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ABSTRACTS



P1/47

Distribution of somatostatin- and neuropeptide Y-immunoreactive neurons in control and epileptic human cortex

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Recently, we have shown that the number of neurons in the cortical white matter is larger in patients with temporal lobe epilepsy than in control subjects. Characterization of these neurons revealed that both excitatory cells and inhibitory neurons expressing calcium binding proteins exist among them.

Since neuropeptide Y (NPY) and somatostatin (SOM) colocalize with GABA, in the present work we map the distribution of NPY- and SOM-immunoreactive neurons in the temporal archi- and neocortex of patients with epilepsy. SOM and NPY-positive neurons were studied in adult control and epileptic hippocampi and temporal cortex, as well as the appearance of those neurons in the temporal, parietal and frontal lobes from an early fetal age (17th week of gestation).

In harmony with previous data, we have found that the number of SOM- and NPY-positive neurons were decreased in the hilus of the dentate gyrus in mesial temporal sclerosis but not in other forms of epilepsies. In contrast, the number of SOM- and NPY-positive neurons were increased in the white matter of the temporal archi- and neocortex compared to controls. It has to be noted that NPY-positive axons abundantly arborized in all cortical layers even in the molecular layer, although NPY-positive somata were mainly visible in the subplate. From the 17th gestational week on, NPY-positive neurons were almost exclusively found in the white matter below layer VI (subplate), whereas SOM-positive neurons were found in smaller numbers in the subplate and appeared in relatively large numbers in the cortical layers II-VI. Ammons's horn and the hilus of dentate gyrus also displayed both SOM- and NPY-immunoreactive neurons beginning at the 17th week. In all of the examined areas NPY-positive neurons were more frequent at 17th week than the SOM-positive neurons.

Our results suggest that early generated NPY and SOM-immunoreactive neurons may play a key role in cortical organization through the subplate, similarly as the calretinin-positive Cajal-Retzius cells do in the molecular layer. Our results indicate that these white matter neurons remnants of the subplate of epileptic cortex that may result in alterations of cortical neuronal circuitry.

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Symp. I. - Thursday

Neuroprotective mechanisms of estradiol: from molecules to behaviour

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It is well-known that estradiol protects cholinergic neurons against neurotoxic insults but the molecular mechanism of this action remains unclear. We identified non-classical estrogen actions on receptors/downstream signalling molecules and explored the role of this non-classical estrogen pathway in estrogen-induced neuroprotection on basal forebrain cholinergic neurons.

In the first series of experiments, we measured the diffusion parameters of TrkA receptor and calculated the incidence of downstream signaling on live adult cholinergic neurons using single molecule detection. We have shown that 17 β -estradiol (E2) rapidly increases TrkA molecule activity enhancing the incidence of downstream signaling processes. We also demonstrated that E2 induces a rapid phosphorylation of downstream signaling molecules such as ERK1/2 and CREB in basal forebrain cholinergic neurons and it was abolished in neuron-specific estrogen receptor α knock-out mice (ER α KO) *in vivo*. Selective blockade of the MAPK pathway completely prevented estrogen-induced CREB phosphorylation in cholinergic neurons.

In the second series of investigation β amyloid (β A₁₋₄₂) was injected to elicit cholinergic cell death in basal forebrain and consequent cholinergic fiber loss in the parietal cortex. Selective activation of non-classical estrogen signaling *in vivo* by estren induced E2-like protective actions after β A₁₋₄₂ lesion on cholinergic fiber density in the cortex and it induced rapid phosphorylation of ERK1/2 and CREB in cholinergic neurons. The cholinergic fiber protection was absent in ER α KO mice. Estren restored the β A₁₋₄₂-induced deficits of learning behaviours.

These findings demonstrate that E2 induces rapid ER α dependent non-classical action on CREB phosphorylation via ERK1/2 signaling pathways activation in cholinergic neurons. These observations suggest the critical role of non-classical estrogen signaling via ER α and ERK1/2 pathways in estrogen-induced neuroprotection in the cholinergic system.

P2/2

Techniques to create visible electrophysiological recording pipettes under 2-photon microscopic illumination

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Technical advances in measuring and manipulating activity in single neurons or neuronal processes raised an increasing need to create glass pipettes that are clearly visible under 2-photon microscopic illumination to facilitate targeted approach of identified cells and subcellular compartments. In particular, a brightly fluorescent pipette that negates the need for ejecting dye to patch fluorescently labeled neurons even in deep tissue would be a great advantage for further exploration of *in vitro* and *in vivo* cellular physiology. Labeling the borosilicate glass pipette with a fluorescent material which is stable, not soluble in living tissue, nor compromises the buffer in the pipette, does not disturb seal formation between pipette tip and cell membrane, and does not cause any type of disturbing thermo effects from the laser illumination has cumulatively made this challenge exceedingly complex. To provide a solution to this problem, we used luminescent semiconductor nanocrystals (quantum dots) that are surface functionalized with an extremely hydrophobic ligand, to fluorescently label recording pipettes. Quantum dot labeling is highly stable and does not interfere with the buffer within the pipette tip. Labeling can include just tip or an extended portion of the pipette and include the inside or outside area of the pipette or probe as desired. We visualized the probes under 2-photon microscopic illumination while performing simultaneous electrophysiological recordings (*in vitro* and *in vivo*) and other measurements such as calcium monitoring, without compromising electrophysiological recording quality and stability. We also used coated pipettes for single cell electroporation *in vivo* with high success rates. The superb visibility of the pipette made it possible to reach and record from cells in deep (~800 μ m) cortical tissue, as well as to perform targeted recordings from thin dendrites of fluorescently labeled cells, guided only by fluorescence. The new technique may be exploited in brain research as well as in other disciplines that call for fluorescent visualization of testing tools.

P1/10**A first step towards studying patient-specific neurons: neural progenitor cells generated from human pluripotent stem cells**

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Human pluripotent stem cells (hPSC) have the potential to generate all tissue types of the human body, thus, hPSCs can provide an unlimited cell source for developmental studies, regenerative therapies, and drug screening. Human pluripotent stem cell-derived model systems overcome several limitations of currently used cellular model systems, including those of cancerous cell lines (tumorous character), animal models (species differences), and ex vivo tissue samples (limited availability and genetic variations of the donors). These limitations are especially apparent for studying human neural cell types, therefore we started to establish methods for the generation of various types of human neural model cells from hPSCs. Differentiation of hPSCs into specific neural cell types can be divided into well-defined stages adding up a several week long procedure. The first step of the differentiation processes is the generation of neural progenitor cell (NPC) populations. We adapted and optimized the previously published differentiation protocols and established NPC cultures for the generation of mixed neural cell types, as well as PROX1 positive hippocampal neurons from hPSCs. The NPC cultures were characterized by morphology and immunostaining of specific marker proteins, including SOX2 and Nestin. For studying calcium signals in these cells, NPCs expressing a Ca-indicator protein (GCaMP3) have also been generated by using a transposon-based gene delivery system. Based on these technologies and using patient-derived iPSCs we plan to generate efficient cellular model systems for studying the cellular basis of various neurological and psychiatric diseases.

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P2/20**Ultrastructural characterization of postcentral somatosensory cortical synapses in primates: a 3D analyses**

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Thalamic feed forward (FF) inputs to the cerebral cortex form large terminals are characterized by multiple synaptic specializations and numerous mitochondria. We hypothesized that FF corticocortical afferents are highly reliable and therefore possess similar synaptic structure to that of thalamocortical ones. In contrast, cortical terminals usually form small boutons, which are associated with feedback (FB), modulatory functions and have less complex structure than large thalamocortical endings. The structural correlates of modulatory FB and FF connections in cortex are not completely clear. Somatosensory cortical areas 3b and 1 are in close anatomical and functional relationships, with area 1 representing a higher processing stage than primary somatosensory cortical area 3b. We studied the FF and FB characteristics in the connectivity of the two areas by neuronal tract tracing both at the light and electron microscopic levels following BDA injections of area 3b and 1 of the squirrel monkey by: i) comparing laminar and horizontal distribution patterns of labeled terminals of area 3b and 1 and ii) quantitative 3D ultrastructural characterization of synapses formed by labeled terminals using serial section electron microscopy. Our light microscopic observations identified small (<1 µm) and large (>1 µm) terminal like structures. Notably, large bouton like structures appeared in both areas either as intrinsic or interareal antereograde labeling. Also, both types of axonal enlargements were found in the supra- and infragranular layers of areas 3b and 1. Small and large terminals were compared using different ultrastructural features. The average volume and surface area of the large terminals were 10-17 times larger than that of small boutons confirming the light microscopic observations. 3D reconstruction of large boutons revealed a complex morphology characterized by intrusions and protrusions. There was no significant difference between large and small boutons in regard to the number and size of

postsynaptic densities and that of mitochondria or their relative measures on other quantitative features. Comparisons of interareal terminals of areas 3b and 1 are in progress. The available data suggest that corticocortical terminals of areas 3b and 1 exhibit ultrastructural characteristics that are distinct from thalamocortical ones.

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Symp. IV. - Friday

Juxtacellular recording and labeling of cortical neurons in freely moving rodents during natural sleep.

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Natural sleep in rodents has two main stages, slow wave sleep (SWS) characterized by delta waves and spindles and REM sleep with desynchronized state or theta oscillations. Previous experiments determined the temporal structure of firing of putative pyramidal cells/fast spiking interneurons during various sleep stages, but firing behaviour of identified neurons in spontaneously sleeping, freely moving animals is missing. We developed a method not requiring anaesthesia prior to recording sessions and performed juxtacellular recording and labelling of dorsal cortical neurons in freely moving rats during natural sleep. Anatomically identified supragranular pyramidal neurons fired sporadically at low rates during SWS. The activity of interneurons was more regular and locked to different phases of SWS oscillations. The firing of fast spiking (FS) interneurons was heterogeneous. The first group showed a strong burst of activity at the beginning of spindles preceded by delta waves with K-complex and then switched to tonic firing at a relatively lower rate without significant phase preference to spindle waves. The second group of FS cells fired during the descending phase of spindle waves and reached maximum firing rates before the trough regardless of the presence of a K-complex. The third group expressed strong activity near the spindle through. Some FS cells could not be classified into these three sub-groups. In general, supragranular FS interneurons showed no phase locking preference to theta cycles during REM sleep. Anatomical analysis of FS cells confirmed the presence of parvalbumin in all three groups. Reconstructions of examples from each group showed perisomatic baskets formed by axon terminals with heterogeneity in the overall distribution of axonal clouds. Our results reveal functional heterogeneity in parvalbumin expressing basket cells and identify delta wave/K-complex associated and unassociated groups suggesting preferential involvement in corticothalamic/spindle inducing and corticocortical/spindle maintaining interactions, respectively.

