurons of hypothalamic paraventricular nucleus. Nesfatin and pTRH were also colocalised in neurons of the nucleus raphe pallidus and obscurus in the medulla. These caudal raphe neurons act as sympathetic premotor neurons and activate autonomic preganglionic efferents regulating heat production in brown adipose tissue in rats. In summary, nesfatin may affect the energy balance not only by reducing food intake, but also by increasing the body temperature. Supported by: NKTH-OTKA 80180 (M. P.).

P4.17.

Projections of the hypothalamic paraventricular region to the ventral tegmental area in the rat: the integration of neuroendocrine and reward signals

¹Imre Kalló, ¹Erik Hrabovszky, ¹Csilla S. Molnár, ²Barbara Vida, ²Csaba Fekete, ¹Zsolt Liposits

¹ *Endokrin Neurobiológiai Laboratórium, IEM-HAS, Budapest, Hungary;* ² *Laboratory of Integrative Neuroendocrinology, Institute of Experimental Medicine, Budapest, Hungary*

Neurons residing in the hypothalamic paraventricular nucleus (PVH) and its surrounding areas, the peri-PVH and the perifornical region (PF), play a pivotal role in the regulation of stress, food intake and energy expenditure. The maintenance of these functions also requires the contribution of the central reward system. The present study was undertaken to reveal the putative communication between the hypothalamic paraventricular

complex and the main reward centre, the ventral tegmental area of the brain stem. Neurons in the rat PVH complex were labelled with the anterograde axonal tracer, Phaseolus vulgaris leucoagglutinin (PHA-L), and their descending projections to the VTA studied. The retrograde tracer cholera toxin B (CTB) was applied to the VTA, to map and phenotypically characterise hypothalamic neurons projecting to the VTA. Immunohistochemistry and in situ hybridization were used to label the neuronal tracer and the pre-and post synaptic markers of different neuronal phenotypes. The results provided evidence for a direct projection of neurons located in the PVH complex to the dopaminergic and GABAergic neurons of the VTA. Most of the CTB immunopositive neurons in the rat PVH complex that projected to the VTA were glutamatergic and expressed VGLUT2 mRNA, although GABAergic neurons expressing GAD65 mRNA were also observed, especially in the peri-PVH. The VTA-projecting neurons showed a variable peptidergic phenotype, including corticotrophin releasing hormone-, oxytocin-, thyrotrophin releasing hormone- and cocaine and amphetamine-regulated transcript-containing neurons. These data demonstrate the existence of multiple direct pathways from the PVH complex to the VTA for relaying metabolic and stress signals. Supported by OTKA K101326, OTKA K83710, OTKA K100722 and FP7/2007-2013 Grant Agreement 245009.

P4.18.

Genes with altered maternal expression in the medial prefrontal cortex

¹András Lékó, ²Katalin A Kékesi, ³Éva Hunyadi-Gulyás, ³Katalin Medzichradzsky, ²Gábor Juhász, ¹Árpád Dobolyi

¹Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary; ²Laboratory of Proteomics, Institute of Biology, Eötvös Loránd University, Budapest, Hungary; ³Laboratory of Proteomics Research, Biological Research Centre, Szeged

The medial prefrontal cortex has been implicated in the regulation of emotional and limbic functions including the control of hypothalamic adaptations. Maternal adaptations represent a homeostatic challenge whose counterbalance is expected to include expressional alterations. To determine these alterations, we compared protein levels 14 days after delivery between mothers with litters and mothers continuously deprived of their pups. Changes in protein levels were measured by 2-D gel electrophoresis in groups of 6 rat dams followed by mass spectrometry identification of proteins. A large number of proteins were identified whose expression levels were significantly different between the maternal and non-maternal rats in the postpartum period. To confirm the changes, the mRNA levels of some of the genes were determined by RT-PCR in separate experiments. Furthermore, the distribution of the genes with altered expression levels were addressed by means of in situ hybridization histochemistry. The combination of independent approaches was successful in identifying a number of genes potentially involved in maternal adaptive mechanisms. Interestingly, several cytoskeletal proteins as well as some regulatory proteins were among the genes with altered maternal expression levels. The exact function of these genes in the maternal brain will be tested in functional studies following the experimental manipulation of their expression levels and activities. Indeed, some of them may be involved in altered stress response, reduced anxiety, the control of maternal behaviors, and the increased maternal motivation. Support was provided by Hungarian Scientific Research Funds Research Grant research grant OTKA K100319 and the János Bolyai Fellowship of the Hungarian Academy of Sciences.

P4.19.

Kisspeptin eikozanoid szintézisre kifejtett hatása

¹Zsófia Mezei, ¹Dóra Török, ¹Omid Zamani Forooshani, ¹Ádám Leprán,
¹Krisztina Csabafi, ¹Gyula Szabó, ¹Gyula Telegdy

Bevezetés A kisspeptinek egy a KiSS-1 génről átíródó peptidcsalád tagjai. A különböző szövetekben található GPR és FF2 receptorukhoz kapcsolódva számos folyamatban játszanak szerepet. Sejttypustól függően, a Kisspeptin-13 a GQ/11, az ERK1/2 és a p38 MAP kináz jelutakon keresztül, fejtheti ki hatását. Irodalmi adatokból ismert, a kisspeptin vérárvadási készséget csökkentő és vazóaktív hatása. Kérdés/Hipótezis A vérlemezkék aktiválásában és az érreakciók kiváltásában fontos szerepet játszanak az eikozanoidok. Kísérletünkben ezért arra kerestük a választ, hogy a kisspeptin-13 képes-e befolyásolni a patkány vérlemezkék és az aorta eikozanoid szintézisét. Módszerek Wistar-Kyoto him patkányok vérlemezkéit (2×10^8 sejt/ml) 0, 10^{-8} , $2,5 \times 10^{-8}$, 5×10^{-8} , $7,5 \times 10^{-8}$, 10^{-7} mol/L, míg izolált aortáit (15 mg/ml) 0, 10^{-8} , 5×10^{-8} , 10^{-7} mol/L kisspeptin-13-mal előinkubáltuk, TC Medium 199-ben. A mintákat ^{14}C -arachidonsavval (0,172 pmol/ml) inkubáltuk, majd a keletkezett eikozanoidok mennyiségét (dpm) etilacetátos extrakciót és túlnyomásos vékonyréteg kromatográfiás elkülönítést követően folyadék-szcintillációval határoztuk meg. Eredmények A kisspeptin-13 a vérlemezkék tromboxán szintézisét fokozta, a harang alakú dózis-hatás görbe maximumát, 27%-os növekedést $2,5 \times 10^{-8}$ mol/L koncentrációnál találtuk. A trombociták lipoxigenáz úton képződött termékeinek szintézise a peptid koncentrációjának növekedésével párhuzamosan nőtt 5×10^{-8} mol/L koncentrációig (22%-os növekedés), azonban a dózis fokozásával, további emelkedést nem tudtunk előidézni. Az aorta által szintetizált prosztaciklin stabil metabolitjának, a 6-keto-PGF $_{1\alpha}$ -nak a mennyisége, 56 %-kal növekedett 5×10^{-8} mol/L kisspeptin-13 hatására. Következtetés Az arachidonsav metabolizmus fokozódásának hátterében a kisspeptin receptor aktivációjának eredményeként létrejövő intracelluláris kalcium növekedés állhat. A ciklooxygenáz és lipoxigenáz utak eltérő dózis-hatás görbéje felveti annak lehetőségét, hogy a kisspeptin eikozanoid szintézisre kifejtett hatásában más, mint például az ERK1/2 és a p38 MAP kináz jelátviteli utak is szerepet játszhatnak. Támogatás ETT355-08/2009, TÁMOP 4.2.2.-08/1-2008-0013; 4.2.1./B-09/KONV-2010-005 TÁMOP 4.2.2.2-A-11/1KONYV-2012-0052

P4.20.

Intracellular thyroid hormone availability is system-specifically regulated by type 3 deiodinase in hypophysiotropic neurosecretory neurons during LPS treatment

¹Petra Mohácsik, ²Raffael Arrojo Drigo, ²Antonio C Bianco, ¹Csaba Fekete, ¹Balázs Gereben

¹ *Endokrin Neurobiológia, Kísérleti Orvostudományi Kutató Intézet, Budapest, Hungary;* ² *Division of Endocrinology, Diabetes and Metabolis, University of Miami Miller School of Medicine, Miami, FL, USA*

Thyroid hormones (TH) are key regulators of hypothalamic neurosecretory neurons influencing metabolism, growth, stress and reproduction. We have recently described a novel pathway of TH signaling in parvocellular neurosecretory neurons that involves MCT8-mediated uptake of T3 into axon terminals from the median eminence (ME) followed by regulation of intracellular T3 levels via type 3 deiodinase (D3). We also demonstrated that D3 distribution in axon varicosities in the ME was highly cell-type dependent. In the present study, we assessed how D3-mediated T3 regulation in specific parvocellular neurons of rats responds to bacterial lipopolysaccharide (LPS), a factor known to induce hypothalamic T3 generation. In the ME, D3 activity was not significantly changed but showed a tendency to increase, triggered by increased local T3 generation by hypothalamic tanycytes, that was in striking contrast to the 10-fold drop in cortical D3 activity to compensate for falling cortical T3 levels. Analysis of D3 distribution in hypophysiotrop axons revealed, a significant decrease in the number of D3 positive CRH- (45%) and GHRH-immunoreactive (IR) (10%) varicosities in the external zone of the median eminence, while the presence of D3 in GnRH- and TRH-IR varicosities did not change. Our data suggest that hypophysiotropic neurons can system-specifically influence intracellular T3 levels by regulating their axonal D3 content to allow cell-type specific response to LPS challenge.

P4.21.

Orexinergic input to ventral tegmental area dopaminergic neurons in the human

¹Csilla Molnár, ²Beáta Á. Borsay, ²Kálmán Rácz, ²László Herczeg, ¹Zsolt Liposits, ¹Erik Hrabovszky

¹ *Endokrin Neurobiológiai Kutatócsoport, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Igazságügyi Orvostani Intézet, Debreceni Egyetem, Debrecen, Hungary*

The mesocorticolimbic reward pathway arising from dopaminergic (DA) neurons of the ventral tegmental area (VTA) has been implicated in reward processing and drug abuse. In rodents, behaviors associated with this projection are strongly influenced by an orexinergic input from the lateral hypothalamus to the VTA. The existence and significance of an analogous orexinergic regulatory mechanism acting in the human VTA has been elusive. In the present study we hypothesized that orexinergic neurons provide an anatomically similar input to VTA DA neurons in humans and rats. We used dual-label immunohistochemistry for the comparative analysis of the orexinergic projection to the VTA and the neighboring substantia nigra (SN) of the human and rat species. Orexin B-immunoreactive (IR) axons apposed to tyrosine hydroxylase (TH)-IR DA and to non-DA neurons were scarce in the SN and the VTA of both species; in the VTA only $15.0 \pm 2.3\%$ of TH-IR perikarya in the human and $3.1 \pm 0.5\%$ in the rat received such orexin B-IR afferent contacts. On average, 0.25 ± 0.09 and 0.05 ± 0.02 orexinergic axonal apposition per TH-IR perikaryon were detected in the human and rat species, respectively. The majority of randomly identified appositions between orexin B-IR axons and TH-IR neuronal elements ($87.3 \pm 4.8\%$ in human and 86.7 ± 1.9 in rat) targeted the dendritic compartment of DA neurons. Finally, we found that in both species, DA neurons in the pars compacta of the SN also received sporadic orexin B-IR afferents. This immunohistochemical study provides the first evidence for a direct orexinergic input to DA and non-DA neurons

of the human VTA which shared neuroanatomical characteristics with the homologous rat pathway. We propose that orexinergic mechanism acting in the VTA may play similar roles in reward processing and drug abuse in the human as in laboratory rodents.

P4.22.

Activation of hypothalamic orexin neurons in response to pain-evoked acute stress

¹Rege Sugárka Papp, ²György M. Nagy, ¹Miklós Palkovits

¹ *Neuromorphological and Neuroendocrine Research Laboratory, Department of Anatomy, Histology and Embryology, Semmelweis University and Hungarian Academy of Science. Budapest, Hungary;* ² *Cellular and Molecular Neuroendocrine Laboratory, Department of Human Morphology, Semmelweis University and Hungarian Academy of Sciences, Budapest, Hungary*

It is generally known that a marked Fos expression is induced in corticotrophin-releasing factor-expressing neurons of the hypothalamic paraventricular nucleus (PVN) by acute painful stimuli. Besides the PVN, several hypothalamic neurons respond to acute pain by Fos activation. One group of them is consisted of orexin (orx)-expressing neurons located in the dorsolateral hypothalamic area (DLH) and participated in the central control of food intake (FI) and regulation of sleep-wake cycle (SW). Previously, based on their topographical location in the hypothalamus in different cellular density, 6 subdivisions of the orx-ir neuronal populations have been classified in the anterior, dorsal, ventral and periventricular (PeVe) subdivisions of the DLH, in the dorsomedial (DMN) and perifornical hypothalamic nuclei (PeF). In the present study, we investigated the time-depending activation of the orexin-containing neurons after subcutaneously injection of 4% formalin. The concentration of plasma ACTH was measured 15 min after injections and the number of Fos-activated orx-, melanin-concentrating hormone (MCH)- and

urocortin3 (urc3)-ir neurons was counted 30, 60 and 90 min after injection. Formalin elicited a significant increase of plasma ACTH level (341.2%) comparing to the control level. Orx-neurons showed a time-dependent activation after injection (~30% at 30 min, ~50% at 60 and 90 min). Although this Fos-activation was present in all investigated areas, the density of double labeled neurons was significantly higher in PeVe, DMN and PeF than in the other areas. MCH-ir neurons, that co-distribute with orexin-expressing cells all over the DLH, and also have significant functional role in FI and regulation of SW, did not respond to formalin-induced pain by Fos-expression. Noteworthy, urc3-neurons in the anterior hypothalamus, that participate (with anorexigenic manner) in the FI regulation, showed a time-dependent activation in response to acute pain (~8%, ~15% and ~25% at 30, 60 and 90 min, respectively). To investigate the possible mechanism of pain-responding action of orx-containing neurons, biotinylated dextran amine (BDA) was injected into those subregions which showed high Fos-activation after formalin injection. BDA-labeled fibers could followed to pain related cell groups in the lower brainstem, like to the locus coeruleus, the A1, A2 and A5 noradrenergic, C1 and C2 adrenergic cell groups, as well as to the serotonergic raphe nuclei. Supported by OTKA CK 80180.

P4.23.

A TRPV1 receptor jelenléte és ösztrogén kezelés hatására történő expresszió-növekedése patkány endometriumban

¹Krisztina Pohóczky, ¹József Kun, ²János Garai, ²András Garami, ¹Anikó Perkecz, ³Zsuzsanna Helyes

¹ Farmakológiai és Farmakoterápiai Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary; ² Kórélettani és Gerontológiai Intézet, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary; ³ Szentágotthai János Kutatóközpont, Pécsi Tudományegyetem, Pécs, Hungary

A Tranziens Receptor Potenciál Vanilloid (TRPV1) receptor előfordulását a központi és perifériás idegrendszer mellett számos nem neurális sejten (hám-és simaizomsejtek, keratinociták) is leírták. A kapszaicin kivül fájdalmas hőinger, protonok és lipid mediátorok aktiválják, amely fájdalom és neurogén gyulladás kialakulásához vezet. A hippocampusban és hátsó gyöki ganglionokban ösztrogén hatására megnő a TRPV1 expresszió, amely nőknél szerepet játszhat fájdalommal járó kórképek gyakoribb és a reprodukív ciklussal változó előfordulásában. Célunk a TRPV1 expressziójának és ösztrogén-függő változásainak vizsgálata volt patkány endometriumban. Négy hónapos felnőtt és négy hetes fiatal patkányokat vizsgáltunk, amelyek egy-egy csoportja dietil-stilbösztrol (DES) kezelés kapott 8, illetve 12 napig (n=6-6). A felnőttek egy csoportjában ovariectomiát végeztünk (n=4). Az uterus mintákból TRI Reagenssel RNS-t izoláltunk, reverz-transzkripcióval cDNS-re írtuk át, majd TRPV1 specifikus primerrel kvantitatív polimeráz láncreakciót (qPCR) végeztünk. Referenciaként glicerinaldehid-3-foszfát-dehidrogenáz (GAPDH), hipoxantin-guanin foszforibozil transzferáz (HGPRT) és peptidilprolil izomeráz-A (Ppia) gének szolgáltak. Az immunhisztokémiai jelölést paraffinba ágyazott metszeteken poliklonális nyúl anti-TRPV1 elsődleges, és torma peroxidázal konjugált anti-nyúl másodlagos antitestekkel végeztük, diamino-benzidin (DAB) előhívással. A TRPV1 receptor jelenlétét mRNS és fehérjeszinten is kimutattuk patkány endometriumban, a fiatal és felnőtt állatok között nem volt szignifikáns különbség. Felnőttekben jelentős immunfestődés volt megfigyelhető a sztrómában és a mirigyekben, csekély az epithelben, ivaréretlen állatokban gyenge pozitivitást láttunk az epithelben. DES kezelés hatására mindkét korcsoportban szignifikánsan, átlagban 3-4 szeresére növekedett a nem-neurális TRPV1 mRNS expressziója mindhárom referenciagénhez viszonyítva. Fiatal állatokban az epithelrétegben, felnőttekben a sztrómában fokozódott az immunpozitivitás. Felnőttekben az ovariectómia, bár a DES-nél kisebb mértékben, de ugyancsak szignifikánsan fokozta az mRNS expressziót és az epithelbeli immunfestődést. Elsőként mutattuk ki a TRPV1 ioncsatorna patkány endometriumban való ösztrogén-függő expresszió-fokozódását, funkcionális jelentőségének felderítésére további vizsgálatokat tervezünk. SROP-4.2.2.A-11/1/KONV-2012-0024, SROP-4.2.1.B-10/2/KONV-2010-0002, SROP-4.2.2.B-10/1/2010-0029

P4.24.

Activation of neurons in the hypothalamic dorsomedial nucleus in a rat food entrainment model

^{1,2}Eva Renner, ¹Marianna Dóró, ¹Árpád Dobolyi, ^{1,2}Miklós Palkovits

¹*Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology;* ²*Human Brain Tissue Bank, Semmelweis University, Budapest, Hungary*

We previously reported that a high number of neurons in the ventral subdivision of the hypothalamic dorsomedial nucleus (DMv) express c-fos in response to food intake in fasted rats. Now we established a food entrainment model allowing of food consuming only for 2 h in each day (between 19 and 21 h) with continuous free access to drinking water. Rats were examined at 6 time points: the day before the feeding protocol starts, and on day 1, 2, 4, 8 and 12 days after that. A significant decrease in the apparent body weight of rats (about 10 % of initial weight) was found during the first day of the protocol. Subsequently, the body weight of rats stabilized and increased body weight up to the initial level by the 12th day of food entrainment. Interestingly, the amount of food consumed within the 2 h period elevated markedly, particularly between days 2 and 8 (from 9 to 15 g per animal), but did not change after that day. Rats were sacrificed at each time points followed by perfusion fixation, and the brains were processed for Fos immunohistochemistry. Meanwhile Fos-positive cells were not present before the 2 h feeding period, a high density of labeled neurons appeared when the animals were sacrificed at the end of the feeding period. These neurons appeared almost exclusively in the DMv showing a similar distribution pattern to Fos activation were observed previously in response to unlimited refeeding following 2 days fasting. Interestingly, however, the number of labeled Fos-positive neurons increased from 43 to 89 cells per DMv during the food entrainment protocol. Thus, the Fos activation in the DMv correlated with the amount of consumed food suggesting that this cell group in the hypothalamus

responded very sensitively to the extension of the stomach. This finding further improves our previous results that satiation is one of the important signals that activate DMv neurons. To examine how the signal affects DMv neurons, Fos was co-stained with leptin receptor, glucagon-like peptide-1 (GLP-1) receptor, and neuropeptide Y (NPY) using *in situ* hybridization histochemistry. Double labeling of Fos-positive cells in leptin receptor-, GLP-1 receptor-, but NPY-expressing cells suggest that the signal from the stomach to the DMv could be transferred *via* GLP-1-containing neurons of the nucleus of the solitary tract and as well as by direct signals leptin reaching this nucleus by the known hormonal way through the area postrema. Supported by OTKA CK80180.

P4.25.

Ovariectomy causes increased expression of macrophage-associated genes in the frontal cortex of middle-aged rats

¹Miklós Sárvári, ¹Erik Hrabovszky, ¹Imre Kalló, ²Norbert Solymosi, ³István Likó, ¹Zsolt Liposits

¹ *Endokrin Neurobiológiai Kutatócsoport, MTA, KOKI, Budapest, Hungary;* ² *Állatorvostudományi Kar, Szent István Egyetem, Budapest, Hungary;* ³ *Farmakológiai és Gyógyszerbiztonsági Főosztály, Richter Gedeon Nyrt, Budapest, Hungary*

Complex interactions between the immune, endocrine and central nervous systems shape the innate immune response of the brain. We have previously shown that estradiol suppresses the expression of immune genes in the frontal cortex of middle-aged ovariectomized rats, but not in young ones reflecting elevated expression of these genes in aging, ovarian hormone deficient animals. Here, we explored the impact of the level of ovarian hormones on the microglia phenotype capitalizing on the differential expression of macrophage-associated genes in quiescent and activated microglia. We selected genes encoding phagocytic and toll-like receptors, co-receptors, MHC antigens, which are expressed primarily in

microglia, and measured their mRNA expression by TaqMan-based real-time PCR. We applied four rat models. Young OVX rats for young adults with low E2 levels (Y/OVX group), middle-aged intact female rats (M/CTRL group), middle-aged OVX rats (M/OVX group), and middle-aged OVX rats with chronic E2 treatment (M/OVX+E2 group) were used and compared to reveal the impact of aging and ovariectomy. Aging slightly altered mRNA expression of Cd11b, Fcgr2b, Tlr9, enhanced RT1-Aw2, Cd74 whereas decreased Cd36. Ovariectomy of middle-aged rats robustly increased Cd11b, Cd18, Fcgr2b, Cd45, Cd86, Tlr4, RT1-Aw2, Cd74, resembling to microglial priming. The expression of Cd36 decreased further. Data analysis of publically available human microarray data revealed strikingly similar changes in the postcentral and superior frontal gyrus of postmenopausal women. Estradiol replacement attenuated mRNA expression of Cd11b, Cd18, Fcgr2b, Cd45, Cd86, Tlr4, but didn't rescue Cd36. From these observations we conclude that menopause leads to microglial priming in the cortex of middle-aged females. This acquired microglia phenotype is reversible and in part due to diminishing estrogen signaling. The shift of microglia towards reactive phenotypes can be noxious as primed microglia alter the innate immune response and make the aging female brain susceptible of neuroinflammation. This research was supported by grants from OTKA (100722K) and EU (FP7/245009).

P4.26.