P1/53

AGE-RELATED SHIFTS IN THE ACUTE ANOREXIC EFFECTS OF LEPTIN: THE INFLUENCE OF BODY COMPOSITION

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Introduction: During aging two major trends are observed in the regulation of body weight and body composition: middle-aged obesity is followed at old age by aging anorexia. As similar alterations also appear in mammals, complex regulatory changes may be assumed in the background. Our previous studies revealed well-defined age-related shifts in the responsiveness to catabolic peptide mediators of energy balance (such as the central melanocortin or peripheral cholecystokinin /CCK/ systems) that may contribute to the explanation of both age-related trends. It was also shown that body composition also exerts significant influence on the function of regulatory peptides. Leptin is a catabolic adiposity signal, produced in white adipose tissue acting mainly in the hypothalamus by altering the expression of neuropeptides. Both aging and obesity have been shown to cause leptin resistance. In the present study we aimed to analyze how acute central catabolic leptin effects are influenced by aging and nutritional states.

Methods: Normally fed (NF) male Wistar rats aged 3-, 6- or 12-, 18 and 24 months (young adult, early or late middle-aged, aging and old, respectively) were injected intracerebroventricularly (ICV, 1 microg to test anorexic effects on re-feeding after 48-h fasting) with leptin. A high-fat diet-induced obese (60% fat calories, HF) 12 months old group was also established (HF12). Food intake was recorded in an automated FeedScale system (Columbus).

Results: ICV administered leptin suppressed re-feeding in young adult, aging and old, but not in early or late middle-aged animals. Although all middle-aged rats (NF6, NF12) showed leptin resistance, the 12-months old obese (HF12) group presented significant leptin-anorexia.

Conclusions: Thus, age-related changes in responsiveness to leptin resemble those of peripheral CCK. In both cases, a surprising enhancement of responsiveness of old rats to anorexigenic mediators appear. These alterations may not only contribute to the obesity of the middle-aged animals but also to the anorexia of old ones. Leptin-responsiveness is also influenced by body composition: obesity does not only accelerate the development of leptin-resistance but also the appearance of enhanced central anorexigenic leptin responsiveness of old age. (34039/KA-OTKA/13-02, 13-15)

P2/82

Coordinated thermoregulatory effects of nesfatin in Wistar rats

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The contribution of nesfatin, a recently discovered anorexigenic peptide with hyperthermic effects, to the regulation of energy metabolism has been suggested. Important regulators of body temperature show coordinated actions, e.g. fever-like hyperthermia develops due to a simultaneous rise in heat production and suppression of heat loss. Our aim was to test the potentially coordinated nature of the thermoregulatory effects of nesfatin in rats. Brown adipose tissue (BAT) thermogenesis, skin vasoconstriction as well as elevated metabolism may contribute to hyperthermia. In the first experiment, BAT denervated and control adult male Wistar rats received nesfatin (25 pmol) intracerebroventricularly at room temperature (21 °C). Nesfatin elevated core temperature (T_c), even in denervated rats. In order to clarify the mechanisms behind the effects of nesfatin, next the peptide was applied at cool (15 °C) or thermoneutral (27 °C) ambient temperatures (T_a). Oxygen consumption (VO₂), heat loss (tail skin temperature, T_s) and T_c of habituated, semi-restrained conscious rats (in cylindrical cages) were recorded in an indirect calorimeter (Oxymax, Columbus) complemented by thermocouples linked to a Benchtop thermometer (Cole-Parmer). Upon nesfatin injection (in both T_a-s) an immediate rise in T_c was observed approaching 0.5 °C by 90 min and persisting for at least 180 min. VO₂ increased significantly at both T_a-s, but this was transient at 15 °C. An unexpected reduction in heat loss was observed at thermoneutrality instead of a compensatory rise in T_s despite the significant hyperthermia enhancing the effect of elevated VO₂ on T_c. At 15 °C constant skin vasoconstriction occurred, and T_s did not change upon nesfatin injection. The discrepancy between the long lasting T_c elevation and the transient VO₂ increase at 15 °C raised the possibility of contribution of BAT thermogenesis in cold environment. Therefore, finally we used cold-acclimated (4 °C, CA) rats that received a similar dose of nesfatin at their thermoneutral (25 °C) T_a under similar conditions. In these tests interscapular BAT temperature was also recorded. Nesfatin-induced heat production and hyperthermia was more pronounced in CA rats – the rise in TBAT exceeded the T_c rise, suggesting enhanced thermogenesis in BAT. Nesfatin appears to elicit a coordinated rise in T_c also associated with BAT thermogenesis only in a cold environment. (34039/KA-OTKA/13-02, 13-15)

P2/86

Changes of efficacy of the centrally applied alpha-melanocyte-stimulating hormone (alpha-MSH) or neuropeptide Y (NPY) and peripherally administered cholecystokinin (CCK) in male and female rats of various ages

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Introduction: The obesity of the middle-aged and the anorexia of aged individuals leading to frailty and sarcopenia have severe health-related consequences. The results of our previous studies confirmed that decreased anorexigenic effects of peptides in middle-aged animals followed by enhanced anorexigenic and poor orexigenic efficacy of peptides in old rats may play a basic role in the background of these processes in male Wistar rats.

Aim: Obesity and sarcopenia appear to develop differently in men and women, therefore we intended to compare the efficacy of intracerebroventricularly administered alpha-MSH or NPY or peripherally injected CCK on energy metabolism in female rats.

Methods: First, the extent of alpha-MSH (5 µg) responsiveness was tested and compared on each day of the estrus cycle in 3 months old female Wistar rats. The food intake was measured in a Feedscale system. Thereafter the effects of NPY (5 µg) was compared on each and every day of the estrus cycle. The stage of the estrus cycle was determined by May-Grünwald Giemsa staining of the vaginal smear. After that, age-dependence of neuropeptide sensitivities were measured in different age groups (2-, 4-, 6-, 12-, 18-, 24-month-old). The anorexigenic effect of alpha-MSH or CCK injection (5 µg) was measured on re-feeding after 48-h food deprivation. The efficacy of NPY was evaluated on daytime food intake.

Results: The spontaneous nighttime food intake of female animals depended on the stage of the estrus cycle. Alpha-MSH and NPY significantly altered food intake on various days of the estrus cycle, but we did not find significant estrus cycle-dependent differences in the responsiveness to these neuropeptides in 3 months old female rats. The age-dependent changes of the responsiveness to these peptides were similar in female and male rats.

Conclusions: Our data suggest that age-related changes in the efficacy of peptides are similar in female and male rats, therefore the role of sex hormones rather than that of the tested neuropeptides seems to be more important in the background of age- and gender-related changes in body weight and body composition. (34039/KA-OTKA/13-25)

P1/108

Assessment of working memory performance of aged rats as a potential natural model of cognitive impairment

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One of the most challenging fields of drug-development is to enhance cognition and slow down neurodegeneration in various forms of dementia. For this purpose, novel animal models of memory impairment with better predictive validity are needed for the preclinical testing of drug-candidate compounds. Especially interesting are those models in which the memory deficit derives from natural causes. Age is the most evident risk factor of dementia and cognitive impairment, therefore, it has long been suggested that aged rats may be useful models of memory deficit. Our aim was to compare behavioral characteristics and cognitive performance of young adult and aged Long-Evans rats to decide whether aged Long-Evans rats could be used as a memory deficit model.

Locomotor activity was measured in an ordinary open field box, and elevated plus maze was used to assess anxiety-like behavior of the rats. Depression-like tendencies were studied in the forced swim test. Spatial working memory of the rats was tested in the T-maze spontaneous alternation task.

In the open field test, aged rats showed decreased locomotion compared to young adult rats and, in addition, aged rats also entered the inner circle less frequently. In the elevated plus maze, no significant differences were found between the two age-groups in the time spent in the open arms, therefore no increased anxiety was observed in the aged rats. In the forced swim test, aged rats showed increased depression-like behavior as they spent significantly less time swimming and were longer immobile compared to young adult rats. As no differences were found in the alternation performance between the young adult and aged rat groups, aged rats were not considered as deficient in spatial working memory.

To sum up, our results indicate that definite behavioral changes can be characterized in aged rats compared to young adult rats, e.g., in the locomotor activity and in the depression-like behavior. On the other hand, no decline of memory was found in the spontaneous alternation task which suggests that the aged rats do not naturally serve as models of spatial working memory impairment. One possible explanation of these results may be that in rats – similarly to humans – aging is not a uniform process and may diversely affect cognitive functions in each individual. However, aging related changes of other memory and cognitive domains such as long-term memory, object recognition or attention should be assessed in the future.

P2/70

Neurotensin: revealing a novel neuromodulator circuit in the nucleus accumbens - parabrachial nucleus projection of the domestic chick

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Lower brainstem projections from nucleus accumbens (Ac) subregions to the parabrachial complex (PB), the nucleus of the solitary tract and the vagal motor nuclei have been described previously in the domestic chick by our group. Such projections, particularly those from the core and rostral pole regions of Ac have not been found in mammals or pigeons. Here we report on the presence of neurotensin (NT) in the neurons projecting from different Ac subnuclei, and also from the bed nucleus of stria terminalis (BSTl), to the PB in the domestic chicken. The study is based upon correlated retrograde tracing (using Fast Blue) and NT immunohistochemistry, supplemented with regional charting and quantitative analysis of double-labeled neurons. The density of retrogradely labeled cells in Ac subdivisions reflects the size of FB tracer deposit, and the degree to which it extends to the medial PB. Of all Ac subregions, the core contained the largest amount of double-labeled cells. The findings demonstrate that the anatomical pathway through which the Ac can directly modulate taste responsive neurons of the PB employs neurotensin as a neuromodulator. The observed anatomical difference between mammals and birds is either a general taxonomic feature or it reflects feeding strategies specific for the domestic chick. The results are also relevant to a better understanding of the role of NT in food intake and reward-related behaviors in birds.

P1/77

Retrograde Endocannabinoid Signaling is Involved in the Mechanism whereby Low Concentration of 17 β -Estradiol Decreases Frequency of Postsynaptic Currents in GnRH Neurons of Metestrous Mice

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Gonadotropin-releasing hormone (GnRH) neurons regulate reproduction centrally. In vivo, 17 β -estradiol (E2) controls GnRH release in concentration and estrus cycle dependent manner. In vitro, patch-clamp electrophysiological data on GnRH neurons of ovariectomized female mice demonstrated that low concentration (10 pM) of E2 decreased spontaneous firing rate which was eliminated by blocking fast synaptic neurotransmission (Chu et al., J. Neurosci., 29:5616, 2009). In the present study, we examined the effect of low concentration of E2 on postsynaptic currents (PSCs) in GnRH neurons of acute brain slices obtained from metestrous female mice and analyzed the putative involvement of endocannabinoid signaling in the evoked effect of E2. Whole-cell patch clamp recordings revealed that 10 pM E2 significantly diminished frequency of the PSCs (49 $\pm$ 7.6% of the control) in 9 from 18 GnRH neurons, which could be abolished by the application of the ER α / β blocker Faslodex (77 $\pm$ 5.8%). Pretreatment of the brain slices with the cannabinoid receptor type-1 (CB1) antagonist, AM251 (1 μ M) significantly attenuated the effect of E2 on the PSCs (86 $\pm$ 4.1%). The intracellularly applied endocannabinoid synthesis blocker THL (10 μ M) also eliminated action of E2 (67 $\pm$ 5.8%). E2 remained effective in the presence of TTX (51 $\pm$ 11.5% of the control frequency with no change in the amplitude) in 8 from 12 GnRH neurons.

These data suggest that endocannabinoids are involved in the mediation of the effect of E2 on suppression of fast neurotransmission onto GnRH neurons. Our results also indicate direct action of E2 in GnRH cells. In addition, involvement of the retrograde endocannabinoid machinery is revealed in this effect. The interaction of estradiol and endocannabinoid signaling mechanisms represents novel regulatory machinery in the execution of the negative estrogen feedback to GnRH neurons.

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P2/69

Network Analysis of Spread of Low Frequency Activity Induced in Ex vivo Horizontal Cortical Slice Preparations

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During slow wave sleep (<1Hz) membrane potential of cortical neurons spontaneously fluctuates between two states: UP-state is the active phase, when the plasma membrane is depolarized and action potentials can be elicited; DOWN-state is the inactive phase, when neurons are hyperpolarized. Earlier, we identified that the entorhinal (ERh) cortex exhibits this low frequency oscillation in rat brain slices. Our further goal was to define through what connections the activity spreads in the connected cortical regions, and also to identify network connections between cells firing during activation.

We studied slow oscillation using microelectrophysiological methods in rat brain slice preparations of limbic cortical regions. Multi- and single unit activity was extracted from our field recordings to find correlation between field events and single cell firing. We studied vertical spread between cortical layers using current-source density analysis and horizontal spread between adjacent cortical regions by cutting different connections between ERh and perirhinal (PRh) cortices.

UP-states that we registered ex vivo have similar appearance as those registered in vivo; field potential events were in strict correlation with multi-unit activity and single cell firing.

In most cases, the activity initiated in the infragranular layers and then spread upwards. We found network connections between identified cells that also showed similar pattern.

The frequency of the slow oscillation registered in both areas was identical. In most cases UP-states registered from the PRh cortex followed those generated in the ERh cortex with specific latency, whilst cutting the supragranular layers and the corpus callosum altered the area of initiation and the spread of the activity.

Our results indicate that this activity could be the ex vivo model of cortical UP and DOWN activity. In vertical plane, layer V activates the supragranular layers and single cells constitute complex networks thanks to their extensive dendritic arborization. Under normal circumstances, in horizontal slices ERh cortex may trigger the activity, which then spreads from the ERh cortex toward the PRh cortex mostly through supragranular and extracortical connections. However, in the absence of connections between ERh and PRh cortices, the activity of PRh cortex disengages from that of ERh cortex, thus the initiation area may be changed.

P2/59

Sampling in a hierarchical model of images reproduces top-down effects in visual perception

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Sensory perception can be understood as inference of latent causes underlying stimuli. This requires animals to learn the statistics of the environment in terms of a generative model with latent variables corresponding to features with hierarchical organisation, which is characteristic of efficient probabilistic models (Hinton, 2006). While layers of probabilistic image models map intuitively to the hierarchical structure of the ventral stream, details of the correspondence have remained elusive.

Mean activity at the lowest level of visual hierarchy, V1 simple cells, are well covered by linear filters adapted to the statistics of natural scenes (Olshausen, 1996) and by a more elaborate model describing interactions between filters (Schwartz, 2001). Effects of top-down interactions on mean responses have been formalised in terms of covariance components of latent variables (Karklin, 2008). However, thorough understanding requires that other aspects of the rich response statistics can also be linked to computational principles.

A computationally appealing and neurally feasible method to perform Bayesian inference is the construction of stochastic samples (Lee, 2003). Sampling establishes a link between the neural response distribution and probability distributions in Bayesian models. We explore sampling in a hierarchical model of vision and its consequences on neural response statistics in V1.

We have built a hierarchical model of the visual system that bears similarities with the covariance model of Karklin, and used sampling to perform Bayesian inference in it. Activity of model neurons were regarded as samples from the posterior distribution resulting from inference. Top-down effects in the visual hierarchy are particularly noticeable in the phenomenon of illusory contours, thus we used synthetic images with the model to predict the neural response to such stimuli. The sampling algorithm reproduces the magnitude ratio and temporal evolution of neural responses to real and illusory contours, measured from V1 and V2 (Lee, 2001).

P1/81**Morphological evidence for synaptic communication between GnRH axons and dopaminergic neurons in the mouse preoptic area and arcuate nucleus.**Zsuzsanna Bardóczy^{1,2}, Zsolt Liposits^{1,3}, Imre Kalló^{1,3}¹*Institute of Experimental Medicine Hungarian Academy of Sciences, Department of Endocrine Neurobiology, Budapest, Hungary;* ²*Semmelweis University, School of PH.D. Studies, Budapest, Hungary;* ³*Faculty of Information Technology, Pázmány Péter Catholic University, Department of Neuroscience, Budapest, Hungary*

Besides forming the final output pathway regulating the ovarian cycle via the anterior pituitary gland, gonadotropin-releasing hormone (GnRH) neurons also provide neuronal input to hypothalamic and extrahypothalamic loci involved in the regulation of diverse, reproduction-related functions. Among those, the preoptic area (POA) and the arcuate nucleus (Arc) have particular significance due to their critical role played in the control of GnRH and prolactin secretion. These areas contain the A15 and A12 dopaminergic cell groups. As dopamine can directly influence the function of GnRH neurons (Liu et al, 2013), as well as exert indirect effects on GnRH secretion (Goodman et al, 2012), a putative GnRH afferentation to dopaminergic neurons could contribute to the fine regulation of the known lactation-related switch in GnRH secretion.

The aim of our study was to determine whether GnRH neurons establish synaptic connections with dopaminergic neurons in the mouse POA and Arc. To enhance the detectability of GnRH processes, ovariectomised, estrogen-treated female mice were used. The brain sections were immunohistochemically double-labelled to trace juxtapositions between GnRH-immunoreactive (IR) varicosities and the dopamine-secreting, tyrosine hydroxylase (TH)-IR neurons. Confocal and correlated light- and electron microscopic studies were carried out; regions with candidate appositions underwent serial ultrathin-sectioning and electron microscopic analysis.

At light microscopic level, GnRH processes were seen to course through and form varicosities in the POA and Arc. Confocal microscopic analysis and 3D reconstruction of the labelled cellular profiles identified GnRH-IR axon varicosities juxtaposed to TH-IR perikarya and dendrites. At the ultrastructural level, GnRH-IR axon terminals were observed, with multiple small clear vesicles some of which were associated with the presynaptic membranes, and a few heavily labelled dense core granules. Similarly to our previous results (Kalló et al, 2013), showing synaptic contacts between GnRH axon terminals and kisspeptin neurons, GnRH-IR axons formed asymmetric synapses with TH-IR neurons in both the POA and the Arc. These findings indicate direct influence of GnRH neurons on dopaminergic cells in both critically important regulatory centers of reproduction. The co-transmitter involved in the synaptic communication and the functional subpopulations of the target cells in the POA and the Arc, however, need to be determined.

P1/68**Comparison of neuronal activities and visual response characteristics of caudate nucleus neurons in halothane anesthetised and awake, behaving animals**Balázs Barkóczy¹, Diána Nyujtó¹, Rozalinda Pose¹, Tamás Nagypál¹, Balázs Bodosi¹, György Benedek¹, Attila Nagy¹¹*University of Szeged, Department of Physiology, Szeged, Hungary*

This study focuses on an important question, whether the brain activities recorded from anesthetised, paralyzed animals are comparable to those recorded from awake, behaving animal models. In order to answer to the question raised above we compared the neuronal activities recorded during visual stimulation from the caudate nucleus (CN) a subcortical sensory-motor structure in two halothane anesthetised, paralyzed and two awake, behaving cats.

Multiscale multielectrode recordings were performed from the CN during static (random dot kinematogram) and dynamic (moving visual noise) visual stimulation. During the recording session the anesthesia was maintained by a gaseous mixture of air and halothane (1.0%). In the behavioural paradigm the visual stimuli were presented when the trained animals maintained visual fixation.