Kisspeptin immunoreactivity in human GnRH neurons

¹Máté Sipos, ²Beáta Á. Borsay, ²Kálmán Rácz, ³Stephen R. Bloom, ³Mohammad A. Ghatei, ³Waljit S. Dhilló, ¹Zsolt Liposits, ²László Herczeg, ¹Erik Hrabovszky

¹ Endokrin Neurobiológiai Kutatócsoport, MTA-KOKI, Budapest, Hungary; ² Department of Forensic Medicine, Faculty of Medicine of the University of Debrecen, Debrecen, Hungary; ³ Department of Investigative Medicine, Hammersmith Hospital, Imperial College London, London, UK

Central kisspeptin signaling to gonadotropin releasing hormone (GnRH) neurons plays a critical role in mammalian reproduction. While most information about this communication has been obtained from rodents, recent immunohistochemical experiments on post mortem human hypothalami identified neuroanatomical characteristics in this communication that are specific to the human. Our objective in the present study was to address the possibility that human GnRH neurons synthesize kisspeptins, in addition to receive kisspeptin-immunoreactive neuronal inputs. Coronal hypothalamic sections were prepared from autopsy samples of male and female individuals (N=21) who died from sudden causes of death. The immunohistochemical detection of kisspeptins revealed the largest population of cell bodies in the infundibular nucleus; these cells were typically lightly-labeled. Darkly stained fusiform neurons also occurred occasionally in various hypothalamic regions, with morphological characteristics reminiscent of GnRH neurons. Double-immunofluorescence experiments were carried out to verify that these cells are GnRH neurons. Confocal microscopic analysis established that about one tenth of the GnRH-immunoreactive somata (men below 50 ys: $6.5 \pm 2.72\%$; men above 50 ys: $16.4 \pm 10.78\%$; postmenopausal women above 56 years: $9.4 \pm 2.70\%$; N=390) and their proximal dendrites showed kisspeptin immunoreactivity. The double-labeled neurons showed no preferential localization to any particular preoptic or hypothalamic site. The GnRH-immunoreactive axons however rarely contained kisspeptin signal and kisspeptin labeling was also absent from the GnRH-immunoreactive axon terminals in the infundibular stalk. This study reveals an interesting colocalization phenomenon between kisspeptins and GnRH in about 10% of the human GnRH neurons, which is absent in rodents. This observation raises the possibility that endogenous kisspeptin is released as a cotransmitter of GnRH to activate neighboring neurons (including GnRH cells) that contain the kisspeptin receptor. Such autocrine/paracrine regulatory mechanisms may contribute to the synchronized electric and secretory activity of the human GnRH network.

The maternal induction of the novel neuropeptide amylin depends on TIP39-containing posterior thalamic neurons

¹Éva Rebeka Szabó, ¹Melinda Cservenák, ¹Dominika Domokos, ¹Árpád Dobolyi

¹ *Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, Hungary*

We previously identified amylin in a microarray study as a novel neuropeptide expressed exclusively in the preoptic area of mother rats. It was also shown that amylin neurons are activated in response to pup exposure. The discovery of the brain expression of amylin helped to explain the presence of amylin receptors in the central nervous system. In the present study, we used in situ hybridization histochemistry to reveal a marked increase in amylin expression around parturition in the preoptic area, and showed that amylin mRNA level remained elevated as long as the pups are not removed from mother mice in the postpartum period. Since we previously described that tuberoinfundibular peptide of 39 residues (TIP39) is induced in the posterior intralaminar complex of the thalamus with similar time course in mother rats, we investigated the topographical relationship between TIP39 fibers, its receptor, the parathyroid hormone 2 receptor (PTH2 receptor) and amylin neurons in the preoptic area. A very similar distribution was found with high density of both TIP39 and amylin immunoreactivity and PTH2 receptor expressing neurons (X-gal labeling in mice, in which the beta-galactosidase is driven by the PTH2 receptor promoter) in the medial preoptic nucleus, some parts of the medial preoptic area, and the ventral subdivision of the bed nucleus of the stria terminalis. High magnification confocal images revealed that TIP39 terminals closely appose amylin neurons suggesting their innervation by TIP39 neurons. Injection of the retrograde tracer cholera toxin B subunit into the medial preoptic nucleus revealed that TIP39 fibers arise from the posterior thalamus as a large percentage of TIP39 cell bodies located there were retrogradely labeled. Subsequently, amylin expression was investigated in mice lacking PTH2 receptor to reveal a functional relationship between amylin and TIP39. The maternal induction of amylin levels was markedly reduced in the absence of PTH2 receptor based on

quantitative in situ hybridization histochemistry. These results suggest that the maternal induction of amylin is driven by posterior thalamic TIP39-containing neurons that have been suggested to convey suckling information from the spinal cord towards limbic and hypothalamic maternal centers. Support was provided by Bolyai János Fellowship of the HAS, OTKA NNF2 85612 and K100319 research grants.

P4.28.

A PACAP38-szerű immunoreaktivitás mérése tejmintákban és tehéntej alapú tápszerekben

¹Andrea Tamás, ¹Katalin Csanaky, ¹Eszter Bánki, ²Ibolya Tarcai, ³László Márk, ⁴Zsuzsanna Helyes, ⁵Tibor Ertl, ⁵Judit Gyarmati, ¹Kitti Horváth, ¹Luca Sántik, ¹Dóra Reglődi

¹ *Anatómiai Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;* ² *ÁNTSZ, Pécs M. J. V. Egyesített Egészségügyi Intézmények, Pécs, Hungary;* ³ *Biokémiai és Orvosi Kémiai Intézet, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary;* ⁴ *Farmakológiai és Farmakoterápiai Intézet, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary;* ⁵ *Szülészeti és Nőgyógyászati Klinika, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary*

A hipofízis adenilát-cikláz aktiváló polipeptid (PACAP) egy neuropeptid, mely fontos szerepet tölt be az újszülöttek fejlődésében és a reprodukciós folyamatok szabályozásában. Két aktív formája ismert: a 27 és a 38 aminosav hosszúságú formák. Kutatócsoportunk írta le először a PACAP38 jelenlétét és a PACAP38-szerű immunoreaktivitás(LI) magas szintjét kérődző állatok és az ember anyatejében. Kimutattuk, hogy a tejben mérhető PACAP38-LI 5-20-szorosa a vérben mérhető értéknek, és szintje nem változik a szoptatás első hat hónapjában, de később a tej mennyiségének csökkenésével fokozatosan emelkedik. Az anyatej összetétele azonban nem csak a szoptatási periódus teljes időtartama alatt

változik, hanem különbséget mutat a szoptatás elején (előtej) és végén (utótej) termelődő tejmintákban is, ezért jelen vizsgálatunk első felében célul tűztük ki a kismamák elő- és utótej mintáinak összehasonlító vizsgálatát. A szoptatás kiemelkedően fontos az újszülöttek megfelelő növekedéséhez, azonban ha ez valamilyen ok folytán gátolt, az anyatejet a tehéntej alapú tápszeres táplálás helyettesíti. Jelen vizsgálatunk második részének célja a PACAP38-LI mérése volt különböző tehéntej alapú tápszerekben, bolti tejben és friss tehéntejben, emellett bizonyítani kívántuk a PACAP38 jelenlétét a tápszerekben tömegspektrometria segítségével. Vizsgálataink során azt találtuk, hogy a PACAP38-LI szintje hasonló az előtejben és az utótejben, a friss tehéntejben, a pasztörizált bolti tejekben és a tápszerekben is. Szignifikánsan magasabb PACAP38-LI mérhető a hipoallergén tápszerekben a nem hidrolizált tápszerekhez képest, amiből arra következtethetünk, hogy a PACAP38 stabil az anyatejben, és ellenáll a tápszergyártás során alkalmazott gyártási folyamatoknak. A tejmintákban mérhető magas PACAP szint jelentősége pontosan még nem ismert, de fontos szerepe lehet az újszülöttek idegrendszerének fejlődésében, a gasztrointesztinális rendszer immunitásának kialakításában és az emlőmirigy működésének szabályozásában is. Jövőbeni kísérleteink során célunk ezen hatásmechanizmusok pontos feltérképezése. (OTKA K104984, TAMOP 4.2.1.B-10/2/KONV-2010-002, 4.2.2.B-10/1-2010-0029, 4.2.2.A-11/1/KONV-2012-0024, Arimura Foundation, Szentágothai János Kutatóközpont, PTE-MTA „Lendület” Program)

P4.29.

Hypothalamic sidedness in mitochondrial metabolism

¹Attila Zsarnovszky, ¹Virág Somogyi, ¹István Tóth, ¹Dávid Sándor Kiss, ¹László Frenyó, ²Frederick Naftolin

¹ *Élettani és Biokémiai Tanszék, Szent István Egyetem, Állatorvostudományi Kar, Budapest, Hungary;* ² *Obstetrics and Gynecology, New York University, New York, USA*

Hypothalamic functional asymmetry had been described decades ago. Yet, since then, most studies in hypothalamic research continued to investigate this brain area as a morphologically and functionally compact midline regulatory center. One of the major neural mechanisms involved in the orchestration of integrated, hypothalamus-driven homeostatic functions is the cyclic synaptic reorganization on hypothalamic neurons. Such morpho-functional changes are highly energy dependent and rely on mitochondrial ATP-production. Therefore, mitochondrial respiration/metabolism plays a permissive role in hypothalamic regulatory events. Here we provided evidence for the functional sidedness of the neuroendocrine hypothalamus of rats by measuring a general metabolic parameter, the mitochondrial respiration, in isolated left and right sides of rat hypothalami. We demonstrated that hypothalamic mitochondrial oxygen consumption, an indicator of mitochondrial respiration and metabolism, shows an asymmetric lateralization during the estrous cycle. Mitochondrial respiration rates, during state 1-5 mitochondrial respiration, were measured in hypothalamic synaptosomes and mitochondria from normal cycling female rats in each phase of the estrous cycle. Observed sidedness in estrous phases and estrous phase-dependent fluctuations in left-right sidedness were analyzed. Besides mitochondrial metabolism, results also indicate that the oxygen supply of the left and right sides of the hypothalamus is unequal. Since patterns of lateralization depended on the estrous phase, our present results suggest that sidedness in mitochondrial respiration may indicate a more general unilateral dominance in the hypothalamus, probably related to the regulation of GnRH secretion and/or energy homeostasis.

Session 5. Disorders of the nervous system, P5.1. – P5.20.

P5.1.

Reorganization of calbindin-positive interneurons in the CA3 and CA2 regions of the human hippocampus in temporal lobe epilepsy

¹Izolda Biró, ¹Kinga Tóth, ¹Blanka-Krisztina Németh, ²Werner Doyle, ³György Buzsáki, ¹Tamás Freund, ¹Zsófia Maglóczky

¹ *Celluláris és Hálózat Neurobiológiai Osztály, Agykéreg Kutatócsoport, MTA, Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Division of Neurosurgery, New York University, School of Medicine, New York, United States;* ³ *Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, United States*

A significant number of patients suffer from temporal lobe epilepsy and show resistance to recent pharmacological therapy. Developing new and efficient ways of treatment requires an improved and deeper understanding of morpho- and physiopathological events caused by epilepsy. The present study demonstrates the changes in the morphology and number of calbindin (CB)-positive interneurons in the CA2 and CA3 regions of surgically removed human hippocampi of patients with temporal lobe epilepsy compared to samples originating from deceased persons with no history of neurological disease. Hippocampi of control and epileptic subjects were immunostained for CB. Interneurons expressing calcium-binding protein CB, have been described as dendritic inhibitory GABAergic neurons in the mammalian cerebral cortex, including the hippocampus. Patients were classified as sclerotic and non-sclerotic on the basis of the degree of CA1 pyramidal cell loss. Numerous CB-positive interneurons are well-preserved in both CA2 and CA3 regions of sclerotic hippocampi especially in strata radiatum, oriens and lacunosum-moleculare. In non-sclerotic epileptic samples and several sclerotic hippocampi the density of CB-positive cells is increased compared to control samples. Light microscopic examination of sclerotic samples shows that morphological features of individual CB-positive interneurons are altered: the size of somata is increased, dendrites are shortened, they become thwarted, distorted and in some cases display spines. Our results show a significant difference in cell density between control and epileptic hippocampi with an excess of CB-positive interneurons in both sclerotic and non-sclerotic epileptic samples. The increased number of CB-positive neurons in the hippocampus of epileptic patients indicates that new cells begin to express CB, or that the expression level of CB is enhanced in cells

which previously produced this calcium binding protein under the detection level.

P5.2.

Streptococcus pneumoniae infection drives atherogenesis and augments cerebrovascular pathologies in ischaemia via IL-1- and platelet-mediated systemic inflammatory mechanisms

¹Ádám Dénes, ²Jesus Pradillo, ²Caroline Drake, ²Katie Murray, ³Peter Warn, ⁴Bazaz Rohit, ⁴David Dockrell, ⁴Sheila Francis, ⁵Bernhard Nieswandt, ²Nancy Rothwell, ²Stuart Allan

¹ *Molekuláris Neuroendokrinológia Laboratórium, MTA KOKI, Budapest, Hungary;* ² *Faculty of Life Sciences, University of Manchester, Manchester, UK;* ³ *Manchester Academic Health Science Centre, University of Manchester, Manchester, UK;* ⁴ *Department of Cardiovascular Science, University of Sheffield, Sheffield, UK;* ⁵ *University Hospital and Rudolf Virchow Center, University of Würzburg, Würzburg, Germany*

Common bacterial infections contribute to the development of diverse non-communicable diseases and impair outcome after myocardial infarction or stroke. Infection by *Streptococcus pneumoniae* (*S. pneumoniae*) is a major cause of prolonged hospitalization and death of patients worldwide, but the mechanisms by which it influences cardio- and cerebrovascular pathologies are not understood. We show that persisting pulmonary infection by *S. pneumoniae* in rodents triggered atherogenesis, led to systemic upregulation of the cytokine interleukin-1 (IL-1) and profoundly (by 50-90%) exacerbated ischaemic brain injury in mice and rats. Infection-mediated cerebrovascular inflammation after experimental stroke was more severe in combination with old age and atherosclerosis. Systemic blockade of IL-1 with IL-1 receptor antagonist (IL-1Ra) fully reversed infection-mediated exacerbation of brain injury and impairment of sensorimotor function after cerebral ischaemia. Infection also facilitated platelet

activation and microvascular coagulation in the brain after cerebral ischaemia. Blockade of GPIIb/IIIa, a major adhesion molecule on platelets, reduced brain injury after experimental stroke in infected mice. Both GPIIb/IIIa blockade and IL-1Ra reversed the induction of microglia associated IL-1 α in response to cerebral ischaemia in infected animals. No evidence for induction of plaque rupture acutely by *S. pneumoniae* in atherosclerotic animals was found. Thus, *S. pneumoniae* augments atherosclerosis and exacerbates experimental ischaemic brain injury via IL-1 and platelet-mediated systemic inflammation. These mechanisms may contribute to diverse cardio- and cerebrovascular pathologies in humans.

P5.3.

Mechanism of Action of Clonidine in the WAG / Rij Model of Absence Epilepsy Evaluated Based on a Data-Driven Computer Model

¹János Tibor Fekete, ¹Zsolt Kovács, ¹Péter Molnár

¹ *Biológia Intézet, Nyugat-Magyarországi Egyetem, Természettudományi Kar, Berhida, Hungary*

Computer simulations became important tools in epilepsy research. Mathematical models are regularly used to explain the often controversial results of in vivo experiments, to interpret data or to integrate partial information into a complex theoretical framework. Current understanding concerning seizure generation in absence epilepsy is largely based on computer models of the thalamo-cortical neuronal network. In the present study we compared in vivo and in silico models of absence epilepsy in terms of in which extent the simulation can reproduce the results of laboratory experiments. The in silico model of absence epilepsy included in this study have already been successfully applied by others to investigate the effects of GABAergic compounds. The in vivo EEG data were obtained from WAG/Rij rats with symptoms of absence epilepsy. The goal of this study was to investigate whether the model can be used to determine the mechanism of action of clonidine, an α_2 -adrenergic agonist,

in absence epilepsy. We found that the number, duration and frequency of the seizures could be fit to the experimental data by systematical variation of the model parameters. The effect of clonidine was modeled by varying IT current amplitudes and GABA concentrations. Decrease in the amplitude of the IT current resulted in a decrease in the total length of seizures and duration of individual seizures whereas it caused an increase in seizure frequency. An increase in this parameter caused a rise both in the total duration and individual length of seizures, whereas the frequency decreased. Effect of GABA concentration changes were examined in the thalamocortical population. After we enhanced GABA concentration we observed an increase in the total duration of spike-wave discharges while the SWD peak frequency decreased. I summary: with systematic variation of the model parameters we successfully reproduced in vivo control measurements, but we were not able to completely reproduce the action of clonidine. In order to clarify the role of the noradrenergic system in absence epilepsy we have to include further details and mechanisms in the model.

P5.4.

Investigating the schizophrenic brain: A network connectivity analysis concerning visual working memory

¹Florian Gesser, ¹Mihály Bányai, ²Jejoong Kim

¹ *Department of Theory, MTA Wigner RCP, Konkoly-Thege út 29-33.;* ² *Department of Psychology, Vanderbilt University, USA*

Schizophrenia is a mental disorder manifesting itself through impairments in various cognitive realms such as (self-)perception, emotions and memory performance. However the pathophysiology foundations and underlying causes of the disease remain still unclear. Partly to the rise of functional magnetic imaging (fMRI) as a prime technique for studying cognitive function there has been proposed the so called “(functional) disconnection hypothesis”. In this framework the cause of schizophrenia is

being attributed to a dysfunctional integration of neural circuitry. Disrupted connectivity of cortical areas responsible for cognitive processes, e.g. decision making, such as the prefrontal cortex, and areas responsible for memory formation based on sensory processing, such as the hippocampus and other areas in the temporal lobe are key candidates for explaining the underlying mechanisms of schizophrenia. One of the major objectives in research on schizophrenia consists in finding sound biological markers which characterize the disease. Through drawing on connectivity analysis methods such markers can be obtained as neuroimaging markers describing differences in network connectivity. In the context of this framework we evaluate fMRI data from an earlier study on a phonological delayed-matching-to-sample-task which aimed at further elucidating the relationship between cognitive components of working memory and brain activation patterns. Working memory deficits are considered as a potential marker for schizophrenia hence we are interested in the differences between schizophrenic (SZ) patients and healthy controls (CO). Thereto we use independent component analysis (ICA), a multivariate analysis technique. Unlike conventional techniques used in fMRI analysis, ICA allows a model-free exploratory data driven approach, separating the signal into maximally independent components and can thus be used to detect functional networks in fMRI data. We use the GIFT matlab-toolbox which allows to analyze whole groups (here SZ vs CO) by combining data sets of individual subjects to a single data set. In order to separate signal from noise (e.g heart beat) we visually inspect the power spectrum of the components time-courses. The so selected components serve as basis from which connectivity analysis methods can be applied either in a with- in or between-component manner. The comparison of the SZ and CO groups reveals functional differences underlying the impaired memory performance.

P5.5.

MIA-690, a newly developed GHRH analog, exerts beneficial effects in different models of Alzheimer's disease

¹Miklós Jászberényi, ¹Krisztina Csabafi, ²Andrew Schally

¹ *Kórélettani Intézet, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary;* ² *Endocrine, Polypeptide, and Cancer Institute, Veterans Affairs Medical Center, Miami, United States*

Alzheimer's disease is the most frequent debilitating disorder of the central nervous system. Neuroendocrine mechanisms appear to play an important role in this insidiously developing disease. In the present study, the effects of a recently developed growth hormone-releasing hormone (GHRH) antagonist (MIA-690) were evaluated in vivo observing the behavior of genetically modified "Alzheimer's" ⁵XFAD mice in a Morris water maze (MWM). The effects of the antagonist were also evaluated in vitro using HCN2 human cortical cell cultures treated with amyloid- β 1-42. In vivo, the indices of cognitive performance (latency, cumulative index etc.) were followed up for ⁶ months. In vitro, the formation of reactive oxygen species, markers of inflammatory and neurohormonal signaling were measured by fluorescent detection, PCR, and ELISA. Accumulation of amyloid- β 1-42 rafts and τ filaments in necropsied brain samples was verified with the help of ELISA and immunohistochemistry. In the MWM experiments, MIA-690 decreased escape latency, and, in the brain samples, it inhibited the concentration of amyloid- β 1-42 and τ filaments. In cell cultures, the GHRH analog showed anti-oxidative and neuro-protective properties and inhibited the GHRH-growth hormone-insulin like growth factor axis. Our data strongly suggest the merit of further studies with GHRH analogs in models of Alzheimer's disease and in elementary clinical trials.

P5.6.

Pilocarpine-evoked seizures result in species-dependent intrahippocampal changes in rodents

¹Norbert Károly, ¹Endre Dobó, ¹András Mihály

Pilocarpine (PILO)-induced epilepsy evokes cell death and sprouting of mossy fibres (SMF). Effects of PILO-induced seizures on selected neuronal markers were studied in rodents after a 2-month survival period, using NPY for a subset of inhibitory neurons, synapsin-I (Syn-I) for axon terminals, calretinin and CGRP for mossy cells (MCs) in mice and rats, respectively. Immunoreactivity of the MCs in the hilum and their processes in the supragranular layer (SGL) was greatly reduced in rats, but remained unchanged in the PILO-responsive mice. Increased levels of NPY immunoreactivity were observed in both species. Intense staining appeared in the hilum, stratum lucidum (SL) and the SGL. We presume that the enhancement of the NPY immunoreactivity may be ascribed to the GABAergic densely-spiny hippocampal cells projecting to the medial septum. Species differences were found in Syn-I immunoreactivity. In rats, the distribution of Syn-I was similar to that of NPY. In mice, the Syn-I level was increased in the SL, but decreased in the hilum. Direct evidence of a close relationship between the SMF and the loss of MCs in the PILO model of rats was provided. Our findings suggest survival of the MCs and loss of the interneurons after PILO treatment in mice. It was concluded that similar PILO seizures may cause different changes in the neuronal circuits of the two species, and these changes most likely include extrahippocampal areas, too. Grant: TÁMOP 4.2.2-A-11/1/KONV-2012-0052

P5.7.

Mechanisms involved in kainate-induced postconditioning in ischemic hippocampus

¹Kitti Kocsis, ¹Levente Knapp, ²Tamás Kovács-Öller, ¹Anikó Berkó, ¹Anikó Pósa, ¹Zsolt Kis, ¹Csaba Varga, ²Béla Völgyi, ¹Tamás Farkas, ¹József Toldi

¹ *Élettani, Szervezettani és Idegtudományi Tanszék, Szegedi Tudományegyetem, Szeged, Hungary;* ² *Kísérletes Állattani és Neurobiológiai Tanszék, Pécsi Tudományegyetem, Pécs, Hungary*

Previously we described a novel form of postconditioning evoked by low-dose kainate (KA) treatment in a two vessel-occlusion (2VO) model of global ischemia. As a result, damaged LTP returned to pre-ischemic values and the structural basis for this was the restoration of dendritic spines of CA1 pyramidal cells. However, the underlying mechanisms involved in kainate postconditioning is still unclear. Here, we investigated some molecular mechanisms, that could be responsible for spine degeneration and plasticity after global hypoperfusion. A real-Time quantitative PCR (RT-qPCR) was performed by using a combination of stem-loop, forward and reverse primers to specifically detect and measure mi-RNA132 levels. The inflammatory effect of ischemic insult was examined by measuring myeloperoxidase (MPO). In the hippocampi taken from the control, 2VO, KA-treated and KA-postconditioned groups, the levels of MPO and the mi-RNA132 were measured. The miRNA132 level of 2VO was higher than in controls, while it was about thousand times higher at the KA-postconditioned group. This significant difference indicates spine genesis and plasticity. MPO activity was at the same value in control, KA-postconditioned and 2VO groups, while it was elevated in KA-treated group. The above results demonstrate that KA postconditioning following ischemic stress induces intracellular mechanisms that result in a supernormal level of protein expression, which eventually leads to the plastic restoration of dendritic spines (synaptic surfaces) and LTP. In addition, we also found that similar to other previously described plastic cortical changes, this KA induced rescue is under the control of mi-RNA132 based post-transcriptional mechanisms. Kainate treatment also interferes with the inflammatory effect of ischemic insult revealed by MPO measurements, which phenomenon needs further examination. Acknowledgement: This work was supported by TAMOP 4.2.2-A-11/1/KONV-2012-0052 as well as OTKA KI05077 and OTKA K105247. A.P. and B.V. were Bolyai Fellows.