The recorded CN neurons were separated into three different classes, i.e. phasically active, fast firing and tonically active neurons based on the shape of their autocorrelogram, background discharge rate and the ratio of proportional interspike interval values >2sec. Single cell activities were then compared during different epochs of the visual paradigm. The background activities of each CN neuron groups was significantly higher in awake animals ($p < 0.01$). Although halothane anesthesia could significantly decrease the background activity of any types of CN

neurons, the most noteworthy finding was that the background activity of all phasically active neurons was less than 1 Hz in anesthetized animals. Similarly the stimulated activities of each group and the net firing rates to static and dynamic visual stimulation were also higher ($p < 0.01$) in awake animals. Furthermore, in the case of anesthetized animals the majority of the recorded CN neurons possessed no visual responses, i.e. their net discharge rate was close to zero.

Our results demonstrated strong difference between the multiscale neuronal activities in the CN of anesthetized, paralyzed and awake, behaving cats. The halothane gas anesthesia significantly suppressed the neuronal activity and the visual responsiveness in the CN. Moreover our results suggest that the analysis of phasically active CN neurons (most probably these are the medium spiny projection CN neurons) is impossible in anesthetized model because of their extremely low activity. Based on the presented results above we suggest the benefits of working with behaving animals in visual electrophysiological experiments.

P2/62

Chronic elevation of 2-AG alters spinal nociceptor circuitry in the mouse superficial dorsal horn

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Chronic pain afflicts ~5% of the global population, and represents a considerable burden on society. Currently available analgesics lack efficacy, and thus alternative strategies present an important target for pain research. One promising approach is modulation of the endocannabinoid (eCB) system. Endocannabinoids play a key role in the regulation of pain sensation. Enhancing levels of the eCB 2-arachidonoylglycerol (2-AG) via blockade of its degradative enzyme monoacylglycerol lipase (MGL) produces CB1 receptor-mediated analgesia in the absence of cannabinoid-like side effects. However, despite initially promising reports, recent evidence reveals a loss of analgesia following chronic MGL inhibition, and functional antagonism of CNS eCB signalling which may even potentiate tonic pain states. To investigate the possible mechanism underlying this effect, we studied changes in CB1 expression in spinal cord dorsal horn pain circuitry in mice lacking MGL.

Using immunofluorescence staining, we observed a dramatic region-specific loss of >50% of CB1 staining in the spinal superficial dorsal horn of MGL^{-/-} versus MGL^{+/+} animals. To determine the cellular localization of this change, we probed spinal cord sections for expression of CB1 in key cell types of the nociceptive circuitry; peptidergic (CGRP⁺) and non-peptidergic (IB4⁺) nociceptive primary afferents, inhibitory (VIAAT⁺) and excitatory (vGluT2⁺) interneurons, and astroglial (GFAP⁺) and microglial (IBA1⁺) cells. Surprisingly, we observed only a small reduction of CB1 expression in axon terminals of the investigated neuronal cell types. However, we did observe significant decreases in CGRP⁻, IB4⁻, and vGluT2-immunostaining, and an increase in GFAP-labelling in the absence of MGL.

These data demonstrate a significant alteration in organisation of the nociceptive circuitry in the presence of chronically elevated 2-AG levels. We show reduction in markers of excitatory, pro-nociceptive axon terminals, with a complementary increase in astroglial profiles, possibly representing a compensatory structural adaptation. These data point to a potential developmental role for 2-AG/CB1 signalling in synapse formation, and/or an ongoing role for MGL in maintaining synaptic stability. In either case, a functional eCB system may be critical for the maintenance of normal nociceptive neural circuitry in the spinal cord.

P2/89

The role of P2Y₁₂ receptors in chronic inflammatory pain

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Previous studies of our group indicate that genetic deletion and pharmacological antagonism of P2Y₁₂ receptors can alleviate mechanical allodynia in acute inflammatory pain. In the present project we investigated the role of P2Y₁₂ receptors in chronic inflammatory pain. For this study we have introduced CFA-induced chronic

inflammation model in mice. By the injection of Complete Freund's Adjuvant (CFA) in the plantar surface of the right hind paw, we could induce local inflammation persisting at least 14 days after the CFA injection. During this 2 week period, mechanical sensitivity was evaluated at several time points using dynamic plantar von Frey aesthesiometer.

In this model the effect of two different P2Y₁₂ antagonists were tested: cangrelor, the non-pro-drug P2Y₁₂/P2Y₁₃ receptor antagonist and PSB-0739, the selective and potent P2Y₁₂ receptor antagonist. To obtain further evidence on the role of P2Y₁₂ receptors in chronic inflammatory pain, in a set of experiment P2Y₁₂R gene deficient (p2y12^{-/-}) mice were used.

72 hours after CFA injection, mechanical allodynia developed in P2Y₁₂ receptor gene deficient mice, but the decline in the paw withdrawal threshold (PWT) was significantly lower than in wild type mice.

Both tested compounds were able to attenuate the inflammatory pain in wild type mice in the acute and chronic phase too, although in P2Y₁₂^{-/-} mice, whilst no effect by cangrelor was detected during the acute phase of hyperalgesia, cangrelor increased, but not decreased mechanical hypersensitivity at the chronic phase indicating the activation of non-P2Y₁₂ receptors at this time point.

It has been revealed that in CFA-induced acute inflammation model, P2Y₁₂ receptor inhibition can change the production of cytokines in the hind paw. Therefore these parameters were tested in our chronic inflammation model too. On the 14th day after the CFA-injection samples of inflamed hind paw were collected and utilized for cytokine assays (IL-1 β , TNF- α , IL-6, IL-10, CXCL1). Our studies revealed that genetic deletion of P2Y₁₂ receptors has anti-inflammatory effect in the acute, but proinflammatory effect in the chronic phase.

In conclusion our findings indicate that P2Y₁₂ receptor inhibition is a potential therapeutic approach in chronic inflammatory pain, although its exact mechanism of action needs further investigation.

P2/5

3D engineered neural tissue from human induced pluripotent stem cells

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Differentiation of pluripotent stem cells into a 3D engineered neural tissue (ENT) provides advantages to study the pathophysiology of neurodegenerative and neurodevelopmental disorders compared to 2D cell culture systems. ENT also allows preclinical analyses of selected neural drug candidates.

In this study fibroblasts and mononuclear blood cells were isolated from genetically and clinically well-characterized patients, reprogrammed into induced pluripotent stem cell state (iPSCs) and differentiated into neurons. The neural maturation was controlled using an air-liquid interface that allowed three-dimensional cell expansion without added growth factors (Krause et al., 2009). After 6 weeks of differentiation the ENTs were characterized with electrophysiology (multi-electrode array, MEA), calcium imaging technique and immunocytochemical methods.

MEA recordings and calcium imaging revealed that ENT neurons exhibited spontaneous firing activity and the evidence of functional synapses. Immunocytochemistry analyses confirmed these results with the presence of various glial and neuronal markers such as GFAP, beta-III Tubulin, MAP2, NF200kD and the pre- and postsynaptic markers synaptophysin and PSD-95. The majority of cells differentiated into mature neurons, but we found some neural tube-like structures with strong nestin positivity, which indicates that further optimization of differentiation would be beneficial.

In conclusion, these findings demonstrate that 3D ENTs neurons might be suitable for drug development and studying the pathophysiology of different neurological and psychiatric disorders, such as Alzheimer's disease.

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P1/59

Possibilities of state space reconstruction from spike-trains

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Telling the connectivity of neurons from spike-trains based on correlations is widely used in neuroscience since extracellular multi-electrode array measurements became available, however correlations fail to capture the nonlinear relationships between the elements of a system.

In the last decade new possibilities emerged on the horizon of multivariate time series analysis namely the Transfer Entropy (TE) and Cross Convergence Mapping (CCM), both capable to detect nonlinear coupling of variables.

These two methods were successfully used on continuous data, but the application is somewhat missing for point-processes like spike-trains.

A reason for this situation is that a good state-space reconstruction is a vital step in the calculation both of TE and CCM, but the reconstruction of the state-space is not a well defined procedure in the case of spike-trains.

In this poster we sketch up the current attempts of state space reconstruction for spike-trains, to make TE and CCM computable.

P2/9

Short- and intermediate-term stability of single-unit activity recorded with silicon probes with electronic depth control

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Action potentials (APs) are the basic computational elements of neuronal networks. With high contact density silicon probes we are able to record the activity of up to several hundred neurons simultaneously, allowing to investigate the operation of these neuronal networks. However, it is crucial that the recorded AP waveforms are stable in time, especially if we examine complex brain functions where changes in the neuronal activity happen over longer timescales (e.g. learning, memory consolidation). The AP waveform of a single neuron can change over time due to several reasons (tissue damage caused by the movement of the probe, electrode drift, glial reaction), therefore it is important to minimize these effects. In this study we evaluated the stability of the single-unit activity (SUA) recorded with a novel silicon probe on short-term (minutes) and intermediate-term (hours) timescales. The probe has an innovative feature called electronic depth control (EDC) which makes it possible to adjust recording site positions independently (with some restrictions) inside the brain without physical movement of the probe, thus minimizing the tissue damage during the experiments. The user can select 32 contacts from the more than 1000 potential recording sites electronically (four shafts, 8 contacts/shaft) with a software running on a conventional computer. We recorded the SUA from the neocortex, hippocampus and thalamus of ketamine-xylazine anesthetized rats with the EDC probe. The probe was implanted 7mm deep into the brain. Spike sorting was performed on the recorded data, then the peak-to-peak amplitude (PPA) and half-amplitude duration (HAD) were calculated from the averaged AP waveform of the sorted units. Time-dependent changes of these properties were used as the indicators of stability. We also investigated depth-dependent changes of the PPA and HAD between the three brain regions from which we recorded. Our results showed that in the case of short-term recordings there was no significant change in the measured properties. Analysing the SUA recorded for hours showed a minor change in the PPA ($7.86 \pm 8.03\%$, mean absolute change $\pm$ SD, $n = 113$ cells) and HAD ($5.85 \pm 6.86\%$). Our results indicate that action potentials belonging to the isolated neurons have stable waveforms without major changes in their shape after hours of continuous recording. Therefore, we can conclude that the EDC probe is a suitable device to record SUA on short- and intermediate-term timescales.

P1/29

Subcellular Ca²⁺ dynamics in Deiters' cells in the Organ of Corti

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Despite of their recognized importance in both physiology and pathophysiology of hearing, precise function and regulation of supporting cells of the Organ of Corti (OC) are largely unexplored. We have developed a method of fluorescence imaging in acute hemicochlea preparation of hearing mice (>P15) to investigate the regulation of intracellular Ca²⁺ by ATP in Deiters' cells (DCs). Loading the DCs with the Ca²⁺ probe Oregon Green 488 BAPTA by single cell electroporation provides the signal to noise ratio suitable for subcellular measurement of the second messenger. The polar DCs are innervated and supposed to play a role in both cochlear amplification and endogenous cochleoprotective mechanisms like decrease of the endocochlear potential, e.g. during noise exposure. Extracellular ATP and intracellular Ca²⁺ are believed to regulate these processes.

In our experiments ATP was applied to the perfusion and the fluorescent signals were detected by a cooled CCD based imaging setup. The evoked elevation of intracellular Ca²⁺ was dependent on the concentration of ATP in the 10 – 100 µM range tested. Measuring the Ca²⁺ transients in different subregions of the polarized DCs, showed that the response in the phalangeal process always preceded by seconds the response at the soma. This delay in response was detected in all three concentration of ATP tested (10, 50 and 100 µM) and was not dependent on the direction of the perfusion flow. We tested the phenomenon in three different mouse strains (in the inbred BALB/c and C57BL/6 and in the outbred CD1) with similar results. Omission of Ca²⁺ from the perfusion buffer decreased only partially the Ca²⁺ transient, but abolished the delay. This suggests the presence of functional Ca²⁺ permeable ionotropic P2X receptors on the phalangeal process, besides the presence of metabotropic P2Y receptors. We hypothesize that intracellular Ca²⁺ regulation of DCs are under complex control of ATP that may underlie the widespread role of this cell in physiological and pathophysiological processes of hearing.

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P2/17

Functionally different organization of the extracellular matrix in the prepositus hypoglossi nucleus of the rat

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Preservation of ocular stability during the head and body displacement requires a very complex sensorimotor integration provided by the vestibulo-ocular reflexes (VOR). The prepositus hypoglossi nucleus (PHN) in the brainstem is regarded as the neural integrator for the VOR. It plays a pivotal role in the vestibulo-cerebello-ocular network via the activation of cerebellum through the mossy fiber system. In our previous work we observed a heterogeneous distribution of hyaluronan (HA), various chondroitin sulfate proteoglycans, tenascin R (TN-R) and HAPLN1 link protein in the different vestibular nuclei of the rat. The aim of the present work was to reveal the distribution of various molecular components of the extracellular matrix (ECM) in the PHN by using histochemical and immunohistochemical methods. We detected conspicuous differences between the magno (PHNm)- and parvocellular (PHNp) divisions of the PHN: the perineuronal net (PNN) was well developed around the neurons in the PHNm, whereas the pericellular positivity was almost absent in the PHNp. In the magnocellular part of the PHN the perineuronal net explored the most intense staining with the aggrecan, neurocan, and TN-R antibodies followed by the HA then the WFA and HAPLN1 reactions. The PNNs were not observed with the versican and brevican staining.

Based on previous physiological studies it is very likely that the PNN-bearing neurons are mostly glutamatergic cells, whereas the majority of cells without PNN are GABAergic neurons. The variable ECM staining and distribution pattern presented here is in agreement with the previous observations revealing a continuum rather than discrete types of neurons in the PHN.

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P1/15

Bidirectional modulation of synaptic integration by dendritic voltage-gated K⁺ channels and NMDA receptors in cortical layer 3 pyramids

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The neuronal distribution of voltage-gated K⁺ channel subtypes and their contribution to shaping the time-window for summation of excitatory synaptic inputs in dendrites is paramount to the fine-tuning of cortical excitability. Here I will present compelling evidence that A-type K⁺ channels act as a powerful brake on membrane depolarisation caused by highly synchronised synapses formed onto layer 3 pyramidal cells (L3 PCs). I will also show that A-type K⁺ channels and NMDA receptors exert a bidirectional regulation of the gain of dendritic integration in these neurones.

We recorded from mouse L3 PCs in current-clamp mode at 32 degrees. Cells were filled with biocytin for post-hoc analysis of the dendritic tree and with a fluorescent dye (Alexa-594) for 2-photon laser scanning imaging of basal dendrites and apical obliques. In a typical experiment, focal uncaging of caged-glutamate was performed in control condition at individual spines and at a number of combined neighbouring spines (2-8), and the resulting depolarisation was recorded at the soma. The potassium channel blockers 4-aminopyridine, barium, and phrixotoxin-2; a sodium channel blocker (TTX); and the NMDA receptor antagonist D-AP5 were bath-applied to examine the relative contribution of individual conductances to glutamate-evoked responses. Furthermore, a computational model based on the detailed anatomy of L3 PCs that included a mechanistic description of an A-type potassium conductance was used to evaluate the range of A-type channel densities and passive cellular properties that may explain the effect of pharmacological manipulations.

We found that NMDA receptors conferred a large degree of supra-linear summation of inputs at distal dendritic locations, whereas Na⁺ channels had a preferential influence at proximal sites and elicited dendritic spikes in basal dendrites. We also found that Kv1.4, Kv4.2, Kv4.3 subunits contributed to A-type K⁺ currents in basal and apical oblique dendrites and that their density did not follow a steep gradient. Finally, we demonstrate that A-type K⁺ channels reduced the amplitude and duration of summed excitatory synaptic inputs. Thus, A-type K⁺ channels exert a robust and dynamic modulation of signal integration in single L3 PC fine dendrites.

P1/39

Evoked high frequency oscillations in the human hippocampal formation

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Background

Cortical electrical stimulation (CES) and evoked potentials (EP) are widely used to study neural networks. High frequency oscillations (HFO, ripple) participate in memory consolidation and epileptogenesis in the hippocampal formation (HcF).

Methods

Evoked HFO (evHFO) were studied in the HcF under general anesthesia in 8 temporal lobe epilepsy (TLE) patients. We used laminar multielectrodes to record local field potential, and spectral activation elicited by CES (0.1 ms, 5-15 mA; 0.5 Hz). Ripples were detected by automatic methods. Spontaneous multiple unit activity and evoked spectral power was calculated. Hippocampal regions were reconstructed based on histological assessment of the removed HcFs, and subregions were determined: cornu ammonis (CA), dentate gyrus (DG), subiculum (Sub).

Results

Significant HFO activity was evoked in 7 cases (87%). Subregion distribution of evHFO is the following (significant HFO (ripple)/ case number): CA2: 1(1)/2, CA3: 1(1)/4; DG: 3(3)/6, distal Sub 5(4)/6, proximal Sub and proSub 2(2)/2. Sub had lower stimulus amplitude threshold (5 mA) than DG (10 mA). Stimulation closer to the temporal pole evoked HFOs with higher probability. Maximal power frequency mean values are the following: DG: 137±79 Hz, CA2 and CA3: 144±79 Hz, proximal Sub and proSub: 118±71 Hz, distal Sub 129±77 Hz. In 7 cases (87%) there were no overlap between evoked HFO activities and evoked multiple unit activities.

Conclusion

EPs in the HcF contain abundant amount of HFOs. The most active region is the Sub, but DG may also contain evoked HFOs. Further investigation is needed to determine the diagnostic role of evHFO in TLE.

P2/28

The M-current contributes to high threshold membrane potential oscillations in a cell type-specific way in the pedunclopontine nucleus of mice

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The pedunclopontine nucleus is known as a cholinergic nucleus of the reticular activating system, participating in modulation of sleep and wakefulness. Besides cholinergic neurons, it consists of GABAergic and glutamatergic neurons as well. According to classical and recent studies, subgroups according to the neurotransmitters are not homogenous, but more functional subgroups exist.

The PPN neurons are not only providing cholinergic inputs but also targets of cholinergic neuromodulation. Although cholinergic neuromodulation has been already investigated in the nucleus, the contribution of the M-type potassium current has not been known yet.

Using slice electrophysiological methods, we provide evidence in the present work that cholinergic neurons possess M-current, whereas GABAergic neurons lack it. The M-current contributes to certain functional differences of cholinergic and GABAergic neurons, as spike frequency adaptation, action potential firing frequency or the amplitude difference of medium afterhyperpolarizations.

Furthermore, we showed that high amplitude, high threshold membrane potential oscillations around 20 Hz frequency is a functional property of most cholinergic cells, whereas GABAergic neurons have only low amplitude oscillations. Blockade of the M-current abolished the oscillatory activity at 20 Hz, and largely diminished it at other frequencies.

Taken together, the M-current seems to be a hallmark of PPN cholinergic neurons. It provides a possibility for modulating gamma band activity of these cells, thus contributing to neuromodulatory regulation of the reticular activating system.

P1/64

Distinct firing patterns of axo-axonic and basket cells in the medial prefrontal cortex of freely moving rats.

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Two prominent cell types among parvalbumin containing neurons are the axo-axonic (AAC, sometimes Chandelier) and the Basket (BC) cells. They are both considered to be fast spiking cells with narrow spikes measured in brain slices as well as in vivo. Basket cells are present in all layers of the cortex except layer 1, innervating and controlling a wide range of neurons via somatic inhibition. The cell bodies of AACs are usually located in layer 2 or layer 5. These neurons make synapses specifically onto the axon initial segments of pyramidal cells exerting unique impact on the contacted cell's output. In vitro studies suggested occasional excitatory role for AAs beside their widely accepted inhibitory action.

We aim to connect the in vivo firing patterns of these neuron types with high resolution anatomical examinations to achieve insight into their participation in cortical computations. We recorded and labeled AACs and BCs in freely behaving rats, followed by neurochemical identification and detailed anatomical examinations.

After morphological evaluation and classification, we compared the firing patterns of identified AACs and BCs. In addition we analysed neurons with similar firing patterns recorded with tetrodes. Our results indicate that AACs and BCs have distinct firing patterns in the medial prefrontal cortex of freely moving rats.

P2/61

Cardiotoxic doses of doxorubicin cause early impairment of cutaneous nociceptors

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Previous studies in our laboratory have revealed an interplay between doxorubicin and chemosensitive afferent nerves which express the transient receptor potential vanilloid type 1 (TRPV1) and transient receptor potential cation channel A1 (TRPA1) receptors. This study examined the effects of doxorubicin on the morphology and function of nociceptive afferent fibers of the rat skin.