The pathophysiological role of P2X7 purinergic receptors on alteration of gene expression in the phencyclidine induced animal model of schizophrenia

¹Cecília Csölle, ²Bence Koványi, ³József Haller, ¹Beáta Sperlágh

¹ *Molekuláris Farmakológia, MTA KOKI, Budapest, Hungary;* ² *Molekuláris Farmakológia Kutatócsoport, MTA-KOKI, Budapest, Hungary;* ³ *Magatartás Neurobiológia, MTA KOKI, Budapest, Hungary*

Schizophrenia is a complex mental disorder that affects 1% of the population. Besides the dysregulation of serotonin and dopamine in the pathomechanism of schizophrenia, glutaminergic hypofunction was also demonstrated in the cortical region of patients with schizophrenia. P2X7 receptors which are ATP sensitive ion-channels are expressed in the neuronal, glial and immune cells. P2X7 receptors are well-known for their prominent role in microglia mediated neuronal death, secretion of IL-1 β and other inflammatory mediators, and non selective pore forming property in apoptosis. On cerebrocortical glutamatergic nerve terminals the pathological activation of P2X7 receptors could contribute to ATP-evoked glutamate and subsequent GABA release, and glutamate mediated excitotoxicity. The aim of this study was to explore the alterations of schizophrenia related genes expression in the phencyclidine induced animal model of schizophrenia in mouse prefrontal cortex. All animals (P2X7 receptor knock-out (P2rx7^{-/-}) and wild type (P2rx7^{+/+}) mice) were given an intraperitoneal injection of an NMDA receptor antagonist, phencyclidine (PCP; 2mg/kg) or saline. PCP produced "schizophrenialike" symptoms such as positive and negative deficits symptoms of schizophrenia, after 1 hour PCP and saline treatment mice were decapitated. We used SYBR Green real-time PCR to identify the changes in the mRNA expression levels in prefrontal cortex of P2rx7^{-/-} and P2rx7^{+/+} mice. We demonstrated that PCP treatment significantly affected the levels of mRNA expression of neuregulin-1 and metabotropic glutamate receptors (mGluR2, mGluR5), which data correlates with previous studies from the literature. P2X7 receptor deficiency caused

remarkable significant alterations in levels of mRNA expression of NMDA-type ionotropic glutamate receptors (NR1, NR2A, NR2B) and as well as GABAA $\alpha 1$ receptor subunit. Our results showed that P2X7 receptors deficiency caused detectable alterations of schizophrenia related gene expression. The mapping of functional consequence of effects on alteration of gene expression are mentioned above, can be the new line of research, which can help to better understanding of the pathomechanism of schizophrenia, in addition it can be a new start point of the discovery of new antipsychotic drugs.

P5.9.

A prenatális dohányzás hatása a patkányok korai idegrendszeri fejlődésére

¹Timea Kvárik, ¹Barbara Mammel, ¹Gréta Bodzai, ¹József Farkas, ¹Attila Matkovits, ²István Szitter, ²Zsuzsanna Helyes, ³Judit Gyarmati, ³Tibor Ertl, ¹Dóra Reglödi, ¹Andrea Tamás, ¹Péter Kiss

¹ *Anatómiai Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;* ² *Farmakológiai és Farmakoterápiás Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;* ³ *Szülészeti és Nőgyógyászati Klinika, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary*

Számos vizsgálat mutat a terhesség során történő dohányzás káros hatásaira. A dohányzás károsítja a fejlődő magzatot, melynek eredményeképp a kognitív és motoros fejlődés késhet, károsodhat a locomotoros viselkedés, valamint emelkedhet a pszichiátriai rendellenességek előfordulása. Viszonylag kevés adat áll rendelkezésre állatkísérletes vizsgálatokból a prenatális dohány-expozíció korai idegi fejlődésre gyakorolt hatásáról. Ezért célul tűztük ki annak vizsgálatát, hogy hogyan hat a prenatális dohányzás a reflexek és a motoros koordináció fejlődésére. Wistar patkányokat (1 hím és 3 nőstény) naponta 40 percre egész testre terjedő dohányfüst-expozíciónak tettünk ki a

megtermékenyítéstől a szülésig. Ehhez zárt rendszerű dohányoztató készüléket és alkalmanként 2db kutatási célokra kifejlesztett cigarettát használtunk. A kontroll csoport (1 hím és 3 nőstény) nem dohányzott. Az újszülött patkányokat postnatális 1. naptól a 21. napig vizsgáltuk egy standardizált neurológiai tesztrendszerrel, melyben a fizikális jelek (súly, szemnyitás, metszőfog kinövés, fül kiegyenesedés), neurológiai jelek és reflexek (fülrángás, szemhéj reflex, első-, hátsó láb ráhelyezési és fogóreflex, akusztikus megrettenési reflex, keresztezett extenzor reflex, felegyenesedési reflex, negatív geotaxis, gait) megjelenésének napját jegyeztük fel. 5 hetes korban vizsgáltuk a motoros koordinációt lépéshiba teszt segítségével, valamint open-field teszttel viselkedés analízist végeztünk. Eredményeink azt mutatják, hogy a prenatális korban dohányzásnak kitett újszülöttek egyes, az idegrendszer érettségét jelző reflexe később, viszont néhány fizikális jel szignifikánsan előbb mutatkozik a kontroll állatokhoz képest. A motoros viselkedésben nem találtunk eltérést a csoportok között. Korábban több perinatális patológiás folyamatban írtunk le drámai fejlődésmaradást, mint pl. perinatális asphyxiában és hypoxiában, ahol a legtöbb fejlődési jel több nap késést mutatott a kontroll állatokhoz képest. Jelen eredményeink alapján összességében elmondható, hogy a prenatális dohányzás ilyen drámai módon nem befolyásolta az idegrendszeri fejlődést. Annak eldöntése, hogy ez vajon a későbbi életkorban okoz-e eltérő érzékenységet különböző káros behatásokra, további vizsgálatunk feladata.

P5.10.

Hyperglycaemia and insulin-induced alterations in the retina of rat pups

¹Barbara Mammel, ¹Tímea Kvárik, ²Judit Gyarmati, ²Tibor Ertl, ³Krisztina Szabadfi, ¹Dóra Reglödi, ³Róbert Gábel, ¹Andrea Tamás, ¹Péter Kiss

¹ *Anatómiai Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;* ² *Szülészeti és Nőgyógyászati Klinika Neonatológiai Tanszék, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs,*

Hungary; ³ *Kísérletes Állattani és Neurobiológiai Tanszék, Pécsi Tudományegyetem, Természettudományi Kar, Pécs, Hungary*

Rat pups are applicable to investigate specific role of the factors which are implicated in the pathogenesis of retinopathy of prematurity (ROP) including hyperglycaemia and insulin treatment. The aim of our study was to investigate specific effect of streptozotocin-induced hyperglycaemia, insulin-treatment and intravitreal injection of a potential retinoprotective agent, pituitary adenylate cyclase activating polypeptide (PACAP) on the rat pups' retina. We made a comparative analysis between the following treatment-groups: controls (Stz-/Ins-), insulin-treated (Stz-/Ins+), hyperglycemic (Stz+/Ins-), insulin-treated hyperglycaemic (Stz+/Ins+); all animals were treated with intravitreal PACAP or vehicle. Blood glucose levels were monitored. After decapitation (P21) retinas were processed for routine histology and immunohistochemistry for glial fibrillary acidic protein (GFAP), GLUT1 and tyrosine hydroxylase (TH). Standard histological methods revealed no major differences between the groups. Elevated expression of GFAP - as an aspecific marker of metabolic insults in the retina- was detected from the inner retina in the Stz-/Ins+ group, although hypoglycaemia didn't develop. Similar alteration of the GFAP staining was found in the hyperglycaemic (Stz+/Ins-) and insulin-treated hyperglycaemic (Stz+/Ins+) groups. Intravitreal PACAP resulted in suppression of the elevated GFAP expression in the Stz-/Ins+ group, but not in the Stz+/Ins-, and Stz+/Ins+ ones. None of the groups showed alteration in the anti-TH immunoreactivity (dopaminergic amacrine cells) or GLUT1 expression of pigment epithelial cells. In our model hyperglycaemia or insulin did not induce ROP, however, sign of metabolic insult was detected in the neural retina, which was partly prevented by intravitreal PACAP application.

P5.11.

A trigeminális rendszer aktivációjának és centrális szenzitizációjának modulálása kombinált L-kinurenin és probenecid előkezeléssel orofaciális formalin tesztben

¹Gábor Nagy, ¹Annamária Fejes-Szabó, ²Zsuzsanna Bohár, ¹Lilla Tar,
²László Vécsei, ¹Árpád Párdutz

¹ *Neurológiai Klinika, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary;* ² *Neurológiai Klinika, MTA-SZTE Idegtud. Kutatócso., Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary*

Az elsődleges fejfájásokban alapvető a trigeminális rendszer aktiválódása és szenzitizációja, amelyben szerepe van a glutamát és az $\alpha 7$ -nikotinos acetilkolin ($\alpha 7$ -nACh) receptoroknak. Állatkísérletes vizsgálatára az orofaciális formalin tesztet tartják az egyik legalkalmasabb modellnek. L-kinureninből (L-KYN) keletkező endogén kinurénsav (KYNA) glutamát és $\alpha 7$ -nACh receptor antagonistá hatású, így az elsődleges fejfájásokban is moduláló hatása lehet. Mivel a KYNA csak kis mennyiségben képes átjutni a vér-agy gáton, ezért helyette az L-KYN és probenecid (PROB) kombinált kezelést alkalmazzák. Kísérleteinkben azt vizsgáltuk, hogy az L-KYN és PROB kombinált előkezelés képes-e modulálni az orofaciális formalin tesztben a caudális trigeminális magban (TNC) megemelkedett c-Fos és kalcium-kalmodulin-függő protein kináz II alfa (CaMKII α)-expressziót. Vizsgálatainkban a felnőtt hím Sprague-Dawley patkányok, először placebo vagy kombinált L-KYN és PROB előkezelést kaptak. Ezután egy órával unilaterális subcutan formalin injekciót adtunk a bajuszpárnába. Négy órával később az állatokat transzkardiálisan perfundáltuk, a TNC-t eltávolítottuk, majd immunhisztokémiai módszerrel c-Fos és CaMKII α festést végeztünk, és végül a pozitív sejteket megszámoltuk. Az L-KYN és PROB kombinált előkezelés hatásosan csökkentette az aktiválódására utaló c-Fos és a szenzitizációra utaló CaMKII α expressziót a TNC-ben, így a KYNA potenciális terápiás lehetőség lehet az elsődleges fejfájások kezelésében.

P5.12.

Synthetic somatostatin receptor agonists inhibit hyperalgesia in mouse models of chronic postoperative and toxic neuropathic pain

¹Péter Nagy, ¹Dániel Nagy, ¹Ian O'sullivan, ²Erika Pintér, ¹János Szolcsányi, ³Zsuzsanna Helyes

¹ *Farmakológiai és Farmakoterápiai Intézet, Pécsi Tudományegyetem, Orvostudományi Kar, Pécs, Hungary;* ² *PharmInVivo Kft., PharmInVivo Kft., Pécs, Hungary;* ³ *Molekuláris Farmakológia, Szentágotthai János Kutató Központ, Pécs, Hungary*

We described earlier that most anti-inflammatory and analgesic effects of somatostatin are mediated by type 4 receptors (sst4) expressed on capsaicin-sensitive sensory nerves. We investigated the role of sst4 in meniscectomy-induced chronic postoperative pain and oxaliplatin-induced neuropathy models with the help of genetic modifications and synthetic agonists. In both models sst4 deficient (sst4^{-/-}) mice and wildtype controls (sst4^{+/+}) were used. Postoperative pain was induced by medial meniscectomy, toxic polyneuropathy by oxaliplatin injection (3 mg/kg i.p.). Mechanonociceptive threshold of the paw was measured by dynamic plantar aesthesiometry. Mediolateral knee diameter was determined with a caliper, in the neuropathy model cold threshold with decreasing temperature cold plate, and motor coordination with rotarod during 40 days. The heptapeptide sst1/4 agonist TT-232 and the peptidomimetic sst4 agonist J-2156 were tested 10 min after administration (100 ug/kg i.p.) between days 18 and 30. In both sst4^{-/-} and sst4^{+/+} mice meniscectomy induced a 60-70% mechanical hyperalgesia 3 days after the operation, which gradually decreased and thresholds returned to the preoperative values 50 days later. The mediolateral knee diameter increased by 20-30% during the study. Both TT-232 and J-2156 administered between days 17 and 21 evoked significant anti-hyperalgesic actions in both mice. Oxaliplatin resulted in a 35% mechanical hyperalgesia on day 3, which was stably maintained during 40 days. Motor function impairment, weight and hair loss, or visible signs of distress were not observed. There was no difference in any parameters between sst4^{-/-} and sst4^{+/+} mice. However, acute administration of TT-232 significantly decreased hyperalgesia and J-2156 converted it to hypoalgesia in both groups. It is concluded, that a single oxaliplatin injection is appropriate to induce sensory polyneuropathy without severe systemic toxicity and motor impairment. Although endogenous activation of sst4 receptors plays a predominant role in neither

joint damage-evoked nor neuropathic chronic mechanical hyperalgesia, exogenous agonists have potent inhibitory effects in both models. Since these agents similarly inhibited hyperalgesia in case of lacking the sst4 receptor, sst1 is likely to mediate these anti-hyperalgesic effects. Support: SROP-4.2.2.A-11/1/KONV-2012-0024, SROP-4.2.1.B-10/2/KONV-2010-0002, SROP-4.2.2.B-10/1/2010-0029, Piers Emson (Babraham Institute, Cambridge).

P5.13.

Decreased number of parvalbumin-immunostained cells in CA2 and CA3 regions of the human hippocampus in temporal lobe epilepsy

¹Blanka-Krisztina Németh, ¹Kinga Tóth, ¹Izolda Biró, ²Werner Doyle, ³György Buzsáki, ¹Tamás Freund, ¹Zsófia Maglóczy

¹ *Celluláris és Hálózat Neurobiológiai Osztály, Agykéreg Kutatócsoport, MTA, Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Division of Neurosurgery, New York University, School of Medicine, New York, United States;* ³ *Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, Newark, United States*

Interneurons expressing calcium binding protein parvalbumin (PV) have been described as perisomatic inhibitory GABAergic neurons in mammalian cerebral cortex including the hippocampus. Alteration of perisomatic inhibition is known to be a crucial phenomenon in epilepsy. Perisomatic inhibitory cells containing PV control the output of the principal cells. The two main types of PV-immunostained cells are the basket cells - giving inhibitory synapses to principal cells' somata and proximal dendrites - and axo-axonic cells, also known as chandelier cells, targeting the axon initial segment of the principal cells. In the present study the density of PV-immunostained perisomatic inhibitory cells has been investigated in surgically removed hippocampi of patients with temporal lobe epilepsy. The patients were classified as „sclerotic” and „non-sclerotic” on the basis of the degree of CA1 pyramidal cell loss. We analyzed the

PV-positive cell density of CA2 and CA3 regions in 3 control, 6 non-sclerotic and 5 sclerotic hippocampi. In the CA3 region of epileptic samples a significant reduction of PV-positive cell number has been detected. We can assume a correlation between the degree of the hippocampal sclerosis and PV-immunostained cell loss in the CA3 region. Thus, the most dramatic PV-positive cell loss was found in the sclerotic samples. In CA2 region, independently of the degree of the CA1 cell loss, the number of PV-immunostained cells seemed to be somewhat reduced, although the result was not statistically significant. The decrease of PV-immunostained elements does not necessarily mean cell death. It might be the result of the loss of PV-immunogenicity, causing lack of PV-immunoreaction whereas the interneurons are preserved.

P5.14.

Sérült motoneuronok megmentése indukált pluripotens őssejt transzplantációval

¹Krisztián Pajer, ²Csilla Nemes, ²Sára Berzsenyi, ²Krisztián A. Kovács, ²Melinda K. Pirity, ²András Dinnyés, ³Antal Nógrádi

¹ Szemészeti Klinika, Neuromorfológiai Laboratórium, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary; ² R&D, Biotalentum Kft., Gödöllő, Hungary; ³ Szemészeti Klinika, Neuromorfológiai Laboratórium, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary

A mellső gyökér sérülések következményeként a gerincvelői motoneuronok túlnyomó többsége elpusztul. Az egyik jövőbeni terápiás lehetőség olyan indukált pluripotens őssejtek (iPS) transzplantációja a sérült gerincvelőbe, melyek képesek a károsodott motoneuronok túlélését és regenerációját előmozdítani a sérülést követően. Kísérleteink során Sprague-Dawley nőstény patkányok lumbalis 4-es (L4) mellső gyökerét kihúztuk, majd a mellső gyökér visszaültetését követően 3 x 10⁵ egér iPS-t ültettünk be az L4 szegmentumba. Kontroll állatok esetén csupán az L4

mellső gyökeret húztuk ki és ültettük vissza iPS beültetés nélkül. A morfológiai ill. a funkcionális reinnervációt retrográd jelöléssel és izomerő méréssel igazoltuk. A beültett iPS-eket egér-specifikus immunhisztokémiai markerekkel térképeztük fel. Az őssejtbeültetés hatására szignifikánsan több sérült motoneuron élt túl és innerválta a visszaültetett mellső gyökeret és a végtagizmokat, mint kontroll állatok esetében. A transzplantációt követő első 7 napban az iPS-ek egyaránt termeltek neurotrofikus faktorokat valamint pro- és anti-inflammatorikus citokineket. A beültetett sejtek differenciálódása során a 7. napon iPS eredetű neuronok és asztrociták jelentek meg a graft és a gazdaszövet határán. A 10. posztoperatív naptól kezdve megnőtt a microglia reakció a grafton belül és a transzplantációt követő 16. napra az iPS-ek és származékai eltűntek a gerincvelőből. Eredményeink bizonyítják, hogy iPS-ek transzplantációjával előmozdítható a sérült motoneuronok túlélése és regenerációja.

P5.15.

Comparison of dendritic impulse propagation in pyramidal neurons of normal and human amyloid precursor protein over expressing (Tg2576) transgenic mice

¹Attila Somogyi, ¹Zoltán Katonai, ¹Ervin Wolf

¹ *Department of Anatomy, Histology and Embryology, University of Debrecen, Debrecen, Hungary*

The Alzheimer disease (AD) is the most frequent neurological degenerative disorder; which is irreversible, progressive, and incurable at present. During the course of the illness accumulations of amyloid-beta peptide appear in the brain tissue in the form of insoluble extracellular plaques, and neurons and synapses show signs of degeneration. Earlier morphological changes have been detected in the layer II/III pyramidal neurons of the somatosensory cortex in human amyloid precursor protein (hAPP) over expressing Tg2576 transgenic mice. On the other hand,

physiological measurements could not differentiate between the mutant and control principal neurons based on their passive membrane properties and action potential generation. This means that we need to investigate the diseased neurons at a subcellular level to identify changes in their function. We aimed to investigate dendritic impulse propagation in mutant pyramidal neurons (n=29) of hAPP over expressing mice and compare them to the control, healthy neurons (n=29). We used morphologically detailed passive segmental cable models of these cells within the NEURON (Duke University, USA) simulation environment. Current was injected to various dendritic points of mutant and healthy neurons to simulate local activity of synapses and the current and voltage transfers to the soma were investigated as a function of path distance of injection site from the soma. Distributions of dendritic surface area in the normalized geometrical and the electrotonic space were also studied. We found no significant difference in the rates of voltage- and current transfers in the basal and apical dendrites from the most path distance ranges except for the current transfer in the apical dendrites (Mann-Whitney test, $p<0.05$). Distributions of the dendritic membrane surface area of the mutant and control neurons were the same in the normalized electrotonic space (where distances of dendritic points and the soma were measured by log attenuation of PSP). The distributions of dendritic surface area differed (Mann-Whitney test, $p<0.05$) in the middle and distal regions of the normalized geometrical space in the two groups of pyramidal neurons. We conclude that in AD only the apical dendrites change their current and voltage transfer properties relative to the control. The authors are grateful to Dr. Alán Alpár for the files containing the reconstructed morphology of neurons and for the OTKA K67747 and ETT 025/2006 for the financial support.

P5.16.

Serum levels of C reactive protein (CRP), human serum albumin (HSA), and glycosylated hemoglobin A1c (GhbA1c) in Alzheimer's dementia

¹Andrea D Székely, ³Iltaf Shah, ³Andrea Petróczi, ^{4,5}Naji Tabet, ⁵Anthony Klugman, ⁵Mokhtar Isaac, ^{1,2}Árpád Dobolyi, ³Declan P Naughton

¹ *Department of Anatomy, Histology and Embryology and* ² *MTA-SE Neuromorphology Research Group, Semmelweis University, Budapest, Hungary*

³ *School of Life Sciences, Kingston University, Kingston-upon-Thames, Surrey, UK*

⁴ *Institute of Postgraduate Medicine, Brighton and Sussex Medical School, Brighton, UK.*

⁵ *Cognitive Treatment and Research Unit, Sussex Partnership NHS Foundation Trust, East Sussex, UK*

Background A growing body of evidence suggests that Alzheimer's disease (AD) is linked to low-grade chronic inflammation and impaired energy metabolism, due to insulin/IGF deficiency. Thus, the cognitive impairments might also partially be explained by 'diabetic' microvascular changes leading to cerebral hypoperfusion, gliosis, tissue atrophy and, extra- and intracellular protein deposits. Although the exact mechanism is still unknown, both conditions share certain abnormalities (i.e. impaired glucose metabolism, increased oxidative stress, deposition of amyloidogenic proteins) and epidemiological risk factors such as obesity or Vitamin D deficiency.

Serum levels of glycosylated hemoglobin (GhbA1c), C-reactive protein (CRP) and human serum albumin (HSA) are the key markers of inflammation (e.g. Type 2 diabetes, T2D), hence they might play a role in the diagnosis/prognosis of AD.

Methods Blood samples were collected from 105 AD patients (37 men and 68 women) with a mean age of 80.7 ± 7.7 (range= 62-95). Patients were non-smokers with no acute illnesses or gastrointestinal problems that may affect adsorption. No further medication, including anti-inflammatory agents, were administered. Patients were grouped as A: untreated (n=26), B: treated with donepezil, rivastigmine or galantamine (n=44) and C: control (n=35) with no cognitive impairment. Cognitive function was assessed using the MMSE. GhbA1c, C-reactive, CRP and HSA were measured with ELISA.

Results Mean HSA values were significantly different for all 3 groups with Group A (2.6130769 g/dL) being the lowest and C (3.8516667 g/dL) the highest, (with B = 3.3686364 g/dL). Mean CRP values in group C (0.2556667 mg/L) were significantly higher than in groups A (0.2005385 mg/L) or B (0.1751591 mg/L), with no significant difference between A and B. Mean GHbA1C values were significantly different for all groups, with Group A (5.7623077) expressing the highest and Group C (4.4127778 %) the lowest levels, while Group B (4.7197727 %) was in the middle range. The pattern of Vitamin D levels (25OHD, nmol/mL) followed HSA concentration with group A = 14.79 +/- 13.31, group B = 32.89 +/- 28.58 and controls = 40.50 +/- 23.46.

GHbA1C levels higher than 5,6 % carry a high risk for T2D and correlate with a higher risk to AD. All treated (B) AD patients and controls (C) were in the normal range, whereas in the untreated (A) group, there were 10/26 in the upper end of the normal range and 15/26 in the high risk group with 1/26 in the 'diabetic' group (6.59 %).

Conclusions HSA and GhbA1c levels show a statistically significant correlation with the condition of the patients thus suggesting the relevance of routine laboratory testing for early detection of AD.

P5.17.

Pathological and functional network connectivity analysis in the human brain using single pulse electrical stimulation

¹Emília Tóth, ²László Entz, ³Corey J. Keller, ⁴Dániel Fabó, ³Stephan Bickel, ⁵Lajos R. Kozák, ²Lóránd Eröss, ⁶István Ulbert, ³Ashesh D. Mehta

¹ *Interdiszciplináris Doktori Iskola, Pázmány Péter Katolikus Egyetem Információtechnológiai Kar, Székesfehérvár, Hungary;* ² *Funkcionális Idegsebészeti Osztály, Országos Idegtudományi Intézet, Budapest, Hungary;* ³ *Department of Neurosurgery, NSLIJ Health System, Manhasset N.Y. United States;* ⁴ *Epilepszia, Országos Idegtudományi Intézet, Budapest, Hungary;* ⁵ *MR Kutató Központ, Semmelweis Egyetem,*

Mapping of functional and pathological areas in the human brain is crucial in epilepsy and tumor surgery. In this study we used single pulse electrical stimulation evoked late responses to analyze pathological networks in epilepsy surgical candidates undergoing intracranial electrode placement. We performed systematic bipolar stimulation of subdurally implanted electrodes by administering single pulse electrical currents (Amp:10 mA, F:0.5 Hz, PW:0.2 msec) on 28 patients undergoing intracranial monitoring for localization of seizure foci. Cortico-cortical evoked potentials (CCEP) were measured. Electrodes were localized using preoperative high resolution MRI and post-operative CT and MRI. Significant CCEP responses marked functional connections between the underlying cortical areas and a graph model was used to display them. Electrodes showing epileptiform discharges were labeled according to the clinical report of the epileptologists. Areas involved in motor, sensory and language functions were identified with clinical stimulation. These well known functional networks were reliably identified using our method in all of our patients. We investigated the number of significant evoked potentials according to the distance between the stimulated-recording sites. Stimulating electrodes over the seizure onset zone showed significantly more CCEPs 1-4 cm away from the stimulating electrodes than stimulating non-pathological electrodes after normalization. We also analyzed connectivity and the z-score of the amplitude of the CCEP, which both decrease with increasing the distance from the stimulation site in both networks. Our results suggest that single pulse electrical stimulation evoked potentials correlate well with clinically defined functional networks and show higher connectivity within pathological networks. Single pulse electrical stimulation is a promising technique in delineating eloquent cortex and might be a useful tool to identify pathological networks. The research was supported by the following grants: OTKA K81354, OTKA PD77864, ANR-TÉT Neurogen, ANR-TÉT Multisca, TÁMOP-4.2.1.B-11/2/KMR-2011-0002

Alteration of the GABA-A receptor $\alpha 1$ subunit expression in different types of interneurons of the human epileptic hippocampus

¹Kinga Tóth, ²Meinrad Drexel, ¹Lejla Faradzs-Zade, ³Loránd Eröss, ³János Vajda, ¹Péter Halász, ¹Tamás Freund, ²Günther Sperk, ¹Zsófia Maglóczky

¹ *Celluláris és Hálózat Neurobiológia, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Department of Pharmacology, Innsbruck Medical University, Innsbruck, Austria;* ³ *Országos Idegtudományi Intézet, Budapest, Hungary*

GABAergic inhibition is known to play a complex role in the pathomechanism of epilepsy. Beside the interneuron loss and fiber reorganization, other factors like changes in the subunit composition of certain transmitter receptors also contribute in the pathological changes affecting the function of the preserved interneurons. In this study we examined the changes of the expression of GABA-A receptor $\alpha 1$ subunit ($\alpha 1$) in different functional types of interneurons: parvalbumin (PV)-containing- and calbindin (CB)-containing cells. Surgically removed hippocampi of drug-resistant temporal lobe epileptic patients were examined and compared with control samples. The sections were immunostained against $\alpha 1$ and the changes in the density and synaptic coverage of the immunopositive cells were analyzed. The coexpression of $\alpha 1$ and calcium binding proteins – PV and CB – in interneurons was studied using immunofluorescent double labeling. In control, $\alpha 1$ -positive cells were found throughout the entire hippocampus with largest densities in the CA1 region and the hilus of the dentate gyrus. The density of the immunopositive cells decreased in the hilus of patients with hippocampal sclerosis, in the CA1 region an increased density of immunoreactive cells was found, which may be partially explained by the tissue shrinkage. The colocalization studies revealed that the percentage of $\alpha 1$ -positive-neurons also expressing CB is reduced significantly in the sclerotic CA1. In the hilus of the dentate gyrus, the percentage of $\alpha 1$ -positive neurons containing CB increased, and significantly more CB-positive interneuron showed $\alpha 1$ -expression. In contrast, a decreased amount of PV-positive cells expressed the $\alpha 1$ subunit in the hilus of epileptic patients. The electron microscopic examination showed that both asymmetric- and symmetric synaptic

coverage of the $\alpha 1$ -positive dendrites was increased in the sclerotic epileptic samples, probably reflecting the sprouting of excitatory pathways and inhibitory cells. These data suggest pronounced differences of co-expression of $\alpha 1$ with interneuron-markers in sclerotic epileptic human hippocampal tissue, as well as regional differences between the hilus and CA1 region. Changes in the subunit composition can lead to an altered response to GABAergic inputs and influence the sensitivity to certain drugs. The observed changes in the $\alpha 1$ -expression may contribute to the pharmacoresistancy of these patients.

P5.19.

The Early Effect of β -amyloid on the Proteome of Synaptic Mitochondria in APP/PS1 Mice Brain

¹Katalin Völgyi, ¹Blanka Kellermayer, ¹Péter Gulyássy, ¹Attila Simor, ¹Viktor Kiss, ²Eszter Tóth, ¹Krisztina Hádén, ³Katalin Kékesi, ⁴Botond Penke, ¹Gábor Juhász

¹ Proteomikai Csoport, Eötvös Lóránd Tudományegyetem, Természettudományi Kar, Budapest, Hungary; ² Immunológiai Tanszék, Eötvös Lóránd Tudományegyetem, Természettudományi Kar, Budapest, Hungary; ³ Élettani és Neurobiológiai Tanszék, Eötvös Lóránd Tudományegyetem, Természettudományi Kar, Budapest, Hungary; ⁴ Orvosi Végyszertár, Szegedi Tudományegyetem, Szeged, Hungary

Synaptic dysfunction is an early event in Alzheimer's disease which has also been detected in transgenic mouse models. Synaptic mitochondria are vital for the maintenance of normal synaptic function, and associated with beta amyloid pathology. In our study we analysed the synaptic mitochondria protein pattern in APP/PS1 transgenic and wild-type mice at ³ months of age. We validated the synaptic mitochondria preparation with fluorescence-activated cell sorting, electron microscopy and Western Blot techniques. We investigated the early synaptic mitochondrial protein changes with ²-dimensional gel electrophoresis in combination with DIGE and mass spectrometry. We detected a large number of altered protein

spots which reflects the vast effect of beta amyloid on synaptic mitochondrial proteome. We report here on direct evidences of amyloid induced early changes in synaptic mitochondrial proteome. These results provide a molecular background for early phase of Alzheimer's disease. We also give molecular evidences underlying existing results of FDG imaging technologies showing early changes in Alzheimer diseased patients.

P5.20.

Patterns of synchronous population activity in the neocortex of patients with epilepsy or tumour, in vitro.

¹Kinga Tóth, ²Ágnes Kandrás, ²Csilla Szabó, ³Attila Bagó, ⁴Loránd Eröss, ⁴László Entz, ¹Tamás Freund, ²István Ulbert, ⁵Lucia Wittner

¹ *Celluláris és Hálózat Neurobiológia, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Kognitív Idegtudományi és Pszichológiai Kutatóintézet, MTA Természettudományi Kutatóközpont, Budapest, Hungary;* ³ *Neuroonkológia és Intracranialis Sebészet, Országos Idegtudományi Intézet, Budapest, Hungary;* ⁴ *Funkcionális Idegsebészet, Országos Idegtudományi Intézet, Budapest, Hungary;* ⁵ *Kognitív Idegtudományi és Pszichológiai Kutatóintézet, MTA Természettudományi Kutatóközpont, Budapest, Hungary*

Epilepsy, one of the most common neurological disorders, is thought to be related to hyperactivity of neuronal circuits. Pharmacological treatment is often effective, but significant numbers of patients resist pharmacotherapy. Surgical tissue removal in these patients offers a remarkable possibility to study living human tissue known to be intimately involved in the generation of this neurological disorder. Similar to interictal spikes recorded on the scalp EEG, spontaneous population activity (SPA) has been shown to be spontaneously generated in epileptic human tissue in vitro. Tissue slices were prepared from neocortical tissue of patients with epilepsy and as a control, of patients with tumour but without epilepsy.

Local field potential gradient (LFPg) was recorded with a ²⁴ channel laminar microelectrode. Neocortical neurons were characterized in simultaneous intracellular records. SPA consists of high frequency oscillations and elevated cell firing superimposed on a local field potential transient. SPA could be detected in ⁴⁸% and ⁴¹% of slices derived from epileptic and tumour patients, respectively. Several patterns of SPA could be differentiated both in the neocortex of epileptic and tumour patients, in a similar ratio. About 15% of the slices showed simultaneous multiple SPAs. The most frequently, SPA was generated in the supragranular layers, both in epileptic (68%) and in tumour (56%) tissue. Cell clustering analysis showed that ⁵⁸% and ⁴⁷% of the neurons elevated their firing rate in epileptic and tumour slices, respectively. In general, supragranular cells showed the maximal firing before, whereas infragranular cells discharged after the LFPg peak of the SPA. Different cell firing patterns were found, and we showed that both excitatory and inhibitory neurons participate in the generation of SPA. Our results suggest that spontaneous SPA is generated in both epileptic and non-epileptic neocortical tissue slices. Supragranular layers have a leading role in the initiation of the SPA, in which both excitatory principal cells and inhibitory interneurons participate. The cellular and network properties of SPAs were similar in tissue slices derived from epileptic and tumour patients. This indicates that in vitro occurring SPA cannot be directly related to epileptic processes. The research was supported by OTKA K81354, OTKA PD77864, ANR-TÉT Neurogen, ANR-TÉT Multisca, TÁMOP-4.2.1.B-11/2/KMR-2011-0002, Bolyai Research Fellowship (to LW)

Session 6. Sensory and motor systems, P6.1. – P6.20.

P6.1.

Changes in gamma range coupling oscillatory potentials between retina and primary visual cortex during stimulus variance and isoflurane anaesthesia in rats

Coupling of distant brain areas in the CNS can be performed by time-locking gamma oscillations. In the visual system such kind of timing has been found between the retina and the primary visual cortex (V1). There are already two separately investigated oscillatory responses to flash in the visual system: the retinal oscillatory potentials (OPs) and the visual evoked potential OPs. The aim of the present study is to provide convincing data supporting that retinal and cortical OPs are timing or coupling signals like cortical gamma oscillations. We recorded the OPs from two structures of the visual system, the retina and the V1 of freely moving and isoflurane anaesthetized rats. The effects of stimulus parameter variance, such as stimulus width (0.01 - 1000 ms), stimulus intensity (800 - 5000 mcd/mm²), interstimulus interval (10 - 400 ms) and stimulus frequency (0.25 - 25 Hz), on the responses of the V1 were examined. After that, isoflurane dose effects on OPs were studied with the most adequate stimulus. Using 0.6% and 1.5% isoflurane, OPs (amplitude, latency, correlation) changed dose dependently. Introducing tetrodotoxin (TTX) on V1 surface demonstrated that retinal OPs do not passively spread to the cortex. Our results suggest that the oscillatory potentials serve time-locked coupling of the retina and the primary visual cortex. Moreover OPs follow the depth of the narcosis indicating changes in the level of coupling. Thus measuring oscillatory potentials could provide an effective method for monitoring narcosis progress in clinical practice by tracking changes in visual data processing.

P6.2.

Role of Pituitary Adenylate-Cyclase Activating Polypeptide and Tac1 gene derived tachykinins in sensory, motoric and vascular functions under normal and neuropathic conditions

¹Bálint Botz, ¹András Imreh, ¹Katalin Sándor, ¹Krisztián Elekes, ¹János Szolcsányi, ²Dóra Reglődi, ³John P. Quinn, ⁴James Stewart, ⁵Andreas Zimmer, ¹Zsuzsanna Helyes

¹Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, Pécs, Hungary; ²Department of Anatomy, Faculty of Medicine, University of Pécs, Pécs, Hungary; ³School of Biomedical Sciences, Liverpool University, Liverpool, United Kingdom; ⁴School of Infection and Host Defense, University of Liverpool, Liverpool, United Kingdom; ⁵Laboratory of Molecular Neurobiology, University of Bonn, Bonn, Germany

Pituitary Adenylate-Cyclase Activating Polypeptide (PACAP) and Tac1 gene-encoded tachykinins (substance P: SP, neurokinin A: NKA) are expressed in capsaicin-sensitive peptidergic nerves, but data regarding their role in nociception, inflammation and vascular responses are contradictory. Therefore, we aimed to investigate the function of these sensory neuropeptides, and the NK1 tachykinin receptor (derived from the *Tacr1* gene) in the partial sciatic nerve ligation-induced traumatic mononeuropathy model, using gene deficient (*PACAP*^{-/-}, *Tac1*^{-/-}, and *Tacr1*^{-/-}) mice. The mechanonociceptive threshold of the paw was measured with dynamic plantar aesthesiometry, the motor coordination on a Rota-Rod device, and cutaneous microcirculation with laser Doppler imaging. Neurogenic vasodilatation was evoked by topical application of the selective Transient Receptor Potential A1 (TRPA1) agonist mustard-oil, stimulating sensory nerves. In both wild type groups (*PACAP*^{+/+}, C57Bl/6) a 30-40% mechanical hyperalgesia developed one week after nerve ligation, which persisted during the study. This hyperalgesia was not altered in *Tac1*^{-/-} and *Tacr1*^{-/-} mice, while it was absent in the *PACAP*^{-/-} animals. Motor coordination of the *PACAP*^{-/-} and *Tac1*^{-/-} groups was significantly worse both before and after nerve ligation, compared to their wild types, but it was not altered in *Tacr1*^{-/-} mice. Microcirculation on neither the operated nor intact limbs of the *PACAP*^{-/-} mice differed from the wild types during the postoperative control measurements, but it was significantly lower in the *Tac1*^{-/-} and *Tacr1*^{-/-} groups. The TRPA1 activation-induced neurogenic vasodilating response was significantly smaller in *PACAP*^{-/-} mice, but remained unchanged in *Tacr1*^{-/-} and *Tac1*^{-/-}

/- animals. As a conclusion, partial sciatic nerve ligation does not induce motor impairment, only sensory neuropathy. Both PACAP and SP/NKA participate in normal motor coordination. In contrast, SP/NKA and the NK1 receptor are not involved in these processes, but play a role in maintaining basal cutaneous blood flow. PACAP is a crucial mediator of neuropathic mechanical hyperalgesia and neurogenic vasodilation. Therefore, identifying its target and developing selective, potent antagonists, might open promising new perspectives for the treatment of neuropathic pain and vascular complications. Support: SROP-4.2.2.A-11/1/KONV-2012-0024, SROP-4.2.1.B-10/2/KONV-2010-0002, SROP-4.2.2.B-10/1/2010-0029

P6.3.

The distribution of monoacylglycerol lipase (MGL) immunoreactivity in the superficial spinal dorsal horn of rodents.

¹Klaudia Docová, ¹Zoltan Hegyi, ¹Krisztina Hollo, ¹Greta Kis, ²Ken Mackie, ¹Miklos Antal

¹ *OEC Anatómiai Intézet, Debreceni Egyetem, Debrecen, Hungary;* ² *Department of Psychological and Brain Sciences, Indiana University, Bloomington, United States*

It is generally accepted, that the endogenous cannabionoid ligand, 2-arachidonoylglycerol (2-AG) is an effective modulator of the nociceptive information processing at various levels of the central nervous system including the spinal dorsal horn. It has been demonstrated that 2-AG is mobilized in the lumbar spinal cord following painful stimuli to the hindpaw, and there is close correlation between spinal 2-AG levels and stress-induced antinociception. Although the molecular machinery responsible for the activity-dependent 2-AG biosynthesis have already been described in the spinal dorsal horn, much less is known about the distribution of hydrolytic enzymes which are responsible for the elimination of 2-AG. In the mammalian central nervous system, 85% of 2-

AG is hydrolyzed by monoacylglycerol lipase (MGL). Thus, here we investigated the cellular distribution of MGL in the superficial spinal dorsal horn of rodents by using immunocytochemical methods at the light and electron microscopic levels. We found that MGL is strongly expressed in the spinal dorsal horn, with a heavily stained band confined to laminae I-II. In the superficial spinal dorsal horn (laminae I-II) 57% of the MGL immunoreactive spots were associated with neuronal cell bodies, where MGL may participate in the degradation of metabolically produced 2-AG. 17, 4 % of the MGL-immunoreactive spots were found on astrocytic (GFAP immunoreactive) processes. Much smaller proportion of axon terminals showed immunoreactivity for MGL than expected. Only 10% and 6,3% of MGL immunoreactive spots were associated with axon terminals of CGRP immunoreactive peptidergic nociceptive primary afferents and VGLUT2 immunoreactive putative excitatory interneurons, respectively. Our results suggest, that the postsynaptically released 2-AG is predominantly broken down by astrocytes, and axon terminals of peptidergic primary afferents and excitatory interneurons in the superficial spinal dorsal horn.

P6.4.

Distribution of ECM in brainstem nuclei functionally related to the vestibular nuclear complex

¹Botond Gaál, ²Éva Rácz, ²Szilvia Kecskés, ³Klára Matesz

¹ *Anatómiai, Szövet- és Fejlődéstani Intézet, Debreceni Egyetem, Orvos - és Egészségtudományi Centrum, Debrecen, Hungary;* ² *Anatómiai, Szövet- és Fejlődéstani Intézet, Debreceni Egyetem, Debrecen, Hungary;* ³ *Debreceni Egyetem; Anatómiai, Szövet- és Fejlődéstani Intézet, HAS - UD Neuroscience Research Group, Debrecen, Hungary*

The vestibular nuclear complex (VNC) is functionally related to a number of other brainstem areas, by afferent and efferent synaptic connections. One of the most important outputs is to the oculomotor nuclei, establishing

the vestibulo-ocular reflex. According to our earlier findings those neurons of VNC wear perineuronal nets (PNN), that are in direct synaptic contact with eye moving neurons, located in the superior vestibular nucleus and magnocellular part of medial vestibular nucleus. In the oculomotor nuclei, most of the neurons are singly (SIF) or multiply innervating motoneurons, whereas the interneurons are found in smaller number. Previous studies have showed that only SIF motoneurons wear PNN, however the detailed molecular composition of perineuronal ECM was not yet described. In the present study we examine the extracellular matrix (ECM) composition of the oculomotor, trochlear and abducens nuclei, with special emphasis on the presence or absence of PNNs. For our examinations we used female adult Wistar rats. The removed brains were immersed into St. Marie's fixative, then histochemical and immunohistochemical reactions were performed on the 8 μ m thick cross sections. We applied the following ECM markers: biot. HABP, biot. WFA, and antibodies against aggrecan, versican, neurocan, brevican, tenascin-R and HAPLN1 link protein. All reactions were visualized by DAB. The presented images exclusively show PNN-surrounded neurons. In the oculomotor nucleus HA and WFA staining was shown throughout the neuropil, in the PNN aggrecan and tenascin-R showed the strongest labeling. Brevican labeling appeared intracellularly as well. The neurocan and HAPLN1 appeared to be the faintest in this nucleus. In the trochlear nucleus HA, versican, neurocan and HAPLN1 reactions resulted in more intense staining, although the rest of labelings were fainter than the PNNs in the oculomotor nucleus. In case of abducens nucleus aggrecan, versican, tenascin-R and HAPLN1 appeared to be fainter than the previous nuclei which were predicted by the weak WFA labeling. The brevican and HA showed to be similar to the previous nuclei. As conclusion, this work gives more detailed description on oculomotor nuclei PNN pattern, than previous studies. The WFA staining showed slight difference among the three nuclei, which may be corresponding with presence of various amount and molecular composition of ECM in the PNNs. Support: MTA-TKI 255, DE OEC Bridging Fund, TÁMOP- 4.2.2/B-10/1-2010-0024.

Cortical layer 5 control over the motor thalamus of the mouse

¹Nóra Hádinger, ¹Hajnalka Bokor, ¹László Acsády

¹ *Celluláris és Hálózat Neurobiológia, MTA-Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary*

The thalamus can be divided into first and higher order nuclei. The former receive large, multisynaptic excitatory inputs (drivers) from subcortical centers whereas the latter are contacted by drivers originating in layer 5 (L5) of the neocortex. According to our previous data the basal ganglia (BG) recipient thalamic nuclei in primates lack both cortical and subcortical drivers but are known to be innervated by L5. This clearly indicates that these motor thalamic regions receive L5 boutons with significantly smaller in size. This raises the question whether L5 input in BG thalamus can act like drivers. Since these thalamic regions are pivotal in motor execution and planning and are involved in Parkinson's disease their cortical control is of significant interest. Thus, in the present study we investigated the morphological and functional properties of the L5-thalamus connection in the BG thalamus (ventromedial thalamic nucleus VM) in rodents, using a mouse line which selectively expresses channel rhodopsin in L5 (Thy1-ChR). Selective photoactivation of both the ipsi- and, unexpectedly, the contralateral L5 cortical inputs could drive VM neurons. Similar to the primate brain neither anterograde tracing nor immunocytochemical labeling of cortical inputs revealed large cortical terminals in VM, thus the effect was clearly mediated by small L5 terminals. Response probability of VM cells increased with increasing laser intensity suggesting that convergence of several L5 cells may be needed to activate VM cells. To map the cortical input space of VM neurons we used retrograde and anterograde tracing and reconstructed the entire dendritic arbor of VM neurons. The data showed that VM receives L5 inputs from widespread ipsi- and contralateral cortical areas but within VM the cortical inputs from different areas tend to end in different zones. Although these zones likely overlap and the dendritic tree of VM neurons could extend into multiple zones the position of cells within the nucleus can determine the kind of information it receives. Our data indicate that despite the small size of the terminals VM receives a highly efficient

bilateral L5 input which although derives from widespread cortical sources can be specific according to the location of the given cell. Our results contribute to the growing evidence that the rules of operation can fundamentally differ between different thalamic regions.

P6.6.