Adult male Wistar rats were given doxorubicin (cumulative dose: 15 mg/kg) or its vehicle. Capsaicin- and allyl-isothiocyanate (AITC)-evoked cutaneous neurogenic sensory vasodilatation and plasma extravasation, nociceptive thresholds, and carrageenan hyperalgesia were measured with laser Doppler flowmetry, the Evans blue technique and Hargreaves' method, respectively. Capsaicin-induced release of calcitonin gene related peptide (CGRP) was measured in an ex vivo saphenous nerve preparation with ELISA. Cutaneous innervation density and epidermal thickness were assessed by using immunohistochemistry and stereological methods. Experiments were performed within one week after cessation of treatment.

In control rats, applications of capsaicin and AITC onto the dorsal skin of the hindpaw resulted in increases of local blood flow by 224.2 and 121.8%, which were significantly decreased after doxorubicin treatment. Similarly, plasma extravasation induced by capsaicin and AITC was markedly reduced after doxorubicin (for all comparisons $p < 0.05$, $n = 6-8$). In doxorubicin treated rats capsaicin-induced axonal release of CGRP was reduced by about 50%. Nociceptive heat withdrawal latencies were significantly increased (control: 10.65 ± 0.51 s, doxorubicin: 15.03 ± 0.54 s, $p < 0.05$, $n = 6$), and carrageenan-induced heat and mechanical inflammatory hyperalgesia was diminished following doxorubicin treatment. Immunohistochemistry revealed a substantial loss of epidermal TRPV1-immunoreactive axons (1.075 ± 0.04 vs 0.503 ± 0.03 μm total fiber length/ μm^3 epidermal volume), and thinning of the epidermis by about 30%.

The present findings indicate profound changes in the morphology and function of chemosensitive primary afferent neurons following doxorubicin treatment. Since cardiac chemosensitive afferent nerves exert important cardioprotective effects, monitoring of chemosensitive cutaneous nerve functions may well have predictive value to detect early deterioration of cardiac function in the course of doxorubicin therapy.

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P1/17

Bidirectional analog modulation of action potentials in dendrites

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Backpropagating action potentials (bAPs) and the associated Ca^{2+} signals of the dendrites play fundamental roles in neuronal information processing by providing a digital feedback to the input zone of the neurons. Here we investigated whether and how the dynamic variation of the analog parameter, the subthreshold membrane potential can control this digital feedback signal in rat dentate gyrus granule cells. Dual soma-dendritic recordings revealed multiple location dependent modulatory effects of the membrane potential on the bAPs. In the proximal dendrites, the somatic hyperpolarization (from -64.8 ± 0.8 mV to -75.6 ± 0.6 mV) accelerated the repolarization phase of single bAPs, whereas their peak potentials are less affected. In contrast, in the distal dendritic region the bAPs from hyperpolarized voltage reached lower peak potentials. In parallel with the changes of bAPs, somatic hyperpolarization led to distal reduction and proximal enhancement of single spike-evoked dendritic Ca^{2+} signals. Computational simulations and voltage clamp experiments suggested that multiple mechanisms underlie the analog modulation of the dendritic signals in granule cell. First, lower peak of the distal bAP resulted in smaller Ca^{2+} channel activation and influx. Second, the hyperpolarization changed the availability of inactivating Ca^{2+} channels. Consistent with this, blockade of nickel sensitive Ca^{2+} channels eliminated the increase of bAP-evoked proximal Ca^{2+} signals. Third, faster repolarization of bAPs promoted an increased Ca^{2+} influx by enhancing the driving force during the repolarization phase. Ca^{2+} imaging in combination with dynamic conductance clamp showed that faster repolarization alone (i.e. without changes in the preceding membrane potential) was able to enhance Ca^{2+} signals in the proximal dendritic region. Therefore, the above two mechanisms synergistically promote the enhancement of proximal Ca^{2+} signals. Finally, we showed that GIRK channel activation through

dendritic mGlu2 receptors, which evoked physiological hyperpolarization in granule cells, was able to enhance dendritic Ca²⁺ signals in the proximity of the presumed location of the receptors. Altogether, the results demonstrated that steady state membrane potential changes are translated to gradual and location dependent changes in bAP shape and in associated Ca²⁺ signals, providing the potential physiological mechanisms of the analog modulation of digital signaling in granule cell dendrites.

P1/98**Optogenetic control of the prefrontal cortex pathway to the lateral hypothalamus during rat intermale aggression**

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The prefrontal cortex (PFC) is believed to be a key structure in the inhibitory control of aggressive behavior, as chronic structural and functional deficits observed in this brain area were correlated in humans with aggression-related psychopathologies. However, the PFC shows increased activity during aggressive interactions in animals, and increased prefrontal activity was observed in humans involved in aggressive acts while their brains were investigated by brain imaging techniques. This prefrontal paradox has not been resolved so far. We showed earlier that the PFC sends direct projections to hypothalamic structures controlling the execution of aggressive acts and hypothesized that these neurons are responsible for PFC activations observed during aggression. To investigate this hypothesis, we used optogenetic tools to activate the PFC pathway to the lateral hypothalamus (LH), one of the hypothalamic structures involved in aggression control. We expressed channelrhodopsin (ChR2) in infralimbic and prelimbic PFC neurons and then selectively activated their axons in the LH by photostimulation. Stimulation increased the expression of the neuronal activation-marker c-Fos in the LH, confirming that the stimulation effectively activated this hypothalamic region. During aggressive interactions, stimulation increased the share of bites aimed at vulnerable targets of opponents (head, throat and belly), while aggression-related social communication decreased. The number of bites was not affected. These findings suggest that PFC neurons projecting to the LH, surprisingly, facilitate violent forms of aggression without affecting aggressiveness quantitatively. Interestingly, the LH was over-activated by aggressive interactions in several laboratory models of aggression-related psychopathologies. Our study outlines the functional heterogeneity of PFC neurons with respect to aggression control, and demonstrates that the optogenetic technique can be successfully used to disentangle the specific roles of neuron populations residing in this brain area.

P2/35**Investigation of hedonic behavior in a schizophrenia rat model**

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Introduction: There are several lines of evidence that point out deficits in reward and reinforcement in schizophrenic patients. The goal of this study was to reveal the potential alterations in sucrose preference in a new substrain showing disturbances related to schizophrenia.

Methods: Two experimental groups of male Wistar rats were studied (n=8 rats/group): naive rats without any treatment and the 23rd generation of selectively bred animals with social isolation and ketamine treatment at the age of 4-7 weeks. The tail flick, the startle reaction and the simplified holeboard tests were performed at the age of two months to compare the two groups. At the age of 3 months, rats were housed individually with two bottles filled with tap water or sucrose solution (1 or 5 %). During habituation phase, the animals were given two bottles both with water for 6 days, then one of the bottles was filled with 1 % sucrose solution for 3 days. The location of water was switched daily to avoid side preference. After a subsequent 4 day-period, when only water was given for both sides again, one of the bottles was changed to another filled with 5 % sucrose solution for 3 days. During the last session the rats were applied only water for 4 days. Fluid consumption and body weight were measured at each day. The relative daily volume consumption was analyzed (fluid volume x 100/ body weight).

Results: The new substrain showed decreased pain sensitivity, higher degree of startle reaction and anxiety with decreased exploratory activity and disturbed cognitive performance. Regarding the body weight, no significant differences were observed between the two groups during the whole investigation period of sucrose preference test (20 days). During the habituation phase, there were no significant differences in total water consumption, between the sides and groups. Presence of sucrose solutions, independently of their concentrations, enhanced the total fluid consumption due to increased sucrose containing fluid intake in both groups. Additionally, the water intake was slightly lower in the animals of new substrain.

Conclusion: Our results showed that animals with several cognitive and behavioral abnormalities have no anhedonia in sucrose preference test, suggesting that this model is not sensitive to simulate this symptom of schizophrenia.

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P1/101

Hysteresis effect on the speed of processing changes in binocular correlation

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When stereoscopic images are fused, the state of fusion is resistant to changes in binocular disparity that are greater than the disparities that can be fused otherwise. This phenomenon has been described as fusional hysteresis. Here we were interested if a similar hysteresis effect can be observed in transitions between fused and disordered states when binocular disparities are not present. To this end, we measured reaction times (RT) to step changes of binocular correlation in dynamic random dot patterns consisting of dark and light dots that were updated at 60 Hz on a 3D computer screen. Three levels of binocular correlation were defined: in the correlated state (C) all dots had the same contrast in both eyes; in the anti-correlated state (A) all dots had opposite contrast; in the uncorrelated state (U) the probability of same or opposite contrast was equal for each dot. We measured RT to changes from a background having one of these states to targets defined by another state. The targets were horizontal or vertical stripes made up by regions of the screen that had the correlation state to which the change occurred. These stripes were only visible binocularly. Subjects (n = 4), each having normal stereopsis, had to press a button as fast as possible when they detected the change and then indicate the orientation of the target. Four types of changes were compared: anti-correlated to correlated (AC), correlated to anti-correlated (CA), uncorrelated to correlated (UC) and correlated to uncorrelated (CU). We found that the order of RTs in correct trials was typically AC<UC<CA<CU. This points to two effects influencing RT. First, subjects were faster in detecting changes involving the anti-correlated state than those involving the uncorrelated state. Thus, larger changes in binocular correlation can be detected faster. Second, a profound hysteresis was observed whereby the detection of changes toward the correlated state was faster by about 45 ms than detection away from the correlated state. This effect is consistent with the fusional hysteresis effect as it shows longer persistence of the fused state.

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P2/100

A novel stimulus and analysis system for studying the neural mechanisms of natural language processing in the human brain

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Human speech perception during interpersonal communication involves the processing of auditory stimuli with visual stimuli from the lip movements of each speaker. Traditional experiments on language perception in the human brain used tightly controlled sets of stimuli in the experimental design, including short phrases and repetitions of words to analyze the effects on neurological recordings. However, critics, like proponents of corpus-based linguistics, argue that simple language stimuli outside the context of a conversation fail to capture the full spectrum of linguistic complexity during natural speech. We present a novel experimental strategy and data design to study natural language use in epileptic patients undergoing electrocorticography while watching a Hollywood movie that contains many instances of natural interpersonal speech. Detailed analyses were created based on the language components available, involving multisensory speech and linguistic parsing of human language based on phonology, lexical access, semantics, and syntax: the study of the cognitive impacts of sounds, neurological effects of the meanings of words, denotation of the language used, and sentence composition. This experimental system

promotes a more comprehensive understanding of the neural implementation of speech by allowing a shift from studying language in confined experimental conditions and introducing innovative approaches to studying the brain's capacity for understanding multimodal and naturalistic language.