Hemokinin-1 induces hyperalgesia in inflammatory and neuropathic pain models of the mouse

¹Zsófia Hajna, ¹Éva Borbély, ¹Valéria Tékus, ¹István Tóth, ²Alexandra Berger, ²Christopher J. Paige, ¹Erika Pintér, ¹József Kun, ¹János Szolcsányi, ¹Zsuzsanna Helyes

¹ *Farmakológiai és Farmakoterápiai Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;* ² *University Health Network, Ontario Cancer Institute, Toronto, Canada*

Hemokinin-1 (HK-1) encoded by the recently discovered Tac4 gene is present in the central nervous system, with similar expression pattern, structure and immunological characteristics to those of substance P (SP). HK-1 also acts on neurokinin 1 (NK1) receptors, although, based on a range of distinct actions, an own HK receptor has also been proposed. Recent data suggested that HK-1 also might participate in inflammatory and pain processes. Therefore, we investigated the role of HK-1 in inflammatory and neuropathic hyperalgesia using Tac4 gene-deleted (Tac4^{-/-}) mice and their C57Bl/6 wildtypes (WT). Acute neurogenic inflammation of the paw was evoked by the Transient Receptor Potential Vanilloid 1 (TRPV1) agonist resiniferatoxin (RTX). Noxious heat threshold was measured with increasing temperature hot plate 5-20 min after intraplantar RTX injection. Mechanonociceptive threshold was assessed by dynamic plantar aesthesiometry 2-24 h after RTX. Chronic arthritis was elicited by Complete Freund's Adjuvant (CFA). Mechanonociceptive threshold was determined by aesthesiometry and paw volume by plethysmometry 3 times a week for 21 days. Traumatic

mononeuropathy was induced by tight ligation of 1/2-1/3 of the right sciatic nerve. 7-19 days after the operation, mechanonociceptive threshold and motor coordination were measured by aesthesiometry and accelerating rotarod, respectively. In WT_s, RTX induced a remarkable drop of the thermonociceptive and mechanonociceptive thresholds showing the development of thermal allodynia as well as mechanical hyperalgesia. In Tac4^{-/-} mice, both parameters were significantly diminished compared to WT_s. CFA-induced mechanical hyperalgesia was also markedly attenuated in Tac4 gene-deleted mice, while oedema was not altered. Sciatic nerve ligation resulted in a decrease of the mechanonociceptive threshold in WT_s 7-19 days after the operation, while the motor performance was not affected. This neuropathic hyperalgesia was also significantly smaller in Tac4^{-/-} animals. HK-1 plays a predominant role in acute inflammatory thermal allodynia, as well as mechanical hyperalgesia under inflammatory and neuropathic conditions. It is likely to act on the peripheral sensory nerve terminals and also in the spinal cord central sensitization occurs. Identification of its target and mechanisms of action opens new perspectives to develop novel analgesics. SROP grants: 4.2.2.A-11/1/KONV-2012-0024, 4.2.1.B-10/2/KONV-2010-0002, 4.2.2.B-10/1/2010-0029

P6.7.

Resting-state functional connectivity predicts the face selectivity of fMRI responses in the fusiform gyrus

¹Petra Hermann, ²Viktor Gál, ³Zoltán Vidnyánszky

¹ *Információs Technológiai Kar, Pázmány Péter Katolikus Egyetem, Információs Technológiai Kar, Budapest, Hungary;* ² *Szentágothai J. Knowledge Center - Magnetic Resonance Research Center, Semmelweis University, Budapest, Hungary;* ³ *Department of Cognitive Science, Budapest University of Technology and Economics, Budapest, Hungary*

Face processing involves a region of the human fusiform gyrus, the fusiform face area (FFA). fMRI responses in the FFA show the highest face selectivity in the visual cortex and this region might play a primary role in coding the structural information of face stimuli. An important unresolved question is whether and to what extent the functional connectivity between the FFA and other visual cortical regions involved in object processing contributes to the face selectivity of fMRI responses in the FFA. Here we addressed this question by measuring both the face selectivity of fMRI responses and the resting-state functional connectivity between visual cortical areas in the same human participant. The results revealed that the strength of the resting-state functional connectivity between the FFA and a region of the lateral occipital cortex (LOC) involved in visual object processing showed a strong negative correlation with the face selectivity of fMRI responses in the FFA: the stronger the functional connectivity between these regions during rest, the less face selective the FFA responses. These findings suggest that face selectivity in the FFA is determined in part by its functional connectivity with non-face selective visual cortical areas of the lateral occipital cortex.

P6.8.

D1A dopamine receptor containing neurons in the cerebral ganglia of the snail and their possible role in space positioning of the olfactory organs during olfactory orientation

¹László Hernádi, ¹Tibor Kiss, ¹Károly Elekes

¹ *Kísérletes Állattani Osztály, MTA Ökológiai Kutatóközpont, Balatoni Limnológiai Intézet, Tihany, Hungary*

In the snail *Helix pomatia* the main olfactory organs are located on the tip of the paired posterior tentacles. Behavioral studies have shown that in odor conditioned animal the posterior tentacles display a typical horizontal lowering in the presence of conditioned odor by the contraction of tentacle flexor muscles. In this study we described the distribution of D1A

dopamine receptor immunolabeled cell bodies and fibers in the cerebral ganglia as well as in the ganglion and flexor muscles of the posterior tentacles in order to investigate they might have a role in the odor conditioning and the modulation of the sensory-motor responses to conditioned odor. D1A immunolabeled cell bodies are located only in the cerebral ganglia sending axonal projections to the tentacular ganglion and flexor muscles. The labeled cell bodies were located on the ventral-frontal area of the cerebral ganglia where the sensory branches of the cerebral nerves reach the ganglion as well as on the dorsal-caudal area where the connectives of other ganglia reach the cerebral ganglion. Double immunostaining experiments revealed that D1A labeled neurons do not contain either dopamine or serotonin, therefore their transmitter contents remained unknown. D1A immunoreactivity could not be detected in muscle fibers which suggests that DA exerts its excitatory effects exclusively via neuronal elements which are neither serotonergic nor dopaminergic. The present findings suggest that D1A dopamine receptor containing neurons might have a role in the sensory-motor integration during both odor conditioning and olfactory orientation in the presence of conditioned odor. Support: OTKA No. 78224

P6.9.

Characteristics of auditory cortical evoked responses elicited by acoustic and thalamic electrical stimulation in the cat

¹Domonkos Horváth, ¹Richárd Fiáth, ¹Lucia Wittner, ^{1,2}István Ulbert, ^{1,2}György Karmos

¹ *Institute of Cognitive Neuroscience and Psychology, Research Centre for Natural Sciences, HAS, Budapest, Hungary;* ² *Faculty of Information Technology, Péter Pázmány Catholic University*

Changes of auditory cortical evoked potentials depending on vigilance state are well known. However, underlying thalamocortical mechanisms are still elusive at the neural circuit level. We investigated the

simultaneously recorded changes of the click evoked responses in the medial geniculate body (MGB) and in the auditory cortex and also studied the cortical evoked responses induced by single pulse electrical stimulation of the MGB. Recording electrodes were chronically implanted to the surface of the auditory cortex of cats. Intracortical recordings were performed with a 24 channel linear array multielectrode implanted into the A1 area. Similar 24 channel linear array multielectrode was implanted into the medial geniculate body (MGB) for recording and microstimulation. Local field potentials, single unit and multiunit activity (MUA) were recorded and analyzed. Position of the MGB electrodes was verified by recording tonotopic responses and post mortem histology. We presented separated acoustic and MGB stimulations. Mostly early multiunit responses and locally generated potential fields appeared in the MGB with minor changes in the wakefulness-sleep cycle. The configuration of the auditory cortical evoked potentials induced by MGB microstimulation was similar to those elicited by click stimuli but with shorter latency. In slow wave sleep the amplitude of the early components of the click evoked cortical response was not suppressed and the late phase was prevailed by large amplitude positive wave and decreased unit firing. Similarly, late components of the cortical responses evoked by MGB microstimulation displayed increase both in amplitude and latency. The characteristics of the electric microstimulation elicited potentials depended on the stimulation location in the MGB. Stimulating at locations closest to the areas showing maximal MUA evoked the highest amplitude cortical responses. The research was supported by the following grants: OTKA K81354, OTKA PD77864, ANR-TÉT Neurogen, ANR-TÉT Multisca, TÁMOP-4.2.1.B-11/2/KMR-2011-0002

P6.10.

Binocular reaction time and disparity

¹Gábor Horváth, ¹Eszter Mikó-Baráth, ²Béla Török, ¹Gábor Jandó

¹Department of Physiology, University of Pécs, Pécs, Hungary;
²Department of Ophthalmology, Kantonsspital St. Gallen, St. Gallen, Switzerland

According to Worth's theory (1906), binocular fusion and stereopsis are two different stages of binocular visual information processing, however, temporal relationship between stages is not fully understood. Cyclopean stimuli, including dynamic random dot stereogram (DRDS) and dynamic random dot correlograms (DRDC), evoke percept and brain electrical responses only in individuals who have mature cortical binocularity and the stimuli are viewed binocularly. The objectives of the present study was to measure simple reaction time (RT) to different types of patterned cyclopan stimuli and non-cyclopan checkerboard pattern onset in young healthy adults. During non-cyclopan pattern onset a 120' checkerboard appeared from the grey background. In cyclopan stimuli, the same checkerboard pattern appeared from a correlated dynamic noise background. In DRDSs the disparity was varied between 3.75-60 min of arc. Transient perievent brain electrical potentials were recorded simultaneously with RT measurement from O1, O2, P3, P4 positions. Dichoptic viewing was achieved by red green goggles, stimulus contrast and luminance was kept constant for all type of stimulations. Cyclopan RTs were consistently longer compared to non-cyclopan pattern onset. RTs showed a U shaped relation as a function of disparity, lowest RT was measured at around 15' disparity. RTs to DRDC stimuli fell between 20' and 30' disparity. Transient perievent brain potentials did not show a definite positive or negative peak at a consistent latency, both inter and intra-individual variability was high. In conclusion, binocular visual information processing is disparity dependent. RTs for disparities less than 7' and greater than 20' were longer than RTs to patterned DRDC stimulus. However, processing of disparities between 7' and 20' is faster than correlation and anticorrelation. Based on the RTs, processing of correlation and anti-correlation does not seem to be a preprocessing stage of disparity.

P6.11.

Distribution of vestibular, trigeminal and spinal afferent terminals on functionally different hypoglossal motoneurons in the frog, *Rana esculenta*

¹Szilvia Kecskés, ¹Klára Matesz, ¹András Birinyi

¹ *Anatómiai, Szövet- és Fejldéstani Intézet, Debreceni Egyetem OEC, Általános Orvostudományi Kar, Debrecen, Hungary*

The prey-catching behavior of the frog consists of sequence of coordinated activity of different muscles corresponding to various stages of feeding. One component of neuronal network underlying prey-catching behavior consists of the hypoglossal nucleus responsible for the contraction of tongue muscles. The activity of this network is modified by various neuronal elements. Thus, the afferent vestibular fibers terminate not only in the vestibular nuclei but also in the area of the hypoglossal nucleus in the brainstem. Snapping of prey object stimulates the trigeminal afferent terminals in the oral cavity and pharynx which initiates the contraction of muscles innervated by the hypoglossal nerve. During prey-catching behavior the primary afferent fibers of second cervical spinal nerves convey information from the somatosensory receptors of the forelimbs, into the brainstem. Because of the absence of the neck in the frog, a branch of the second spinal nerve emerges as the dorsal ramus of the hypoglossal nerve and coordinates the timing of tongue movements and the mouth opening during feeding. The aim of our experiments was to study whether the afferent fibers establish direct connections with the hypoglossal motoneurons in order to find the neuroanatomical background of the influence of primary afferents on contraction of muscles innervated by the hypoglossal nerve. In anaesthetized frog either the vestibular, trigeminal or second cervical spinal nerves and the hypoglossal nerve were simultaneously labeled with anterograde and retrograde tracers. The spatial distributions of connections between the labeled afferents and the dendrites or perikarya of the XII motoneurons in different subdivisions of the hypoglossal nucleus were shown on Neurolucida reconstruction of the brainstem. Close appositions between vestibular, trigeminal or cervical afferent fibers and somata or dendritic trees of motoneurons of the XII nerve were detected with confocal laser scanning microscopy. These

possible monosynaptic connections may serve as one of the morphological substrates of the fast response during feeding movements of amphibians that gives the reflex-like ability of the prey-catching behavior. This work was supported by OTKA K 67641, MTA-TKI 255 and DE OEC Bridging Fund.

P6.12.

Design and synthesis of opiate-cannabinoid bivalent ligands: A novel approach to prevent chronic opiate-administration induced hyperalgesia, allodynia and antinociceptive tolerance

¹Attila Keresztes, ²Robert Berkecz, ¹Geza Toth, ¹Csaba Tomboly, ¹Eva T. Papp, ¹Szabolcs Dvoracsko, ³Eva Varga

¹ *Biokémiai Intézet, MTA-SZBK, Szeged, Hungary;* ² *Department of Medical Chemistry, University of Szeged, Szeged, Hungary;* ³ *Department of Pharmacology, University of Arizona, Tucson, Az, USA*

Despite enormous research efforts, chronic pain management still remains a challenge. Nowadays, opiates are one of the primary choices for clinical treatment of severe chronic pain. However, unfortunately, sustained opiate-treatment leads to increased pain sensitivity (opiate-induced hyperalgesia) and reduced analgesic efficacy (antinociceptive tolerance). Recent data indicate that sustained opiate (morphine) treatment also activates spinal glia and inhibition of spinal glial activation seems to attenuate opiate-induced hyperalgesia, allodynia and antinociceptive tolerance. Interestingly, CB2-receptor selective cannabinoid agonists were shown to suppress neuropathic and inflammatory pain sensitization and spinal glial activation. Our earlier data indicate that co-administration of morphine and CB2-receptor agonist also efficiently attenuates morphine-induced hyperalgesia, allodynia and sustained morphine-treatment mediated spinal glial activation. Herein, we wish to present a novel approach which combines opiate (oxycodone) and cannabinoid (JWH-018) pharmacophores into a single molecule to overcome the undesirable side-

effects. In addition to a better pharmacokinetic, pharmacodynamic and patient compliance, these new agents are expected to show improved or equivalent analgesic efficacy with the lack of opiate-induced hyperalgesia, allodynia and antinociceptive tolerance.

P6.13.

Neurotransmitter candidates at an excitatory synapse of muscles in the snail tentacle

¹Nóra Krajcs, ¹László Hernádi, ¹Károly Elekes, ¹Tibor Kiss

¹ *Kísérletes Állattani Osztály, MTA ÖK Balatoni Limnológiai Intézet, Tihany, Hungary*

Patterned movements of the olfactory organ occur in many animals, which can improve access of odor molecules to the receptors. In land snails, tentacle movements, like twitching and quivering play an important role in the olfactory orientation. Three fine muscles (M1, M2, M3) of the posterior tentacles of the snail, *Helix pomatia* was described, which can be responsible for the execution of these movements. In the present study electronmicroscopical, immunohistochemical and pharmacological experiments were made in order to investigate the possible transmitters and modulators participating in the regulation of muscle function, with special attention to the role of acetylcholine (ACh) and glutamate (Glu). The tested putative neurotransmitters ACh, Glu, dopamine (DA), glycine and histamine evoked muscle contraction at 10^{-4} M concentration. 5-HT had biphasic effect; it contracted or relaxed the muscle. ACh and Glu had the most remarkable contractile effect; furthermore these contractions were comparable with those evoked by electrical stimulation. The presence of choline acetyltransferase was demonstrated in the cerebral ganglia and in the muscle immunohistochemically and also with Western blot, supporting the notion that ACh could be an excitatory transmitter. DA and 5-HT modulated differently the muscle contractions elicited by the external applied ACh and electrical nerve stimulation. Based on these findings we

suppose that ACh and Glu had direct excitatory effect on the muscles, while 5-HT and DA play modulatory role. Supported by an OTKA grant, No. 78224.

P6.14.

Response characteristics of the striatum to visual motion in freely moving rat

¹Anett Nagy, ¹György Benedek, ¹Attila Nagy, ¹Antal Berényi

¹ *Élettani Intézet, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary*

Earlier electrophysiological studies proved that neurons located in the dorsocaudal subfield of the caudate nucleus respond to moving visual stimuli; however these experiments were performed in anaesthetized animals. These results do not reflect the naturally occurring top down response amplification and filtering mechanisms of the intact brain, nor the interference arising from the sensorimotor feedback in the basal ganglia during self-motion. Moreover the population based information transferring pipelines (e.g. simultaneous phase locking, monosynaptic interactions of single units, etc.) were not investigated due to the lack of simultaneous multichannel recordings. To overcome these challenges, in the current preliminary study a freely moving rat was running for water reward in a linear maze, where the walls and floor were covered intermittently either with a homogenous gray painting, or with black and white sinewave pattern. The grey colored wall was serving as a control condition, while the latter was perceived as a moving sinusoidal grating during the run along the track, resembling the stimulation used in the anesthetized experiments. Multiple, multiscale neuronal activity of the caudoputamen was recorded simultaneously using 4 movable tetrodes. The neuronal responses were compensated with the velocity and position of the animal and then correlated with the temporal frequency of the perceived motion of the visual stimulus. The recorded cells were classified according

to their cell types and the modulation of their firing rate by the oscillation of the local field potential. Our results suggest that 72 out of the 131 recorded cells were sensitive to the visual motion percept (comparing the firing rates against the control condition), while 62 out of the 72 cells showed linear correlation with the visual motion velocity. This work was supported by OTKA/Hungary grants PD75156 and OTKA 83810 and by the EU FP7 PEOPLE 2009 IOF 254780 program.

P6.15.

Responses of various cell types to optic flow in the caudate nucleus of the behaving cat

¹Tamás Nagypál, ²Péter Gombkötő, ³Róbert Averkin, ⁴Tamás Puskás,
⁴Antal Berényi, ⁴György Benedek, ⁴Attila Nagy

¹ *Élettani Intézet, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary;* ² *Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ, USA;* ³ *MTA-SZTE Agykérgi Neuronhálózatok Kutatócsoport, SZTE TTIK, Szeged, Hungary;* ⁴ *Élettani Intézet, SZTE ÁOK, Szeged, Hungary*

The caudate nucleus (CN), one of the input structures of the basal ganglia, is considered to play an important role in the control of oculomotor and skeletomotor processes. The CN neurons receive sensory information, which may represent the surrounding environment and integrate it with the motor inputs to refine the movements during motor tasks. The major novelty of the present study is that we developed an awake, behaving, head restrained, eye-movement controlled cat model where we could investigate the effect of stationary visual stimulus and optic flow as a dynamic visual stimulus on the activity of the CN. Extracellular multielectrode recordings were carried out with 8 implanted platinum-iridium electrodes in the CN. The cat performed fixation on the centre of the monitor and we applied during this visual fixation ‘center in’ and ‘center out’ optic flow stimulation. We categorized the CN neurons upon their electrophysiological

properties (interspike intervals, autocorrelation of firing and firing rate) and investigated their activity during different epochs of the behavioural paradigm. Altogether 113 putative medium spiny projection neurons (MSNs), 41 fast-firing neurons (FFNs) and 28 tonically-firing neurons (TFNs) were recorded from the CN. About half of the recorded MSNs and FFNs responded to stationary visual stimulation as well as to optic flow stimulation. On the other hand the TFNs showed responses only to optic flow stimulus. The large majority of CN neurons were not sensitive to the direction (center-in or center-out) of the optic flow. Furthermore about two third of the FFNs showed responses shortly before and during the reward period after the correct completion of the task. Other 13 CN neurons (8 MSNs, 3 FFNs and 2TFNs) showed clear eye-movement correlated activity. We suggest that the visually active CN neurons may contribute to the control of initiation of reflexive saccades. CN neurons with increased activity enhance while CN neurons with decreased activity discontinue the tonic inhibition of the superior colliculus originating from the substantia nigra pars reticulata and thereby they may help keeping fixation or triggering a saccade. This work was supported by OTKA/Hungary grants PD75156 and OTKA 83810. T Nagypal is a fellow of Talentum Foundation of the Gedeon Richter Plc.

P6.16.

Motor nerve sensitivity changes caused by N-arachidonoyl-dopamine and capsaicin in rats.

²Árpád Sáfrány-Fárk, ¹Zita Petrovszki, ¹Gabriella Kekesi, ¹Peter Liszli, ¹Gyorgy Benedek, ³Katalin Nagy, ¹Gyöngyi Horváth

¹ Department of Physiology, University of Szeged, Faculty of Medicine, Szeged, Hungary; ² Department of Orthodontics and Pediatrics Dentistry, Faculty of Dentistry, University of Szeged, Szeged, Hungary; ³ Department of Oral Surgery, University of Szeged, Faculty of Dentistry, Szeged, Hungary

Introduction: In vitro data proved that capsaicin and the endogenous cannabinoid lipids including N-arachidonoyl-dopamine (NADA) can inhibit the voltage gated sodium channels (VGSC). Since these ligands can also influence TRPV1 and/or cannabinoid receptors, the peripheral motoneurons can be appropriate model for selective influence of VGSC. The goal of this study was to investigate the in vivo potency of these ligands on the facial nerve- induced vibrissal muscle activity. **Methods:** Male Wistar rats were anesthetized with ketamine-xylazine, and facial nerve at both sides was exposed at buccal level. The nerve was wrapped around unipolar wire electrode, and electrically stimulated (1 mA for 250 μ s). Action potentials of whisker muscles were recorded with unipolar needle electrodes placed into the whisker area of the rats, and the maximal amplitude was determined as differences between the highest positive and lowest negative peak. Amplitudes and peak-latencies (if there were any) were analyzed. After baseline measurement the substances were injected in the perineural sack in 10 μ l volume, and repeated responses were detected for 30 min. The amplitude changes in percentage were analyzed. **Results:** The single stimulus produced a visible whisker movement accompanied with EMG activity. Control recordings indicated that the magnitude of action potential was stabile at least for an hour. Injection of a vehicle did not produce significant changes in EMG activity; in contrast, injection of lidocaine (85.4-427 nmol) evoked dose-dependent decrease in EMG activity. The highest dose of lidocaine (427 nmol) produced a prolonged paralysis. NADA (11.4-57 nmol) also produced a dose-dependent inhibitory effect on the EMG activity, and 57 nmol produced a long-lasting inactivity. As regards the capsaicin, it did not influence significantly the amplitudes and latencies of action potentials between 66-1320 nmol doses. **Conclusion:** These results show that intraneural injection of NADA but not capsaicin resulted in a sustained inactivation of vibrissae muscles, which may be related to the reported inhibitory effects of these ligands at the VGSC. This work was supported by OTKA (K83810), TÁMOP -4.2.2.A-11/1/KONV-2012-0052, TÁMOP-4.2.2/B-10/1-2010-0012 grants, and Foundation for Dental Education of University of Szeged.

Az egyensúlyszabályozás vizsgálata élettanilag elkülönülő sagittális és transzverzális komponensek szerint - a vizuális és proprioceptív referencia súlya

¹Simon László, ²Végső Péter, ¹Endrényi Miklós, ¹Garab Sándor

¹ *Anatómiai, Szövet- és Fejlődéstani Intézet, Semmelweis Egyetem, Budapest*

² *Országos Idegtudományi Intézet, Budapest*

Az egyensúlyszabályozást közvetlenül tükröző poszturográfias mérésnél a vizsgált személy súlypont-ingadozásának függőleges vetülete egy ideális egyensúlyi pozíció körül leírt gombolyagszerű pályát ír le. Funkcionális anatómiai megfontolások alapján külön kívántuk vizsgálni a nyílirányú ill. harántirányú komponenseket. Az általunk használt PEP poszturográf (Med-Eval Kft.) illetve a klinikai diagnosztikai célra kifejlesztett Négylépcsős Összehasonlító Teszt kiértékelése lehetővé teszi a szabályozás vizuális és proprioceptív komponensének elkülönített vizsgálatát. A háromszög alakú platformon a vizsgált személy 20 másodpercig az élettanilag megszokott helyzetben, (spontán kisterpesz, nyitott szem), majd 20 másodpercig csukott szemmel egyensúlyoz. Ezután zárt lábbal, de nyitott szemmel történik a mérés, és az utolsó 20 másodpercben, a zárt lábú, csukott szemű mérés követi a Romberg-teszt elvét.

A gyors kiértékelésre alkalmas négymezős, a nyílirányú és a haránt elmozdulásokat feltüntető ábrán két irányban lehet az ingadozások mértékét összehasonlítani. A sokparaméteres off-line kiértékelésben speciális jellemzőket lehet megadni az egyensúlyszabályozás vizuális és proprioceptív komponenseinek súlyára.

Egészséges korcsoportokon mutatjuk be a négylépcsős teszt fázisaiban:

- a, a nyílirányú ill. haránt ingadozások pregnánsan eltérő viselkedését,
- b, a vizuális és a proprioceptív referencia differenciált szerepét,
- c, a szabályozó komponensek súlyának változását a korcsoportokban.

Endogenous and exogenous chemical responsiveness in the medial orbitofrontal cortex

¹István Szabó, ¹Bernadett Nagy, ¹Bettina Csetényi, ¹Edina Hormay, ¹Márk Bajnok Góré, ¹Zoltán Karádi

¹ *Institute of Physiology, Pécs University, Medical School, Pécs, Hungary*

The orbitofrontal cortex (OBF) – important brain region in the frontal lobes for the processing of reward and punishment – is known to play important roles in the central regulation of feeding. The glucose-monitoring (GM) neurons here have already been shown to be intimately involved in these functions. Our main goal in the present experiments was to characterize the endogenous and exogenous chemical sensitivities of these chemosensory cells. Extracellular single neuron activity was recorded in the medial OBF (mOBF) of male Sprague Dawley (SD) rats by means of tungsten wire multibarreled glass microelectrodes, during 1) microelectrophoretic application of various chemicals (such as D-glucose, acetylcholine (Ach), dopamine (DA), noradrenaline (NA)), as well as 2) gustatory stimulations with the five primary taste qualities (NaCl, HCl, monosodium-glutamate (MSG), sucrose, QHCl) and orange juice as complex taste solution. One fifth of all neurons tested changed their activity in response to the microelectrophoretic administration of D-glucose, that is, these cells proved to be elements of the forebrain GM neural network. NA elicited activity changes in 20% of neurons, whereas 40% of the examined cells changed in firing rate during microelectrophoretic application of DA. Of the tested chemicals, Ach was found to be the most likely to alter neuronal activity: it proved to be effective in 80% of cells. More than one fourth of tested neurons changed their activity during gustatory stimulation with sweet and salty taste solutions, while in case of the other primary taste qualities and orange juice this alteration was found to be approximately 60%. Our present findings demonstrate multiple functional attributes of GM neurons in the mOBF. These chemosensory cells, by the integration of signals arising from the endogenous and exogenous environments, appear to play important role in adaptive mechanisms of the organization of feeding. Supported by:

P6.19.

Role of the Transient Receptor Potential Ankyrin 1 (TRPA1) ion channel in nociceptive processes

¹Valéria Tékus, ¹Bálint Scheich, ¹Tamás Kóger, ¹Ádám Horváth, ¹Zsófia Hajna, ¹Éva Borbély, ¹Erika Pintér, ¹János Szolcsányi, ¹Zsuzsanna Helyes

¹ *Farmakológiai és Farmakoterápiai Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary*

Transient Receptor Potential Ankyrin 1 (TRPA1) is a non-selective cation channel expressed mainly on capsaicin-sensitive sensory nerve endings. Besides cold temperature, various pain producing agents can activate the receptor such as mustard oil (allylisothiocyanate: AITC), hydrogen sulphide (H₂S) or formaldehyde. The roles of TRPA1 in cold sensation, as well as neuropathic pain is contradictory, it seems to depend on the activation mechanisms and body regions. Therefore, the aims of the present study was to investigate the involvement of TRPA1 in AITC-induced acute nocifensive behaviours, as well as cold sensation and cold-allodynia in a traumatic mononeuropathy mouse models. Experiments were performed with male TRPA1 gene-deficient mice (TRPA1^{-/-}) in comparison with their TRPA1^{+/+} wildtypes. The acute nociceptive effect of AITC was detected by the tail and paw withdrawal latencies after immersing the tail or the paw into 1% solution (in 30% DMSO) of this potent TRPA1 agonist. Cold sensitivity of the same regions was measured by flick latency in response to 0°C water. Traumatic mononeuropathy was induced by tight ligation of 1/3 of the sciatic nerve. Cold allodynia was determined by the latency decrease of the nocifensive behaviours in icy water, mechanical hyperalgesia (drop of the mechanonociceptive thresholds) was measured by dynamic plantar aesthesiometry. The AITC-induced tail and paw flick latencies were significantly longer in TRPA1^{-/-} mice compared to the wildtypes. Meanwhile, cold-evoked withdrawal

latency was significantly increased in case of TRPA1 deficiency on the tail, but not on the paw. Seven days after sciatic nerve ligation a 40-50% mechanical hyperalgesia developed in both groups. In contrast, 60% cold allodynia observed in the TRPA1+/+ group was significantly decreased in TRPA1-/- mice. It is concluded, that the nocifensive actions of AITC is exclusively TRPA1-related, but it is involved in cold sensation only on the tail. TRPA1 activation plays a role in neuropathic cold allodynia mediated by peripheral mechanisms, but not in mechanical hyperalgesia due to central sensitization. Supported by SROP-4.2.1.B-10/2/KONV-2010-0002, SROP-4.2.2.A-11/1/KONV-2012-0024, SROP-4.2.2.B-10/1/2010-0029; OTKA NK-78059

P6.20.

Motor function changes after centrally or peripherally administered kynurenic acid

¹Gábor Tuboly, ²Zita Petrovszki, ²Gabriella Kekesi, ²Peter Liszli, ³Laszlo Vecsei, ²Gyongyi Horvath

¹ *Neurológiai Klinika, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary;* ² *Department of Physiology, University of Szeged, Faculty of Medicine, Szeged, Hungary;* ³ *Department of Neurology, University of Szeged, Faculty of Medicine, Szeged, Hungary and Neuroscience Research Group of the Hungarian Academy of Sciences and the University of Szeged.*

Introduction: Data suggest that NMDA receptors play an important role in pain-induced responses, and can also influence motor behavior primarily at spinal level. The goal of this study was to reveal the motor effects of spinally and perineurally applied endogenous NMDA-antagonist kynurenic acid (KYNA). Methods: Chronic intrathecal catheterization was performed in ketamine-xylazine anesthesia in one group of Wistar rats. Motor behavior (placing reflex, paw withdrawal behavior) and thermal pain threshold were determined before and after KYNA (10-100 µg). The next

two groups of Wistar rats were involved in electromyographic (EMG) or electroneurographic (ENG) recording studies. Wistar rats were anesthetized with ketamine-xylazine, and facial (motor) nerve at both sides was exposed at buccal level. The nerve was wrapped around unipolar wire electrode, and electrically stimulated. Motor responses of whisker muscles were recorded with unipolar needle electrodes placed into the whisker area of the rats. The sciatic (mixed) nerve activity was investigated in other group of rats. The proximal end of nerve (near to the hip) was electrically stimulated, and the nerve activities were registered from the distal end of the nerve. After baseline measurements KYNA (100 μ g) was injected in the perineural sack, and repeated responses were detected for 30 min. The amplitude changes in percentage were analyzed. Lidocaine was used as positive control. Results: Intrathecal administration of KYNA caused dose-dependent motor impairment and antinociception, and the largest dose caused a prolonged paralysis at both sides up to 106 $\pm$ 5 min. Regarding the EMG or ENG activities, the single stimulus produced a visible whisker/limb movements accompanied with action potentials (AP). Injection of a vehicle did not produce significant changes in amplitudes of APs. Lidocaine evoked dose-dependent decrease in the amplitude of APs. In contrast a high dose of KYNA (100 μ g) did not cause any effects on these nerves. Conclusion: Our results showed that spinal KYNA treatment caused dose-dependent antinociception and motor paralysis. In contrast, it had no effect on the peripheral nerve activity, suggesting that inhibition of voltage gated sodium channels do not have role in the anesthetic effect of KYNA at spinal level. This work was supported by TÁMOP-4.2.2.A-11/1KONV-2012-0052 grant.

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P7.1.

The effects of fatty acid amide hydrolase blockade induced enhanced anandamide signaling in the modulation of behavioral responses to environmental stimuli

¹Manó Aliczki, ¹Dóra Zelena, ¹Éva Mikics, ¹Ottó Pintér, ¹Katalin Gyimesiné Pelczer, ¹József Haller

¹ *Magatartásneurobiológiai Osztály, Kísérleti Orvostudományi Kutatóintézet, Magyar Tudományos Akadémia, Budapest, Hungary*

It was previously shown, that endocannabinoid signaling affects emotional behavior, however, effects seem to depend on the environmental context. In the present study, we investigated the behavioral effects of enhanced anandamide (AEA) signaling via the blockade of AEA degrading enzyme fatty acid amid hydrolase (FAAH) in the rat elevated plus-maze and mouse forced swimming test, respectively, in differentially aversive environmental conditions. Our results indicated that FAAH blockade dampened the behavioral responses to changes in environmental aversiveness in both paradigms and animal species, respectively. The effects were cannabinoid receptor type-1 dependent and were not the effects of the putative anxiolytic actions of FAAH inhibition. We also showed that FAAH blockade did not only decrease behavioral reactivity, but altered the strategy how animals were coping with environmental challenges as both in the rat tail-pinch test and in the mouse back test, subjects tended to cope with the test challenge showing an active instead of a passive strategy. These results suggest that endocannabinoid signaling does not have specific effects on different forms of behavior, but rather modulates the intensity and the pattern, how the individual is reacting to environmental changes. These findings might show a new perspective in the therapy of psychiatric disorders, where dampening abnormal responses to environmental stimuli can be crucial, such as in posttraumatic stress disorder or phobias.

P7.2.

Anxiolytic/neuroleptic 2',3'-benzodiazepines are potent blockers of phosphodiesterase 10A2 (PDE10A2)

¹Judit Bíró, ¹Géza Szabó, ¹Szabolcs Udvari, ¹Adrienn Pálvölgyi, ¹Omar Abdul-Rahman, ¹Péter Kiricsi, ¹Erika Gösi, ¹Éva Szabó, ²Gábor Szénási, ¹István Gacsályi, ¹Gábor Gigler, ¹Ferenc Antoni

¹ *Preklinikai Főosztály, Egis Gyógyszergyár NYRT, Budapest, Hungary;* ² *Kórélettani Intézet, Semmelweis Egyetem, Budapest, Hungary*

2',3'-Benzodiazepines (HAI) (Horváth et al(2000) Progress in Neurobiology 60:309-342) were reported as psychoactive agents that target an unidentified, high-affinity binding site largely restricted to the striato-nigral system of the brain. In the current study we examined PDE10A2 as the potential target of HAI compounds. Recombinantly expressed human PDE10A2 or particulate rat striatal PDE were potently inhibited by HAI compounds. The PDE10A2 inhibitory potency closely correlated with the ability of the compounds to displace 3H-girisopam from the high-affinity, striatal binding-site reported previously. When transfected into human embryonic kidney (HEK293) cells, variously mutated versions of PDE10A2 showed differential partitioning between the particulate and soluble fractions. However they were invariably inhibited by HAI compounds, suggesting that post-translational modifications have no major impact on the pharmacology of inhibition by HAI compounds. In mouse striatal slices, enhancement of the level of S845-phosphorylated GluR1 (pS845GluR1) by HAI compounds correlated with the potency to inhibit PDE10A2. Similarly, in HEK293 cells overexpressing striatal adenylyl cyclases 2, 5 or 9 the Ki of PDE10A2 inhibition correlated closely with the potency to enhance cAMP levels. Administration of HAI compounds to C57/Bl6 mice increased striatal pS845GluR1 levels, and this appeared to correlate with the neuroleptic potency of the compounds rather than the Ki for PDE10A2. However, pharmacokinetic analysis revealed major differences in the brain levels of individual HAI compounds. Overall, low brain penetration was associated with an anxiolytic profile, whilst higher brain penetration produced an antipsychotic effect. In summary, it appears that inhibition of PDE10A2 underlies all of the psychotropic actions of

HAI compounds. The data also suggest that moderate inhibition of PDE10A2 is a useful principle for an anxiolytic therapeutic profile.

P7.3.

Szelektív CRF receptor antagonisták hatásai az alkohollal kezelt és megvont patkányokban

¹Zsolt Bagosi, ¹Miklós Palotai, ¹András Buzás, ¹Péter Bokor, ¹Krisztina Csabafi, ¹Miklós Jászberényi, ¹Gyula Szabó

¹ *Kórélettani Intézet, Szegedi Tudományegyetem, Szeged, Hungary*

Az akut alkohol bevitel fokozza a dopamin felszabadulást a striátumban és a GABA felszabadulást az amygdalában. Ezeket a változásokat a corticotropin-releasing factor (CRF) közvetíti, mely alkohol hatására hypothalamikus és extrahypothalamikus magvakból egyaránt felszabadul. Kísérleteinkben azt vizsgáltuk, hogy a krónikus alkohol bevitel és az azt követő akut alkohol megvonás miként befolyásolja a striatális dopamin és az amygdaláris GABA felszabadulást, valamint azt, hogy CRF receptorok (CRFR1 és CRFR2) közül melyik közvetíti az extrahypothalamikus neurotranszmitterek kidobását. Az alkohol hatásainak vizsgálatához hím Wistar patkányokat használtunk, melyeket 5 napon át, napi 4 alkalommal intraperitoneálisan 2 g/kg alkohollal (kontrollként fiziológiás só oldattal) kezeltünk, majd 1 napon át megvontuk tőlük az alkoholt. A CRF receptorok blokkolásához CRF antagonista antalarmin (0,1 mcg/2 mcl) vagy szelektív CRFR2 antagonista astressin 2B-t (1 mcg/2 mcl) használtunk, mellyel az állatokat 30 perccel a dekapitalásuk előtt intracerebroventrikulárisan kezeltük. A striatális dopamin és az amygdaláris GABA szinteket először a 6. napon (12 órával az utolsó alkohol beadás után), másodszor a 7. napon (24 órával az utolsó alkohol beadás után) mértük meg szuperfúziós rendszer és szcintillométer segítségével. A krónikus alkohol kezelés hatására fokozódott a dopamin felszabadulás a striátumban és a GABA felszabadulás az amygdalában. A fokozott neurotranszmitter kidobást az antalarmin szignifikánsan gátolta; az astressin 2B nem volt rá hatással. Az akut alkohol megvonás következtében a striatális dopamin szint szignifikánsan lecsökkent, továbbá

az amygdaláris GABA szint szignifikánsan megemelkedett a kontrollhoz képest. A neurotranszmitter kibóást az antalarmin a kontroll szintjére emelte, illetve csökkentette; az astressin 2B nem befolyásolta azt. Kísérleteink azt bizonyítják, hogy a krónikus alkohol kezelés okozta dopamin felszabadulás a striátumban és GABA felszabadulás az amygdalában kizárólag a CRFR1 receptoron keresztül történik. Az akut alkohol megvonás következtében lecsökkent striatális dopamin szintet és a továbbra is fentartott amygdaláris GABA szintet egyaránt a CRFR1 up-regulációja magyarázhatja. Kísérleteink a TÁMOP 4.2.2-A-11/1/KONV-2012-0052 pályázat támogatásával készültek.

P7.4.

Synaptoprotective Effect of Diazepam in the Rat Learned Helplessness Paradigm

¹Judith Baka, ¹Eszter Csákvári, ¹Orsolya Huzián, ¹Bálint Kincses, ¹Nikoletta Dobos, ¹László Siklós, ²Tamás Farkas, ¹Tibor Hajszán

¹ *Biofizika Intézet, MTA-Szegedi Biológiai Kutatóközpont, Szeged, Hungary;* ² *Élettani, Szervezettani és Idegtudományi Tanszék, SZTE-TTIK, Szeged, Hungary*

Major depressive disorder (MDD) is predicted to become the leading cause of disability worldwide by the year 2030, representing an enormous financial and social burden. Clinical management of MDD is quite limited due mostly to the fact that the neurobiology of depression and the mechanisms of antidepressant therapy are still largely unknown. Recently, we have confirmed the validity of the new “synaptogenic hypothesis” of depression by demonstrating an inverse correlation between the number of synapses in limbic brain areas and the severity of depressive symptoms, both in animal models and in human beings. It is hypothesized that loss of synapses in depression is, at least partly, caused by prolonged stress and the resultant glutamate excitotoxicity, which could be prevented by antagonizing glutamate release in response to stress. In addition to their

anxiolytic, anticonvulsant, muscle-relaxant, and sedative/hypnotic effects, benzodiazepines, such as diazepam, strongly inhibit glutamate release at high, pharmacological doses. Our poster presents a series of experiments, investigating whether diazepam is able to prevent helplessness and to protect synapses in the learned helplessness (LH) model of depression. Diazepam, when administered intraperitoneally to ovariectomized female CD(SD) rats (n=24, 200-250g), dose-dependently decreased depressive symptoms in LH and demonstrated synaptoprotective effects in electrophysiological and morphological measurements. These findings further support the synaptogenic hypothesis of depression and suggest that synaptoprotective treatment is able to antagonize the negative effect of stress on mood, which may be useful in the clinical management of patients with recurrent and/or treatment-resistant depression. Supported by Research, Technology and Innovation Fund (KTIA) and Hungarian Scientific Research Fund (OTKA) joint grant OTKA81190, and National Development Agency (NFÜ) grant TÁMOP-4.2.2.A-11/1/KONV-2012-0052.

P7.5.

Csökkent szorongás- és depresszió-szerű magatartás a vazopresszinhiányos hím Brattleboro patkányokban centrális támadásponttal

¹Diána Gabriella Balázsfi, ¹Barbara Klausz, ¹Ottó Pintér, ¹Krisztina Bea Kovács, ¹Anna Fodor, ¹Bibiána Török, ²Mario Engelmann, ¹Dóra Zelena

¹ *Magatartás-neurobiológiai Osztály, Magyar Tudományos Akadémia Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary;* ² *Institut für Biochemie und Zellbiologie, Otto-von-Guericke-Universität, Magdeburg, Germany*

Bevezetés: A só-víz háztartásban betöltött szerepén túl a vazopresszin hatással van a stressz-tengely működésére is, így a stressz indukálta szorongás és depresszió kialakulásában is nagy szerepet játszhat. A spontán mutációval vazopresszin-hiányossá (KO) vált Brattleboro

patkányokban a kezelés akut stressztől mentesen lehet tanulmányozni ezen hormon szerepét. Problémát jelenthetnek viszont a só-víz háztartás felborulása miatt másodlagosan létrejövő hatások.

Cél: A vazopresszin érzelmi zavarokban játszott szerepének kiterjedt igazolása hím Brattleboro patkányokban. Továbbá annak bizonyítása, hogy a vazopresszin magatartási hatásai centrális támadásponttal jönnek létre.

Módszerek: A KO patkányok egy részében bőr alá ültetett V2 receptor agonista (desmopressin) tartalmú ozmotikus minipumpa segítségével helyreállítottuk a perifériás só-víz háztartási zavart. Ezután teljesen KO, centrálisan KO (desmopressin kezelt) és vad típusú (WT) állatokat hasonlítottunk össze. A szorongást megemelt keresztpalló (EPM), üveggolyó-elrejtési (MB) és védekező-kikerülő (DW) teszttel, az anhedóniát a cukorpreferencia alapján, és a depresszióra utaló jeleket a kényszeres úszás (FS) tesztben vizsgáltuk. Az idegsejtek aktivitását c-Fos immunhisztokémia segítségével detektáltuk több agyi területen (laterális szeptum, mediális, centrális, basolaterális amygdala) FS után.

Eredmények: Míg a KO patkányok több időt töltöttek a nyílt karban az EPM során, és a nyílt térben a DW esetén, addig kevesebb időt töltöttek az üveggolyók elásásával és kevesebb lebegő magatartást mutattak a kényszeres úszás teszt során, illetve több cukros vizet fogyasztottak, mint a WT-k. A desmopressin kezelés normalizálta a KO állatok folyadékfelvételét és a testsúlyát. Ugyanakkor a kezelt állatok magatartása megegyezett a teljesen KO patkányokéval, és a legtöbb tesztben jelentősen különbözött a WT állatokétól. A c-fos elemzés a laterális szeptumban egyértelmű eredményt nem hozott, azonban az amygdala területei a KO állatokban (desmopressin kezelt és kezeletlen is) eltérő sejtaktivációt mutattak a WT csoporthoz képest.

Értékelés: Eredményeink megerősítették, hogy a vazopresszin centrális támadásponttal fokozza a szorongó-depressziós viselkedés létrejöttét. Úgy tűnik az amygdala és nem a laterális szeptum játszik szerepet a megfigyelt viselkedés kialakításában. Kísérleteink fontos adatokkal szolgálhatnak specifikus vazopresszin antagonisták terápiás alkalmazásához.

P7.6.

The neural background of spontaneous mouse-killing behavior in rats - a c-Fos study

¹László Biró, ¹Áron Tulogdi, ¹Máté Tóth, ¹Éva Mikics, ¹Beáta Barsvári, ¹Mona Stankovic, ¹József Haller

¹ Magatartás-neurobiológiai Osztály, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary

Callous-unemotional violence associated with antisocial personality disorder is often called ‘predatory’ because it involves restricted intention signaling, hypoarousal, and is gain oriented, which also characterize predatory aggression. This metaphor was recently substantiated by a laboratory study showing that brain mechanisms underlying predatory aggression are activated in social contexts in a rat model of hypoarousal-associated abnormal aggression. In this study, inferences on the mechanisms of predatory aggression were based mainly on electrophysiological studies performed in cats where affective and predatory aggression was studied in detail and comparatively. We studied here the mechanisms of mouse killing (a rat-specific form of predatory aggression) in rats i.e. the subjects of the aforementioned abnormal aggression model, because the brain mechanisms of predatory aggression are poorly known in this species. Spontaneously muricidal rats were compared with non-muricidal ones that spent the same time with mice. Mouse killing occurred suddenly without any threat signals and lasted less than 1 min. Despite its short duration, muricide induced a strong c-Fos activation in brain areas involved in cat predatory aggression, namely the central amygdala, lateral hypothalamus and ventral periaqueductal gray, suggesting that the neural control of this behavior is highly conservative. While this pattern of activations was markedly different from that of normal intra-specific aggression which involves the medial amygdala, mediobasal hypothalamus and dorsal periaqueductal gray, it overlaps with the neuronal background of abnormal aggression in the hypoarousal model. This further supports the notion that hypoarousal-associated abnormal aggression activates mechanisms of both intra-specific and predatory aggression.

P7.7.