P2/91**The role of hematopoietic Syk tyrosine kinase in ischemic brain injury**

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Stroke is one of the most common causes of death worldwide and a leading cause of long-term disability. Immune cells (T cells, B cells, macrophages, granulocytes or platelets) have been shown to contribute to brain injury and outcome after cerebral ischemia. The spleen tyrosine kinase (Syk) enzyme plays an important role in the activation of immune cells, but it has not been investigated, whether Syk contributes to brain injury. We have induced focal cerebral ischemia by middle cerebral artery occlusion (MCAO) in wild type mice and in mice in which Syk has been selectively eliminated from all hematopoietic cells (Syk KO). We found significantly increased infarct volume in Syk KO mice compared to wild type animals. This was associated with markedly impaired neurological outcome in Syk KO. These differences were not due to larger blood brain barrier injury or increased infiltration of blood borne cells into the brain parenchyma. In contrast, increased numbers of activated microglia have been seen in some areas of the ipsilateral hemisphere, such as the hippocampus. Our results suggest that hematopoietic Syk kinase plays a protective role in cerebral ischemia. Further work is required to identify the immune cell populations and the mechanisms through which Syk contributes to brain protection.

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P2/25**The Caskin scaffold protein regulates dendritic spine morphology, learning and memory**

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Caskin (CASK interacting protein) is a scaffold protein which is expressed in the brain in high amounts and is located at synapses. It has two isoforms, namely Caskin1 and 2. Caskin1 can be one of the molecular linkers in the pre- or postsynaptic structures, however its role in the formation and function of the postsynaptic density (PSD) is yet unknown.

We have investigated the effects of Caskin1 on dendritic spine morphology. Caskin1 was overexpressed in transfected hippocampal neuronal cultures and dendritic spines were analysed by confocal and electronmicroscopy. Our data revealed that overexpressed Caskin1 was present predominantly in the somatodendritic region of neurons and was enriched especially in dendritic spine heads. Importantly, overexpression of Caskin1 increased the amount of more mature, mushroom-shaped dendritic spines in the expense of filamentous spines of cultivated neurons.

We also examined the spine morphology of Caskin1,2 double knock out (CaskinKO) mice and their heterozygous littermate controls (Caskin+/-) in the hippocampus CA1 str. radiatum by electronmicroscopy. While the area and the perimeter of the investigated dendritic spines were similar between the two genotypes, the length of the PSD and also the PSD/area ratio were significantly decreased in CaskinKO mice.

Behavioural and memory tests were also carried out to compare CaskinKO and Caskin+/- mice. In the Morris water maze test, Caskin KO animals showed impaired performance, indicating a deficit in hippocampus-dependent spatial

memory. Based on data obtained from the novel object recognition test, Caskin KO mice had deficit in short-term, hippocampus-independent memory formation, as well. While the lack of Caskin did not influence general locomotory activity, Caskin KO animals showed an age-dependent decay in explorative behaviour and became increasingly passive. Electrophysiology and sensory tests are in progress to further characterise the physiological consequences of Caskin deletion. However, our results already prove that the Caskin scaffold protein plays important role in regulating dendritic spine morphology and learning abilities, possibly via interacting with members of the PSD.

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P2/14

Interpretation of Microelectrode Recordings with a Focus on Single Neurons

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As the microelectrode technology is improving and the spatial and temporal resolution of the recordings is getting higher and higher. To harness the benefits of these developments, new methods for analyzing the data are needed. Qualitative and quantitative measures will be presented on the evaluation of our methods for preprocessing and analyzing the data, with respect to frequency filtering, correlation and up- and down-state detection, spike clustering, the calculation of spike-triggered averages and the estimation of current source density distribution of single cells. As an example to the above-mentioned methods, an analysis of parallel cortico-thalamic recordings will be shown. The main motivation of this work is the interpretation of electrophysiological recordings and to show new methods to estimate the current source density distribution on a single cell level.

P1/91

Spatial and temporal patterns of neurons affected by prolactin in the maternal rat brain

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Intracellular signaling pathway for prolactin involves activation of Janus tyrosine kinase 2 (Jak2) and subsequent phosphorylation of the signal transducer and activator of transcription 5 (STAT5) proteins. PhosphoSTAT5 (pSTAT5) proteins translocate to the nucleus and bind to STAT response elements in promoter regions of genes to regulate transcription. We confirmed previous studies that 2 hours suckling resulted in a widespread induction of pSTAT5 in some forebrain regions such as the arcuate nucleus, the para- and periventricular nuclei, the medial preoptic area, the bed nucleus of the stria terminalis, and the medial amygdala. We also demonstrated by double labeling that pSTAT5 appears in oxytocin neurons in the lateral subcommissural nucleus of the preoptic area. Following direct intracerebroventricular injection of prolactin, pSTAT5 appeared in all the nuclei as following suckling suggesting that prolactin mediated the effect of suckling.

To investigate the time course of prolactin response, pups were allowed to suckle for 30 min, 2h and 6h, respectively. At 30 min, only neurons in the arcuate and periventricular nuclei showed significant pSTAT5 labeling. At 6h, all the nuclei we observed in earlier time points, as well as the posterior intralaminar complex of the thalamus (PIL) contained prolactin-activated cells. Most of the responsive cells in the PIL were identified as TIP39 neurons. In contrast, other TIP39 neurons in the brain did not contain pSTAT5 labeling.

Finally, the Fos and pSTAT5 patterns were compared following suckling. Double labeling of pSTAT5 and Fos demonstrated that most pSTAT5-containing neurons in the brain also showed Fos activation 2h after suckling except the PIL, where neurons were Fos-positive but pSTAT5-negative. In contrast, tyrosine-hydroxylase positive dopamine neurons in the arcuate nucleus were labeled only with pSTAT5.

In conclusion, we demonstrated that prolactin affects neurons in a complex spatial and temporal pattern, which overlaps to some degree with the Fos activation pattern following suckling. However, peculiar differences were found including TIP39-containing relay neurons in the PIL, which showed Fos activation immediately after suckling but pSTAT appeared in them with a time delay.

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P1/83

Magnocellular vasopressin released from the supraoptic nucleus of the hypothalamus is an important regulator of the stress reaction

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Arginine-vasopressin (AVP) released from the parvocellular cells of the nucleus paraventricularis hypothalami (PVN) is a well-known regulator of the hypothalamo-pituitary-adrenal (HPA) axis: the secretion of adrenocorticotropin (ACTH) and corticosterone (CS) depends on its level. However, several studies suggested the role of magnocellular (released both from PVN and nucleus supraoptic nucleus (SON)) AVP in the stress process without strong evidence. Furthermore, intra-SON released AVP from somata and dendrites is able to reach other brain areas (e.g. amygdala) through the extracellular fluid and modulate the neuronal activity thereby the behavior. The role of magnocellularly-released AVP was studied by bilateral stereotaxic injection of an AVP-containing adeno-associated virus (AAV) vector to the SON of AVP-deficient Brattleboro rats. These rats have general AVP deficiency due to a spontaneous point mutation resulting in diabetes insipidus phenotype and reduced hormonal stress response. Anatomical restitution of the AVP synthesis was studied by immunohistochemical mapping. To confirm the functional restitution of the AVP deficiency water consumption and body weight were followed for two weeks. Afterwards ACTH and CS level changes to immune (egg white injection) and metabolic (actrapid induced hypoglycaemia) challenges were examined. C-fos expression in response to the metabolic stressor was also measured by immunohistochemistry as a marker of stress induced brain activation. AVP-deficient rats had no AVP positive cells in their SON or PVN, while after AAV-AVP injection the signal appeared in the SON only. AVP restoration in the SON normalized the enhanced water consumption and the reduced body weight of AVP-deficient animals. AAV vector treatment normalized plasma ACTH levels without changes in CS levels. The enhanced c-fos activation in the SON of AVP-deficient animals was diminished after the AVP-treatment (without affecting the activation of PVN neurons). AVP-deficiency resulted in reduced c-fos activation in all three regions (medial, central, basolateral) of the amygdala. AAV-AVP-treated rats restored this reduction in the medial amygdala only. In conclusion, AVP released from the magnocellular cells of the SON stimulates ACTH secretion and controls the activation of medial amygdala. This is the first direct evidence showing the importance of the hypothalamo-neurohypophyseal system in the regulation of HPA axis.

P2/57

Computational modeling of the possible mechanisms of phase precession

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Place cells in the rodent hippocampus fire only in specific segments of the environment (the place field). The firing rate of the place cell increases while the animal moves towards the center of the field, and decreases after leaving the center. In addition, relative to the theta-frequency local field potential oscillation, the firing of the place cell appears earlier and earlier – this is referred to as phase precession.

Recent experiments have revealed the subthreshold membrane potential changes of place cells, which provided important new constraints for possible mechanisms of phase precession. Our aim was to investigate systematically,

by using simplified cell models, what time-dependent changes in synaptic inputs may underlie the observed pattern of membrane potential variation during the traversal of the place field.

First, an analytical approximation was applied to a one-compartment model neuron, and we investigated the effect of changing the parameters of oscillating excitatory and inhibitory conductance inputs – including their phase and the amplitudes of their tonic (DC) and oscillatory (AC) components. We identified two potential classes of mechanisms which may underlie phase precession. In changing input phase models, one input to the cell has a time-varying phase („inherited precession”); in this case, at least one other parameter needs to vary to create the observed amplitudes. Fixed input phase models require three oscillating inputs at different phases to generate an arbitrary range of phase precession and can also fit the amplitudes seen in the experimental data.