Role of hemokinin-1 and NK1 receptors in anxiety, stress and depression-like behaviour in mice

¹Éva Borbély, ¹Zsófia Hajna, ²Liza Nabi, ¹Valéria Tékus, ³Kristóf László, ³Tamás Ollmann, ³Zoltán Karádi, ³László Lénárd, ⁴John P. Quinn, ⁵Alexandra Berger, ⁵Christopher J. Paige, ²Julie Keeble, ¹János Szolcsányi, ¹Erika Pintér, ⁶Zsuzsanna Helyes

¹ *Farmakológiai és Farmakoterápiai Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;* ² *Institute of Pharmaceutical Science, King's College London, London, United Kingdom;* ³ *Élettani Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Ka, Pécs, Hungary;* ⁴ *Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom;* ⁵ *Ontario Cancer Institute, University Health Network, Toronto, Canada;* ⁶ *Molekuláris Farmakológia Kutatócsoport, Szentágotthai János Kutatóközpont, Pécs, Hungary*

The tachykinin NK1 receptor was suggested to be involved in psychiatric disorders. Several antagonists have been tested as potential antidepressants, but despite their efficacy in animal models they failed in the clinical trials. The discovery of novel Tac4 gene-derived hemokinin-1 (HK-1) is present in several brain regions and activates NK1 receptors. Therefore, we investigated the roles of HK-1 and NK1 receptors in anxiety, stress and depression-like behaviours in gene-deleted (Tac4^{-/-}, NK1^{-/-}) mice compared to C57Bl/6 wildtypes (WT). Anxiety was evaluated in the light-dark box (LDB) and the elevated plus maze (EPM) where mice can move freely during 20 and 5 minutes, respectively. Time spent in the dark closed area of the LDB and on the closed arms of the EPM correlates with the anxiety level. Spontaneous locomotor activity in the open field test (OFT) was measured during a 5-min session. The sucrose preference test (SPT) is a model for assessing hedonic-anhedonic behaviour, where mice can choose between water and 0.8% sucrose for 2 days. In the forced swim test

(FST) the mouse was placed in a water-containing cylinder for 6 min. Immobility (floating) referring to the lack of escaping behaviour was assessed during the final 4 min. In LDB and the EPM Tac4^{-/-} mice spent significantly less time in the lit compartment and on the open arm. NK1^{-/-} animals spent significantly more time in the light compartment of the LDB, but no difference was found in the EPM. NK1^{-/-} consumed markedly more, while Tac4^{-/-} less sucrose solution compared to WTs. In the OFT Tac4^{-/-} mice spent significantly less time with moving, while the motility of NK1^{-/-} animals was remarkably greater than that of the WTs. In the FST Tac4^{-/-}, but not NK1^{-/-} mice showed remarkably longer immobility in comparison with WTs. In conclusion, we provided the first evidence for the anti-anxiety and stress-coping role of hemokinin-1. These effects are not NK1 receptor-mediated, and the involvement of a putative hemokinin receptor can be proposed. Identification of these targets might open new perspectives for anti-anxiety and anti-depressant therapy. Support: SROP-4.2.2.A-11/1/KONV-2012-0024, SROP-4.2.1.B-10/2/KONV-2010-0002, SROP-4.2.2.B-10/1/2010-0029, OTKA-NK78059, OTKA-K81984.

P7.8.

Disassociating effect of sleep disruption on memory consolidation

¹Eszter Csábi, ²Mária Schultz-Várszegi, ³Karolina Janacsek, ³Dezső Németh

¹ *Kognitív- és Neuropszichológia Tanszék, Szegedi Tudományegyetem, Bölcsészettudományi Kar, Szeged, Hungary;* ² *Somnocenter, Szeged, Szeged, Hungary;* ³ *Klinikai Pszichológia és Addiktológia Tanszék, Eötvös Lóránd Tudományegyetem, Budapest, Hungary*

The beneficial effect of sleep on memory consolidation by enhancement of neural plasticity is well established, however, previous studies have not addressed the question of how different memory processes are affected by sleep. In the present study we examined the relationship between sleep and different aspects of implicit learning by testing patients with obstructive

sleep apnea syndrome (OSA), a disorder characterized by disrupted sleep patterns. To separate the effects of general skill learning and sequence-specific learning, we used the Alternating Serial Reaction Time (ASRT) task. Our data show differences in offline changes of general skill learning between the OSA and control groups. Control group demonstrated offline improvement from evening to morning, while the OSA group did not. In contrast, we did not observe differences between the groups in offline changes in sequence-specific learning. Our findings suggest that sleep differentially affects memory processes involved in consolidation of skill learning.

P7.9.

A döntési kritérium változásának aránya az unimodális illuzórikus felvillanás kísérletekben

¹Péter Csibri, ¹Péter Kaposvári, ¹Gyula Sáy

¹ *Élettani Intézet, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary*

Az észlelés és az észlelés kontextusának viszonyáról megelőző tanulmányok nagy mennyiségű ismeretet halmoztak fel téri vonásokkal kapcsolatban. Ezek az adatok segítségünkre voltak az alak-háttér elkülönítés és a tárgyak felismerésének jobb megismerésében, amelyek jól értelmezhetőek egy funkcionális vagy ökológiai szemlélet keretein belül. Az idő szerepével, az idői kontextussal azonban eddig meglehetősen keveset foglalkoztak. Ennek a hiányosságnak a pótlására és a területek összekapcsolására a jól ismert keresztmodális double flash illúzió mintájára egy unimodális illúziót hoztak létre. Itt a célinger egyszeri felvillanását többszörös felvillanásként lehet érzékelni, amennyiben a környezetében elhelyezkedő illuzórikus hatást kiváltó inger (inducer) többször villan fel. Ez a hatás nagyon robusztusnak bizonyult. A hatás megfelelő értelmezésének érdekében azonban szükség van a valóban észlelt illuzórikus felvillanások és az inducer, mint zavaró inger hatására

jelentkező döntési kritérium eltolódásának számszerűsítésére, melyre eddig még nem került sor. Munkánk során a szignáldetekciós elméletet alkalmazva mind a döntési kritériumra, mind a percepció szintre sikerült kimutatnunk az inducer hatását.

P7.10.

The role of caffeine and adenosine receptors in a rodent model of impulsive behavior

¹Roland Csurgyók, ¹Hajnalka Horváth, ¹Péter Kardos, ¹Attila Trunk, ¹István Hernádi

¹ Kísérletes Állattani és Neurobiológiai Tanszék, Pécsi Tudományegyetem, Természettudományi Kar, Aranyosgadány, Hungary

Impulsivity is an abnormality of behavior characterized with premature motor responses as well as cognitive abnormalities (choice impulsivity, risk-taking). It is also considered as a major symptom of many psychiatric disorders (ADHD, mania, addictions), and recently became the focus of scientific interest for the impulsivity. It has been previously demonstrated that systemic administration of the adenosine receptor antagonist caffeine (due to its anxiogenic effect) increases motor impulsivity while reduces choice impulsivity in rats. As the major effects of caffeine are exerted by the antagonistic action on adenosine receptors in the central nervous system (CNS), the aim of the present experiments were to investigate the possible involvement of adenosine receptors in the anti-impulsive action of caffeine. Twenty-seven male Wistar rats were used in the experiments. The behavior of the animals was tested in skinner boxes where they had to choose between a small immediate (1 pellet) and a large delayed reward (4 pellets) in a delay discounting paradigm. A1 agonist N6-cyclohexyl-adenosine (CHA, 0.00313, 0.00625, 0.0125 mg/kg) and A2A agonist CGS21680 (CGS, 0.01, 0.03, 0.1 mg/kg), were i.p. injected alone and together with caffeine (10, ²⁵, ⁵⁰ mg/kg). Results indicate that both agonists alone and in combination with caffeine decreased motor impulsivity.

Administration of the A1 agonist CHA (but not the A2A agonist CGS) alone slightly reduced choice impulsivity. However, simultaneous administration of caffeine and CGS enhanced the beneficial effects of caffeine on choice impulsivity. Results suggest that the antagonizing effects of caffeine on A2A receptors may not be responsible for its action on choice impulsivity. Furthermore, we hypothesize, that the decreasing effects of CGS on caffeine induced motor activation can further reduce choice impulsivity. Another possible explanation may be that the CGS decreased the stimulating effects of caffeine on motor behavior, thereby decreased the rate of impulsive choice.

P7.11.

Nőstény Brattleboro patkányok szorongás- és depresszió-szerű magatartásának vizsgálata

¹Anna Fodor, ¹Krisztina B. Kovács, ¹Barbara Klausz, ¹Ottó Pintér, ²Nuria Daviu, ²Cristina Rabasa, ²David Rotllant, ¹Diána Balázsfői, ¹Péter Csikota, ²Roser Nadal, ¹Dóra Zelena

¹ Magatartásélettan és Stressz Kutatócsoport, Magyar Tudományos Akadémia Kísérleti Orvostudományi Kutatóintézete Budapest, Hungary; ² Institut de Neurociències, Universitat Autònoma de Barcelona, Barcelona, Spain

Bevezetés: A vazopresszin részt vesz a hypothalamus-hypophysis-mellékvese tengely szabályozásában, ezért felmerül a szerepe a stressz okozta pszichiátriai zavarokban, így a szorongásban és a depresszióban. A legtöbb preklinikai vizsgálatot hím egyedeken végzik (nemi ciklus hiánya), habár ezek a megbetegedések gyakoribbak nőknél. **Anyag és módszer:** Vizsgálatunkban szexuálisan naiv, vazopresszin hiányos homozigóta (di/di) és vad típusú (+/+) nőstény Brattleboro patkányok szorongás- és depresszió-szerű magatartását hasonlítottuk össze és megvizsgáltuk számos viselkedést szabályozó agyterület aktivációját (pl.: nucleus paraventricularis, prefrontális kéreg, lateralis septum, amygdala,

ventromedialis hypothalamus, nucleus arcuatus). Az állatok szorongás szintjének mérésére a következő tesztek alkalmaztak: emelt keresztpalló teszt (elevated plus maze, EPM), open field teszt (OF), üveggyó-elrejtés (marble burying, MB), szociális elkerülés, új környezetben való táplálkozás (novelty-suppressed feeding). A depresszió-szerű tünetek feltérképezésére: hedónia vizsgálatára szacharin-preferencia tesztet és kényszeres úszás tesztet (forced swim test, FST) végeztük el. A neuronok aktivációját mind nyugalomban, mind 60 perccel a 15 perces FST után c-fos marker segítségével elemeztük. **Eredmények:** Vizsgálataink során a di/di nőstények átlagban ugyanannyit mozogtak, de több időt töltöttek az EPM nyílt karjában és kevesebb golyót ástak el, hamarabb elkezdtek enni a jutalom falatot, több cukros-vizet ittak és kevesebb időt lebegtek, mint +/+ társaik. Mindez arra utalhat, hogy a vazopresszin hiánya a nőstényekben is egy kevésbé szorongó-depressziós fenotípust eredményez. Általánosságban elmondható, hogy nyugalomban a di/di állatokban magasabb c-fos aktivitási értékeket detektáltunk, de stressz (FST) hatására az aktiváció kisebb mértékű volt, mint a +/+ állatokban. **Következtetések:** Eredményeink igazolták, hogy a hímekhez hasonlóan, a nőstényekben is a vazopresszin fontosnak tekinthető a szorongás-depresszió-szerű magatartási változások létrejöttében. A folyamatban a stressz-tengely és az érzelmek kialakulásában releváns agyterületek csökkent aktivációja játszhat szerepet.

P7.12.

Effects of musical environmental enrichment in new born rats

¹Gábor Horváth, ¹Gyongyver Vadasz, ¹Julian Zink, ¹Zsófia Horváth, ¹József Farkas, ¹Péter Kiss, ¹Dóra Reglődi

¹ *Anatómiai Intézet, Pécsi Tudományegyetem Általános Orvostudományi Kar, Pécs, Hungary*

Introduction: Our research group examined several neuroprotective agents and some environmental factors in nervous system lesions previously. We

already described the positive effects of environmental enrichment in some animal models. About musical enrichment we have not found any results on the early neurobehavioral development, so we started to examine it. The aim of the present study was to investigate whether musical enriched environment has any effects on the neurobehavioral and brain development of newborn rats, and is able to attenuate retinal injury induced by neonatal monosodium glutamate (MSG) treatment. Methods: We used two types of musical enrichment: classic and heavy-metal music from ⁶PM till ⁶AM daily. We examined the animals every day to investigate their neurobehavioral development: eye opening, incisor eruption, negative geotaxis, and some reflex performances: placing, grasping, crossed extensor, sensory reflexes. Retina degeneration was induced by early postnatal subcutaneous MSG injection. Results: Our results show that musical enriched environment has no significant effects on the normal development. We found slight differences between the two music groups: the heavy-metal group showed some reflex earlier than the classic. On the other hand we found significant differences between the groups in the retina degeneration: the metal animals had more severe degeneration than the classic rats. In the histological examination of the brain we found differences in myelination and number of oligodendrocytes of the corpus callosum. The results showed the same pattern we found in the neurobehavioral development. Conclusion: These results show that musical environmental factors just slightly influence the early development of the nervous system, but in case of injuries the musical environmental influences will have significant effects in the size of the injury and the dynamism of the regeneration. We hope that our results could be bridge between the rehabilitation, clinical practice and base science in the future.

P7.13.

Behavioral changes in a new substrain developed by selective breeding

¹Gyöngyi Horváth, ¹Zita Petrovszki, ¹Gabriella Kekesi, ¹Gyorgy Benedek, ¹Szabolcs Keri

Introduction: Gene-environment interactions in psychiatric disorders can be modeled using selectively bred rats after receiving complex treatment, i.e. NMDA receptor antagonist administration and social isolation. The aim was to characterize behavioral profiles (sensory gating, pain sensitivity, memory function) in rats selectively bred through 15 generation after the complex treatment. Methods: Four experimental groups of male Wistar rats were studied (n=6-15 rats/group): naive rats without any treatment (NaNo); naive rats with postweaning social isolation and ketamine treatment (NaTr); 15th generation of selectively bred animals without any treatment (SelNo) or selectively bred rats with both isolation and ketamine treatment (SelTr). Groups were matched according to body weight and baseline tail-flick latencies obtained at the age of 3 weeks. The startle reaction, tail-flick and novel object recognition tests were used for comparison of the different groups. Results: As regards the tail-flick latencies, significant differences were observed at the age of 9 weeks between the SelTr and the other groups. The 15th generation of the animals showed a higher degree of the startle reflex amplitude elicited with or without prepulse stimuli, and disturbed prepulse inhibition was also observed. Altered motor activity and decreased differentiation index in the memory test were observed in the new substrain, along with enhanced grooming behavior. Five functional indices (TF latency, startle reaction, prepulse inhibition, differentiation index, and grooming activity) were rated between 0 and 2, and the analysis of the summarized score revealed that the NaNo group had the lowest overall indication of schizophrenic-like signs, while the SelTr animals scored the highest. Conclusion: These data suggest that both heritable and environmental factors were important in the generation of the behavioral alterations. We assume that further breeding after this complex treatment may lead to a valid and reliable animal model of schizophrenia. This work was supported by OTKA (K83810, NF72488;), TÁMOP-4.2.2/B-10/1-2010-0012 and TÁMOP-4.2.2.A-11/1KONV-2012-0052 grants.

Gender-specific differences in cognitive performance in two memory tests

¹Gabriella Kékesi, ²Zita Petrovszki, ²Gyongyi Horvath, ²Gyorgy Benedek, ²Szabolcs Keri

¹ *Élettani Intézet, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary;* ² *Department of Physiology, University of Szeged, Faculty of Medicine, Szeged, Hungary*

Introduction: There are only few data about the gender differences regarding the cognitive functions. We determined the behavioral parameters in two different cognitive tests, comparing recognition (novel object recognition/NOR) and working (hole board/HB) memory functions in rats. **Methods:** Behavioral tests were conducted on the same young adult male (n=6) and female (n=10) Wistar rat groups. The NOR task assesses the spontaneous tendency of rodents to spend more time exploring a novel object than a familiar one. During habituation, the animals were allowed to explore an empty arena for 10 min. One minute following the habituation session, the sample phase began where the animals were exposed to two identical objects (5 min). After a one hour retention interval a 5-minute test phase was conducted in the presence of a familiar and a novel object. Recognition memory was expressed as a differentiation index which is defined as the ratio of time spent exploring the novel object over the total time spent exploring both familiar and novel objects. After a habituation and learning phase, the HB test was applied to determine the learning capacity of the rats to discriminate baited from unbaited holes in the open field for a food reward during the test phase. Working memory error was defined as the repeated visit to a baited hole from which the food reward has already been collected. The motor activities of the animals were also assessed in both tests. **Results:** Regarding the NOR test, there were no significant difference between the genders, however there was a tendency (p=0.06) to a higher activity in the female group. In the learning phase of the HB test the female rats collected significantly more food reward during a shorter period compared to male rats. In case of the test phase this gender difference remained significant. The working memory error was the same in case of both genders; furthermore the learning profile was also similar.

Regarding the activity in the habituation phase only the sniffing activity in the inner part of the arena showed significant difference between the genders. Conclusion: These results suggest that the better results regarding the eaten foods might be due to the enhanced motor activity. We are planning further studies to reveal the gender differences in animals with CNS disorders. This work was supported by OTKA (K83810;NF72488), TÁMOP-4.2.2/B-10/1-2010-0012, TÁMOP-4.2.2.A-11/1KONV-2012-0052 grants.

P7.15.

Effects of intraamygdaloid microinjections of RFRP peptides in conditioned place preference test

¹Anita Kovács, ¹Kristóf László, ¹Noémi Bencze, ¹Olga Zhizhina, ¹Tamás Ollmann, ¹László Péczely, ¹László Lénárd

¹ *Institute of Physiology, Pécs University, Medical School, Pécs, Hungary*

RFRP-1 and RFRP-3 (RFRPs) are members of the RF-amide peptide family. RFRP-positive nerve cells were detected in the rat hypothalamus and RFRP immunoreactive fibers were identified in the central amygdaloid nucleus (CeA) by immunohistochemical methods. RFRP analogues bind with relatively high affinity to the NPFF1 and NPFF2 receptors. RFRPs have potent activity for NPFF-1 that is expressed in the CeA. Our previous data showed that 50 ng dose of RFRP-1, or 50 ng and 100 ng dose of RFRP-3 injected to the CeA resulted in significant decreased in food intake. Furthermore, RFRPs had no effect on locomotor activity of the animals in open-field test. The CeA, part of the limbic system, plays an important role in learning, memory, and reinforcing mechanisms. The rewarding, reinforcing effects of RFRPs in the CeA have not been investigated yet. The aim of our study was to examine in the CeA the possible effects of RFRPs on reinforcement in conditioned place preference test. Male Wistar rats were microinjected bilaterally with 50 ng or 100 ng RFRPs. Other animals received 20 ng NPFF-receptor antagonist

RF9 alone, or RF9 15 min before 50 ng RFRP-1 treatment or vehicle solution into the CeA. Control animals received only vehicle. Drugs were dissolved in sterile saline (0.15 M) and were microinjected bilaterally into the CeA in 0.4 μ l. In the conditioned place preference test 50 ng RFRP-1 significantly increased the time rats spent in the treatment quadrant. Prior treatment with antagonist blocked the effects of RFRP-1. Antagonist in itself did not induce place preference. 50 ng or 100 ng doses of RFRP-3 had no effects in place preference test. Our results show that in the rat CeA, RFRP-1 has positive reinforcing effect via NPFF-1 receptor because the RF9 antagonist could be block this action. Supported by SROP-4.2.2/B-10/1-2010-0029, SCROP-4.2.1.B-10/2/KONV-2010-002 and by the HAS.

P7.16.

Representation of cooperative intention in the human brain based on facial expression

¹Zsófia Kovács-Bálint, ²Anita Deak, ²Peter Papp, ³Gabor Perlaki, ³Gergely Orsi, ¹Istvan Hernadi, ²Tamas Bereczkei

¹ *Biológiai Intézet, Kísérletes Állattani és Neurobiológiai Tanszék, Pécsi Tudományegyetem, Természettudományi Kar, Pécs, Hungary;* ² *Institute of Psychology, University of Pécs, Pécs, Hungary;* ³ *Clinical Neuroscience MR Research Group, HAS-University of Pécs, Pécs, Hungary*

Successful social interactions partly depend on the ability to recognize others' intentions from their faces. Recent investigations about general trustworthiness revealed specific brain modules, which may serve the evolutionary adaptive cognitive mechanism to recognize untrustworthy faces. In this study, our specific aim was to investigate whether specialized brain regions are activated when participants evaluate facial images with cooperative or defective intentions. Twenty-nine healthy volunteers (16 females) participated in our event-related fMRI experiment. Standardized grayscale cooperator and defector facial images (taken in a previous

socioeconomic game) were randomly presented to the subjects for 4sec, followed by a question and a 10sec pause, in two counterbalanced sessions. In the first session, the subjects' task was to answer – with a button press – whether the presented face was a cooperator or not. The task in the second session was reversed – participants had to answer whether the presented face was a cheater or not. Neutral faces and houses (presented before both sessions) served as baseline. Data were collected on a 3T MRI scanner (Siemens Magnetom Trio). BOLD signal analysis indicated that both cheater and cooperator faces caused higher activation in the primary and secondary visual cortices (Br. 17, 18, 19) compared to neutral faces; and that the activation evoked by cooperator faces was higher than the activation evoked by cheater faces. We also found that cooperator faces were distinguished from neutral ones in both left and right fusiform gyri (Br. 37), while cheater faces were distinguished from neutral ones in the left fusiform gyrus (Br. 37). Results also suggest the involvement of the prefrontal cortex (Br. 8, 10) in processing cooperator facial expression. To sum up, present results indicate that the human visual system distinguishes socially relevant facial expressions from neutral ones at an early stage of cortical information processing, however, higher order cortical brain systems may also be involved in the evaluation of possible cooperating. Further studies will elucidate specific features of cooperator facial expression that may lead to its different registration compared to defector or neutral facial cues.

P7.17.

The role of NMDA receptor subtypes in behavioral dysfunctions in an animal model of post-traumatic stress disorder

¹Éva Mikics, ¹László Biró, ¹Boglárka Nagy, ¹Christina Miskolczi, ¹Júlia Sosztarich, ¹Beáta Barsvári, ¹Katalin Gyimesiné Pelczér, ¹Dóra Zelena, ¹József Haller

¹ *Magatartás Neurobiológiai Osztály, MTA Kísérleti Orvostudományi Kutatóintézet, Budapest, Hungary*

Post-traumatic stress disorder (PTSD) is a severe psychiatric condition that develops in a significant portion of humans undergoing traumatic life events. It is generally believed that the inadequacy of treatments is due to a deficient understanding of the pathomechanisms of PTSD. N-methyl-D-aspartate (NMDA)-mediated glutamatergic neurotransmission is strongly involved in the development of trauma-induced behavioral dysfunctions, and indirect evidence suggests that NR2B subunit-expressing NMDA receptors are primarily involved in this process. Here we investigated the effects of the general NMDA blocker MK-801 and specific NMDA NR2A, NR2B and NR2C/D subunit antagonists on the expression of conditioned fear, a frequently used model of PTSD. Furthermore, we studied the effects of MK-801 and the NR2B antagonist Ro25-6981 in the social avoidance test that mimics trauma-induced social anxiety. Rats were exposed to a single series of 3mA footshocks and were tested 28 days later in the conditioned fear test or the social avoidance test. When re-exposed to the traumatic context, rats showed a dramatic increase in freezing behaviour (conditioned fear) compared to unshocked controls. We found that the NR2A antagonist PEAQX and the NR2C/D antagonist PPDA did not affect conditioned fear responses 28 days after traumatic shock exposure. However, both MK-801 and the selective NR2B subtype antagonist Ro25-6981 significantly reduced the duration of freezing in the conditioned fear test. Moreover, in contrast to MK-801, Ro25-6981 did not induce locomotor side effects in the open-field test. In the social avoidance test, rats exposed to trauma avoided the opponent-containing sub-chamber of the testing apparatus. Surprisingly, Ro25-6981 further enhanced social anxiety of rats in the social avoidance test 28 days after trauma, suggesting that different symptoms of PTSD may be underlined by different pathomechanisms. Our results suggest that studying the role of glutamate neurotransmission in a subunit-selective manner opens novel opportunities for understanding the mechanisms underlying trauma-induced behavioral dysfunctions. The findings may contribute to the development of novel treatment strategies for PTSD.

Behavioral changes and general toxicity in rats after subchronic inorganic arsenic and fluoride treatment

¹Kitti Sárközi, ¹Zsuzsanna Máté, ¹Tünde Vezér, ¹András Papp

¹ *Népegészségtani Intézet, Szegedi Tudományegyetem Általános Orvostudományi Kar, Szeged, Hungary*

Nervous system disturbances are among the known consequences of oral exposure of humans to excess amounts of arsenic and/or fluorine. Arsenic excess can cause central and peripheral nervous abnormalities including headaches, weakness and delirium. Neurological deficits were observed also in case of high fluorine intake. Oral arsenic and fluorine exposure was modelled in rats, with open field motility and general toxicity as endpoints. Seven weeks old adult male Wistar rats (160 ± 20 g, 4 groups of 12 rats each) were treated with sodium (meta)arsenite (10 mg/kg b.w.), sodium fluoride (5 mg/kg b.w.) and their combinations, per os by gavage, daily once, 5 days a week for 6 weeks. The 4th group was untreated control. At the end of the treatment, 6 animals in each group were dissected while the other 6 were kept for 6 more weeks without any treatment. Body weight and food consumption as general toxicological parameters were measured daily. Open field motility was tested in the 2nd, 4th, 6th, 8th, 10th and 12th weeks. Arsenic, alone or together with fluorine, caused significant decrease in the time of rearing and ambulation, and increase in immobility and local activity (vs. untreated or fluorine treated) in the 4th and 6th week. By the 12th week, these changes diminished largely in the combined but much less in the arsenic treated group. The speed of ambulation, but not the mean length of one ambulation event, was also decreased by arsenic. Weekly body weight gain was significantly reduced in the arsenic and combined treatment groups from the first week on. This difference was more prominent from the 2nd week and was seen during the whole treatment period, although from the cessation of treatment body weight increased in all groups. Food consumption in the arsenic and combined group was significantly reduced during the treatment period, but from the 7th week it increased also. Chemical analysis indicated strong deposition of arsenic in the treated rats' blood, cortex and liver samples by the 6th week and its partial elimination by the 12th week, which was in fair

agreement with the observed behavioral and general effects. This study revealed significant effects on both general and behavioral endpoints by arsenic, but not by fluorine, which underlines the risk from environmental exposure. Supported by: TÁMOP-4.2.2/B-10/1-2010-0012.

P7.19.

Functional and immunocytochemical evidence for anxiolytic and antidepressant actions of somatostatin receptor subtype 4 activation in mice

¹Bálint Scheich, ²Viktória Kormos, ²Valéria Tékus, ²Zsófia Hajna, ³Balázs Gaszner, ⁴Kristóf László, ⁴László Lénárd, ²Erika Pintér, ²János Szolcsányi, ²Zsuzsanna Helyes

¹ *Farmakológiai és Farmakoterápiai Intézet, Pécsi Tudományegyetem, Általános Orvostudományi Kar, Pécs, Hungary;* ² *Department of Pharmacology and Pharmacotherapy, János Szentágothai Research Center, University of Pécs, Pécs, Hungary;* ³ *Department of Anatomy, University of Pécs, Pécs, Hungary;* ⁴ *Department of Physiology, University of Pécs, Pécs, Hungary*

Somatostatin is a cyclic neuropeptide widely distributed in the brain and playing an important role as an inhibitory neurotransmitter and neuromodulator. The peptide is a well known regulator of stress-related behaviour and its expression is altered in patients with mood disorders, but there is little known about the roles of its Gi-protein-coupled receptors (sst1-sst5). Sst2, sst3 and sst5 are responsible for the endocrine actions, and we earlier showed that somatostatin receptor subtype 4 (sst4) mediates anti-inflammatory and analgesic actions in the periphery. We aimed at investigating the role of sst4 by using gene-deleted mice (sst4^{-/-}) and a selective sst4 agonist (J-2156). Sst4^{-/-} mice showed increased anxiety in the open field (OFT) and elevated plus maze tests (EPM), as well as enhanced depression-like behaviour in the forced swim test (FST), but not in the tail suspension test (TST). J-2156 exhibited an anxiolytic effect in

the EPM but not in the OFT. It also decreased immobility in the TST but not in the FST. We also wanted to elucidate the mechanisms of the antidepressant action of J-2156 in the TST. For this purpose we examined the effect of the agonist and the tail suspension-stress on neuronal activation in several stress-related brain areas by c-Fos immunocytochemistry. J-2156 alone did not influence the c-Fos immunoreactivity but significantly increased the c-Fos response upon stress in the dorsal raphe nucleus, in the lateral septum, in the bed nucleus of the stria terminalis, in the amygdala, in the magnocellular part of the paraventricular nucleus of the hypothalamus, in the central projecting Edinger–Westphal nucleus and in the periaqueductal gray matter but not in the parvocellular part of the paraventricular nucleus of the hypothalamus. In conclusion, sst4 receptor activation exerts anxiolytic and antidepressant effects in stress situations. The antidepressant activity of J-2156 in the TST can be explained by the modulation of neuronal stress-responsiveness in several stress-related brain regions. SROP-4.2.2.A-11/1/KONV-2012-0024, SROP-4.2.1.B-10/2/KONV-2010-0002, SROP-4.2.2.B-10/1/2010-0029

P7.20.

Behavioural effects of cocaine and the ghrelin-associated peptide obestatin in mice

¹Júlia Szakács, ¹Krisztina Csabafi, ¹Nándor Lipták, ¹Gyula Szabó

¹ *Kórélettani Intézet, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary*

Ghrelin, a highly conserved, 28-amino acid peptide, was originally identified as the endogenous ligand of the GHS-R1A (growth hormone secretagogue) receptor (Kojima et al., 1999). Over the last years, ghrelin was found to play a role in multiple physiologic and pathophysiologic processes including appetite regulation, energy homeostasis, and addictive behaviours. Via the activation of the cholinergic-dopaminergic system,

central ghrelin signalling has been shown to mediate the reward from alcohol, cocaine and amphetamine (Jerlhag E. et al. 2010, Dickson S.L. et al. 2011). Obestatin is another preproghrelin-derived peptide, identified in 2005, which was shown to influence feeding, drinking, memory, sleep and anxiety behaviours. In the present study, we investigated the behavioural and locomotor changes in CFLP male mice induced by the acute administration of obestatin (0.5 µg /2 µl aCSF) and cocaine (15 mg /bwkg s.c.) in computerized open field (OF) system. Our results have shown that that co-administration of cocaine and obestatin increased the ambulation distance, the ambulation time and decreased the immobility time. Furthermore, single injection of cocaine also stimulated the locomotor activity of mice compared to control and obestatin groups. Rearing and jumping were not influenced significantly by the different treatments. The anxiety markers, namely the time spent in the center of the OF maze and the percent of center ambulation/ total ambulation distance have shown a decreasing tendency due to the action of cocaine. In conclusion, the behavioural changes induced by drugs of abuse and neuropeptides highly depend on the experimental protocol, route of administration and the laboratory species used. For instance, we earlier experienced a dose-dependent anxiogenic and anxiolytic effect of obestatin in OF and EPM (elevated plus maze) system, respectively. Yet most works have dealt with the pleiotropic effects of ghrelin, but increasing amount of evidence suggests, that obestatin may also have a role in the complexity of neurobiological mechanisms involved in natural and artificial rewards. This work was supported by TÁMOP 4.2.2-A -11/1/KONV-2012-0052 project.

P7.21.

No combined effects of caffeine and 3G mobile phone exposure on predictive coding in the human brain

¹Attila Trunk, ²Norbert Zentai, ³Gábor Stefanics, ⁴Ivett BacsKay, ⁴Attila Felinger, ⁵György Thuróczy, ²István Hernádi

¹ *Biológiai Intézet, Kísérletes Állattani és Neurobiológiai Tanszék, Pécsi Tudományegyetem, Természettudományi Kar, Pécs, Hungary;* ² *Department of Neurobiology and Experimental Zoology, University of Pécs, Hungary, Pécs, Hungary;* ³ *Translational Neuromodeling Unit (TNU), Institute for Biomedical Engineering, University of Zurich & ETH Zurich, Switzerland;* ⁴ *Department of Analytical and Environmental Chemistry, University of Pécs, Hungary, Pécs, Hungary;* ⁵ *Department of Non Ionizing Radiations, National Research Institute for Radiobiology, Budapest, Hungary*

Recent theories suggest that the human brain generates predictions about the nature and the timing of sensory inputs that are likely to happen. Predictions enable perception providing top-down constraints on sensory data thus enhancing signal detection and processing. Such mechanisms can be indexed by brain oscillations. Several neurochemical agents (e.g. caffeine, alcohol, nicotine, etc.) are known to facilitate or attenuate brain information processing. Recent studies indicate that caffeine has positive effect on attention, alertness or performance by modulating brain oscillations in the alpha (8-12 Hz) and theta (4-7 Hz) frequency bands. However, it has not been addressed so far, how environmental factors, e.g., electromagnetic fields (EMFs) may modulate known psychoactive effects of caffeine. In a double blind, placebo controlled study we investigated the possible synergism of caffeine consumption (3 mg/kg) and simultaneous UMTS 3G MP exposure on brain oscillations in a visual target detection task. Twenty-three subjects took part in experimental sessions corresponding to four conditions (double placebo, UMTS, caffeine, combined exposure) while reaction times (RT) and EEG were recorded. In each session, 800 visual stimuli were presented pseudo-randomly (80% squares and 20% dev. circles). Reaction time and 1 s pre-target brain oscillatory activity were sorted by target-target distance resulting in increasing expectancy levels (low and high) as targets were further away from each other. We hypothesized that, pre-stimulus brain oscillations reflecting predictive processing will be sensitive to target-target distance in the stimulus sequence (i.e. local probability of the target). It was found that RTs were highly dependent on the number of standard stimuli preceding the targets, significantly decreasing for high compared to low expectancy targets. Furthermore, pre-stimulus theta amplitude was higher and alpha

amplitude was lower in the high compared to low expectancy conditions. The analysis of the high expectancy alpha frequency showed that the alpha amplitude was significantly decreased in the combined treatment compared to either control or UMTS treatments, but did not differ from the caffeine alone treatment. Although preliminary results indicate no super-additive effects on the brain oscillation further analyses are needed to draw general conclusions concerning synergism of EMFs and neurochemical challenges on cognitive brain processes.

P7.22.

Face representation in the inferotemporal cortex of macaque monkey

¹Györgyi Utassy, ¹Anna Bognár, ¹Péter Kaposvári, ¹Péter Csibri, ¹Péter Gombkötő, ²Gyula Kovács, ¹Gyula Sály

¹ Élettani Intézet, Szegedi Tudományegyetem, Általános Orvostudományi Kar, Szeged, Hungary; ² Kognitív Tudományi Tanszék, Budapesti Műszaki és Gazdaságtudományi Egyetem, Természettudományi Kar, Budapest, Hungary

The identity of an animal, the face and facial expressions have exceptional importance for primates living in hierarchically organised groups. There are limited data about how face-sensitive neurones code different faces. Faces can change in physical appearance while their identity remains unchanged, and two faces can be physically very similar despite belonging to two different individuals. Currently, two popular models of face representation prevail: faces can be coded as belonging to an identity category or as a function of distance and direction from an appropriate mean face in a multidimensional face space based on their physical features. It seems that the human fusiform gyrus - a possible homologue to the monkey inferotemporal cortex (IT) - can be stimulated by the identity of faces and not by the physical features of faces. The cellular mechanism of this representation, however is not known. We used the adaptation paradigm to investigate which particular features a neurone responds to.

Faces were transformed into another in small steps (morphs) having the two original faces at both ends of this continuum. From this stimulus set face-pairs were selected clearly belonging to the same individual but having different physical features, and vice versa: two different individuals having physically similar faces. Neuronal responses given to the second stimulus in a stimulus pair and the degree of adaptation were analysed to see whether the cell adapted due to the similarity in physical features or in identity. Our goal is to further investigate whether the monkey IT responds to faces as the human fusiform gyrus does, i.e., whether it codes the identity or the physical parameters of faces.

P7.23.

Effect of vasopressin on the development of post-traumatic stress disorder in Brattleboro rats

¹János Varga, ¹Barbara Klausz, ¹Dóra Zelena

¹ *Magatartásélettan és Stressz Kutatócsoport, Magyar Tudományos Akadémia, Kísérleti Orvostudományi Kutatóintézet (MTA-KOKI), Budapest, Hungary*

The traumatic experience of wars, disasters and other horrors of life often leads to long lasting psychosomatic disturbances, and the modern medical treatments can only barely reverse the symptoms. Not only the treatment, but the modelling of post-traumatic stress disorder (PTSD) is also a challenge. According to previous observations vasopressin (AVP) may participate in the development of fear response during footshock-induced conditioned avoidance test, which is one of the most accepted PTSD model nowadays. However, there are many components of the fear conditioning and AVP could be involved in many such circuitry. To answer the question, which variable contributes mostly to the AVP-regulated fear response we used the AVP-deficient (AVP-) Brattleboro rat as a model organism and compared them to controls of the same strain (AVP+) in behavioral tests (conditioned avoidance test, ultrasound vocalisation

(USV), hot plate, object discrimination test, elevated plus maze) and measured fear-related stress-axis activity (adrenocorticotropin and corticosterone by radioimmunoassay). The previous shock enhanced the freezing response during the conditioned avoidance test in both groups, but the AVP- rats showed smaller elevation. This behavioral alteration was accompanied by reduced stress-hormone levels. There was a tendency in AVP- rats to start to emit USV later, after more shock exposure, and the duration of USV was significantly reduced. In contrast, the two genotypes did not show any difference in nociception during the hot plate test. The discrimination ability of AVP- animals was worse in object discrimination test, but they spent more time with the investigation of the objects. During the elevated plus maze test the AVP- rats entered less into the closed arms, and spent more time in the open arms. Our results confirmed the role of AVP in the development of PTSD-like symptoms, which went parallel with stress axis activation. We could close out the misleading effect of enhanced locomotion, and nociception does not seem to be important contributor, too. On the contrary, the judgment of the situation, reflected by USV, as well as memory disturbances might be important elements. We suppose that the more active coping of AVP-deficient animals is the most important element of the reduced fear response of this rat strain.

P7.24.

Interactions between 2-arachidonoyl-glycerol signaling and hypothalamo-hypophysis-adrenal-axis activity in the regulation of behavioral responses to environmental aversiveness

¹Zoltán Varga, ¹Zoltán Balogh, ¹Manó Aliczki, ¹Dóra Zelena, ¹Éva Mikics, ¹Ottó Pintér, ¹Nikoletta Venczkóné Bakos, ¹János Varga, ¹József Haller

¹ *Magatartásneurobiológiai Osztály, Kísérleti Orvostudományi Kutatóintézet, Magyar Tudományos Akadémia, Budapest, Hungary*

Previous studies showed that the behavioral effects of enhanced 2-arachidonoyl-glycerol (2-AG) signaling induced by monoacylglycerol-lipase (MAGL) blockade highly depend on the environmental context. Furthermore, it is intriguing that biochemical effects of the newly developed MAGL inhibitor, JZL184, were reported to develop rapidly while the behavioral effects of the compound were only showed much later. In the present study, we aimed to investigate the context-dependency and temporal dynamics of the behavioral effects of MAGL blockade. We found, that mice receiving MAGL inhibitor treatment showed hypermotility and anxiolysis in unfamiliar environment (the open-field (OF) and the elevated plus-maze (EPM) tests, respectively), however, these changes were only developed at least 80 min after treatment. In contrast, using in vivo biotelemetry, we showed that MAGL treatment rapidly and transiently dampened injection induced increases in body temperature and changes in locomotion patterns in familiar environment (home cage). Since environmental stressfulness affected MAGL blockade-induced behavioral changes, we investigated the possible role of the hypothalamus-hypophysis-adrenal-axis (HPA-axis) in these changes. We showed for the first time, as the best of our knowledge, that MAGL blockade rapidly increased basal corticosterone (CORT) levels, while leaving stress-induced levels unaltered. We also found that MAGL blockade-induced increases in basal HPA-axis activity decreased anxiety, as CORT-synthesis inhibitor Metyrapone abolished anxiolytic actions of MAGL blockade in the mouse EPM. We also showed that locomotor activity-increasing effects of MAGL blockade were independent of corticosterone synthesis. Based on our findings, one can assume that 2-AG signaling represents a highly sensitive, fine tuning mechanism in the central nervous system, controlling how environmental changes affect behavioral responses.

P7.25.

Attentional selection during reading

^{1,2}Béla Weiss, ¹István Kóbor, ³Petra Hermann, ³Balázs Knakker, ^{1,2}Zoltán Vidnyánszky

¹Magnetic Resonance Research Center, Szentágotthai J. Knowledge Center – Semmelweis University, Budapest, Hungary ²Department of Cognitive Science, Budapest University of Technology and Economics, Budapest, Hungary; ³Faculty of Information Technology, Pázmány Péter Catholic University, Budapest, Hungary

Attentional selection of the relevant and suppression of the irrelevant part of the text is a critical component of natural reading. In spite of this, very little is known about the neural mechanisms involved in attentional selection processes during reading. Recent studies have shown that alpha-band oscillations are involved in attentional inhibition of cortical regions representing irrelevant and distracting visual information. In accordance with these results we predicted that in horizontal left-to-right reading condition the cortical processing of the part of the text that has already been read will be suppressed and this will be reflected in increased alpha oscillations in the right hemisphere. To address this question, we performed simultaneous recording of electroencephalogram (EEG) and eye-tracking (ET) data during reading of horizontally and vertically presented Hungarian texts. Ongoing alpha oscillations were assessed by calculating the EEG power spectrum in the 8 - 12 Hz frequency band while event-related potentials were estimated by averaging the EEG recordings triggered to the end of saccades (fixation related potentials - FRPs). Saccades were obtained using adaptive analysis of ET data. Beside the detection of component peaks, FRPs were also characterized in the time-frequency domain by applying wavelet transformation. We found significantly increased right hemisphere lateralized ongoing alpha oscillations over the occipito-parietal cortex during horizontal left-to-right reading as compared to vertical reading. This result could not be explained based on the differences in FRP alpha oscillations between horizontal and vertical reading. We propose that this increased alpha oscillation might reflect attentional suppression of the distracting part of the text represented in the right hemisphere. Accordingly, measurement of alpha oscillations might allow to estimate the efficacy of attentional distractor suppression during reading both in normal and dyslexics readers.

P7.26.

Effects of QRFP-26 administration into the central amygdala on food intake in rats

¹Olga Zhizhina, ¹Anita Kovács, ¹Kristóf László, ¹László Lénárd

¹ *Institute of Physiology, Pécs University, Medical School, Pécs, Hungary*

RFamide-related peptides are known to be involved in appetite and body weight regulation in rodents. The recently identified 26-amino acid residue of the QRF neuropeptide (QRFP-26), member of the RFamide peptide family, acts as an endogenous ligand of the orphan G protein-coupled receptor GPR103 and possibly interacts with NPFF2 receptors as well. Available data suggest that QRFP-26, administered i.c.v., has a tendency to increase food consumption, especially intake of the food with high fat content. Some of the RFamide peptides were shown to perform their action on appetite through the amygdaloid body, widely known as an important feeding center. QRFP-26 binding sites were identified in the amygdala using immunohistochemical methods, so we hypothesized that direct intraamygdaloid application of QRFP-26 would influence feeding behavior. Male Wistar rats received bilateral microinjections of vehicle or different doses (50, 100, 200 ng /side, dissolved in 0.15 M sterile NaCl/0.4 µl) of QRFP-26 into the central nucleus of amygdala. After injection milk with either low (1,25 %) or high fat (5%) content was introduced to the animals. Liquid food intake in both groups was measured over 60 minutes period. Consumption of food with low fat content was not affected by the peptide. At the same time, animals injected with QRFP-26 in medium dose (100 ng) have shown significant decrease in high-fat milk intake. These findings suggest dose-dependent effect of QRFP-26 and support the hypothesis that QRFP-26 plays a specific role in regulation of fat consumption. Supported by SROP-4.2.2/B-10/1-2010-0029, SCROP-4.2.1.B-10/2/KONV-2010-002 and by the HAS.

P7.27.

Embryonic and post-embryonic effects of synthetic cathinones as 'designer drugs' on early neurogenesis and learning

¹Csilla Karina Zsedényi, ¹Gergely Zachar, ¹András Csillag, ¹Ágota Ádám

¹ *Anatómiai, Szövet-és Fejlődéstani Intézet, Semmelweis Egyetem, Budapest, Hungary*

The term 'designer drug' refer to a group of synthetic substances that are pharmacologically and structurally very similar to an illegal substance. Due to the easy accessibility via the Internet, they spread rapidly among teenage people and young adults. Although widely abused, there is a conspicuous lack of epidemiological, animal and clinical data concerning designer drugs. Here we present data on the effect of the synthetic cathinone derivatives mephedrone and butylone on neurogenesis, behavior and early adaptive learning of domestic chicks. The key questions in our experiment were as follows: 1. What are the effects of these drugs on the developing embryo, particularly on neurogenesis? 2. What are the effects of synthetic cathinones on behavior? 3. Do the exposure to these drugs affect the learning ability in tasks focused on striatal brain regions? To investigate the effect of prenatal exposure of mephedrone on neurogenesis we injected mephedrone (2 mg/kg egg weight) into incubated chicken eggs at critical stages (day 6 or 10) of striatal development. This was followed by quantitative analysis of post-embryonic proliferating neurons in the subventricular zone of the striatum, based on incorporation of the labeled nucleoside bromo-deoxy-uridine (BrdU), visualized by immunohistochemistry, and quantified by stereological method. A decrease in the density of BrdU-labeled cells in the subventricular zone of ventrobasal telencephalon was observed primarily in those birds injected in ovo in the later phase of striatal neurogenesis. To investigate the effect of mephedrone and butylone on resistance to stress, we implied social isolation and counted the number of distress vocalizations. The birds injected with mephedrone (10 mg/kg b.w. i.p.) elicited an increase in the number of distress calls in the first 5 min of the test, as compared to the control group. In the case of butylone (10 mg/kg b.w.) no significant difference was observed. Butylone (10 mg/kg b.w. i.p.) caused coordination disorders with loss of postural control and was accompanied

by strong hyperventillation. Applying the same dose of mephedrone these effects were not observed. To investigate the effect of mephedrone on learning ability, one-day old chicks injected with mephedrone (10 mg/kg b.w. i.p.) 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.

P7.28.

Investigation of possible effects of 60 minute Wi-Fi electromagnetic field exposure on human cognitive performance in a psychomotor vigilance task

¹N Zentai, ²Á Csathó, ¹A Trunk, ¹I Hernádi

¹ Department of Experimental Neurobiology, Faculty of Sciences, University of Pécs, Hungary; ² Department of Behavioral Sciences, Faculty of Medicine, University of Pécs, Hungary

The aim of the present study was to investigate potential effects of Wi-Fi electromagnetic field (EMF) exposure on human cognitive performance indicated by simple reaction time (SRT) during the performance in a computerized version of the Psychomotor Vigilance Task (PVT). In a double-blind crossover design, 7 healthy right-handed young volunteers participated in two experimental sessions with one week interval between the two sessions. At the beginning of the first session, the Barratt Impulsiveness Scale (BIS-11) was filled in to assess the subjects' general attentional, motor and non-planning impulsivity to exclude possible behavioral confounding effects. Each session contained four 20 min experimental blocks (Exposure1/Exposure2/Exposure3/Post-Exposure). In each block, the subjects' task was to perform in a 15 min PVT, while SRT were recorded. After each block, participants rated on Visual Analogue Scale (VAS) their 1) self-reported tiredness, 2) motivation and 3) capacity to focus their attention on the task. During the first three blocks (Exposure 1 to 3), the participants were exposed to genuine Wi-Fi EMF or to sham

irradiation (no exposure) for 60 min. The fourth experimental block (Post-Exposure) was set up at 20 min after exposure to follow possible later arising exposure effect. Simple reaction time data were analyzed by repeated measures of ANOVA across all conditions. Preliminary results do not support clear effects of genuine Wi-Fi exposure on PVT performance measures, however, further data are needed to provide conclusive evidence on possible acute effects of Wi-Fi EMF exposure on human cognitive performance and on subjective or objective indicators of fatigue.

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