We then applied our generic model to a hippocampal CA1 pyramidal cell, based on the known firing phases of its excitatory and inhibitory inputs. Among models with a changing input phase, a phase-precessing CA3 input with inherited DC and AC amplitudes provides a good fit to the data. In the simplest model with constant phase inputs, the CA3 input transfers dominance to the entorhinal input, while perisomatic inhibition dominates at some intermediate phases. The predictions of the models were then tested in simulations of morphologically realistic cell models with synaptic input, and the results were similar to the one-compartment case.

We found that two different classes of models (or their combinations) could explain the membrane potential changes underlying the double rate and phase coding of CA1 place cells, and suggest some experiments to distinguish between these scenarios.

P2/87

The effect of acute food deprivation on the cognitive performance of obese, calorie restricted and normal fed rats

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Nowadays the diseases arising from obesity and co-morbidities including pathological cognitive decline and dementia pose serious health problems. In addition, several studies suggest causal relationship between obesity and an increased risk of dementia. Our goal was to examine cognitive (memory) function of rats kept on different nutritional regimes. In addition, we also tested 2) the effects of acute food deprivation on cognitive performance. Adult male Wistar rats were used in the experiment (n=49). Three groups were assigned: 1) a diet induced obese (DIO) group of animals who were kept on high fat and carbohydrate containing diet from weaning; 2) a calorie restricted (CR) group where animals were kept on a low-calorie feeding regime (16g standard rodent pellets/day) and 3) a control NF-normal fed (NF) group where animals received standard rodent laboratory diet ad lib. We tested the long-term memory of the rats in novel object recognition (NOR) paradigm. For the examination of short-term memory we used the T-maze spontaneous alternation test (T-maze) paradigm. To test the effects of acute food deprivation, cognitive performance of rats was also tested following 24 and 48-hour food deprivation. During the T-maze test we examined the latency of the arm choices. There was no difference between groups in terms of memory performance in the NOR tests. However, the NF group spent significantly less time with exploring of the novel object. There was no significant difference between groups in spontaneous alternation in the T-maze test. However, after a 48-hour food deprivation, a significant increase of spontaneous alternation was observed in each group, without any effects on arm entry latency. We assume that the decreased exploration of the novel object in the NF group was caused by a decreased general motivation. Neither the calorie surplus nor the chronic calorie restriction caused any observable adverse effect on memory functions of rats. In contrary, acute food deprivation successfully enhanced cognitive performance in all groups without any effect on arm entry latency. Based on these results we hypothesize, that different nutritional regimes do not dominantly alter memory performance of rats compared to the effects of acute food deprivation. We also hypothesize, that, besides the increased motivation, other (hormonal) effects may also play important role in the positive cognitive effects of acute food deprivation.

P2/47

Reduced number of hippocampal GABAergic neurons in an animal model for depression

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Major depressive disorder is a common and complex mental disorder with unknown etiology. GABAergic dysfunction is likely to contribute to the pathophysiology since disrupted GABAergic systems are well documented in depressed patients. Here we studied structural changes in the hippocampal GABAergic network using the chronic mild stress (CMS) model, as one of the best validated animal models for depression. Rats were subjected to six weeks of daily stress and behaviorally characterized using the sucrose consumption test into anhedonic and resilient animals based on their response to stress. Different subtypes of GABAergic interneurons were visualized by immunohistochemistry using antibodies for parvalbumin (PV), calretinin (CR), calbindin (CB), cholecystokinin (CCK), somatostatin (SOM) and neuropeptide Y (NPY). We used an unbiased quantification method to systematically count labeled cells in different subareas of the dorsal and ventral hippocampus. Chronic stress significantly reduced the number of specific interneurons in specific hippocampal subregions. PV+ neurons were reduced in all dorsal subareas, whereas in the ventral part only the CA1 was affected. CR+ neurons were reduced in all dorsal hippocampal subareas, but not in the ventral part. Stress had the most pronounced effect on the NPY+ and SOM+ cells and reduced their number in almost all dorsal and ventral subareas. Stress had no effect on the CCK+ and CB+ interneurons. In most cases the effect of stress was irrespective to the behavioral phenotype. However, in a few specific areas anhedonic animals had fewer SOM+, NPY+ and CR+ neurons compared to the resilient group. Overall, these data clearly demonstrate that chronic stress affects the structural integrity of specific GABAergic neuronal subpopulations and this should also affect the functioning of these hippocampal GABAergic networks.

P1/58

Selective association of voltage gated ion channel clusters with specific axon terminals in the thalamus

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The distribution of voltage-gated ion channels on the somato-dendritic domains of the neurons is homogenous or follows various gradients. Such channel clusters are present mainly in axon initial segments and only occasionally associated with an afferent input.

Here we describe the selective enrichment of Kv4.2 and Kv4.3 voltage-gated K⁺ channel subunits in the proximal dendrites of thalamic neurons in association with axon terminals of specific ascending pathway. These subunits were localized in dense patches postsynaptic to giant vesicular glutamate transporter 2 (vGluT2)-positive terminals in the first order (or lemniscal) somatosensory, auditory, and gustatory thalamic nuclei. In higher order (or paralemniscal) nuclei of the same sensory modalities, Kv channel clusters were weak or absent. According to the electron microscopic analysis, these Kv clusters accumulated specifically in those membrane surfaces of the thalamic neurons which were contacted by the large ascending terminals. During ontogenesis, development of the ion channel aggregates and the enlargement of ascending terminals were temporally correlated. Eradicating the ascending terminals homogenized the distribution of Kv 4.2 and 4.3 subunits, indicating the necessity of functional connectivity for synaptic input-specific enrichment of these ion channels. Enriched environment induced an enlargement of these K⁺ channel clusters.

Our data reveal for the first time a use-dependent accumulation of voltage-gated ion channels in association with the major sensory inputs in the thalamus. We propose that transient K⁺ current plays an active role in setting the time course of sensory stimuli-evoked EPSPs depending on the history of activity in the afferent pathway.

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P2/71

DOXORUBICIN IMPAIRS THE TRPV1-DEPENDENT NEUROGENIC SENSORY VASODILATATION IN THE RAT DURA MATER

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Doxorubicin is a potent antitumor drug with severe cardiotoxic and neurotoxic effects. Previous findings in our laboratory demonstrated an interplay between doxorubicin and chemosensitive primary afferent neurons which express the transient receptor potential vanilloid type 1 receptor (TRPV1). The aim of the present study was to examine the effect of doxorubicin treatment on the functions of meningeal chemosensitive nerves implicated in the pathomechanism of headaches.

Adult male Wistar rats weighing 250-300 g were treated with doxorubicin for 2 weeks (cumulative dose: 15 mg/kg). TRPV1 activation-induced changes in dural blood flow by capsaicin were measured with laser Doppler flowmetry in an open cranial window preparation. Calcitonin gene related peptide (CGRP) release was measured with ELISA in an ex vivo dura mater preparation. Immunohistochemistry was employed to study the innervation of the dura mater. In control rats, stimulation of meningeal nociceptive afferents by capsaicin (10^{-7} M) elicited a significant increase in blood flow by $31.6 \pm 10.4\%$. The vasodilatory effect of capsaicin was reproducible: three consecutive applications of the drug produced similar responses. In doxorubicin-treated animals, the vasodilatory effect of capsaicin was significantly reduced upon repeated administrations yielding increases in blood flow by 12 ± 3.8 , 3 ± 2.1 and $0.9 \pm 0.6\%$. Doxorubicin treatment also resulted in a significant reduction in the vasodilatory effect of CGRP. In doxorubicin treated rats, capsaicin-induced release of CGRP was also diminished as compared to the controls. Immunofluorescence studies showed similar densities of innervation by TRPV1- and CGRP-positive nerve fibers in control and doxorubicin treated rats.

The present findings indicate a profound impairment of vasomotor and nociceptive functions of meningeal chemosensitive afferent nerves following doxorubicin treatment which may contribute to pathologies associated with antineoplastic therapy.

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P2/49

The effect of boswellic acid in diabetic brain of rat model of sporadic Alzheimer's

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Alzheimer's disease (AD) is one of the forms of neurodegenerative diseases that affects more than 6% of people older than 65 years. The disease is characterized by a range of changes in brain anatomy, biology and function. Several genetic and environmental risk factors have been suggested for AD. One of the main pathways that involves in AD is hyperphosphorylation of tau. In fact hyperphosphorylated tau induces neurodegeneration disease through two pathways: (I) microtubule polymerization and dynamics dysfunction could lead to disrupt axonal transportation and (II) occurring the senile plaques (SP) by self-aggregation. In the present study, we investigated the effects of different concentrations of beta boswellic acid (65 µg/kg, 55µg/kg, 35 µg/kg, 25 µg/kg, ICV) in a streptozotocin (STZ-2.25 mg/kg, ICV) induced rat model of sporadic AD. The best concentration of beta boswellic acid was chosen as an effective dose for the following processes. We administrated beta boswellic acid to rat model in two steps; before occurrence Alzheimer's and another one, the following of creating AD in rat model.

Treatment with beta boswellic acid significantly improved STZ-induced cognitive damage as evaluated in Morris water maze apparatus, pathological and molecular tests. Boswellic acid was caused to improve the memory of rat model to find the hidden platform less than 10 second comparison with AD group. In addition, histological observation like Nissl staining confirmed the protective effect of boswellic acid on neuronal degeneration. Tau phosphorylated at serine 404 and threonine 181 resulting from intracerebroventricular streptozotocin injection was decreased the following administration of boswellic acid comparison with control group which was done by Immunohistochemistry test. We have plane to do western blotting to confirm decreased expression of hyperphosphorylated tau in the hippocampus presence of injection boswellic acid. As a consequence, these results provided experimental evidence demonstrating preventive effects of boswellic acid on cognitive dysfunction and hyperphosphorylation of tau in ICV-STZ. In addition, boswellic acid plays as a pivotal drug for treating Alzheimer's.

Keywords: Alzheimer's disease, hyperphosphorylated tau, boswellic acid, hippocampus, streptozotocin

P2/90**SPECT imaging reveals early effects of systemic inflammation on brain injury and changes in peripheral organs after cerebral ischemia**

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Inflammation that develops in the brain and peripheral organs after stroke contributes profoundly to the outcome of patients. Clinical decision making would highly benefit from rapid assessment of central and peripheral inflammatory changes after acute brain injury, but appropriate medical imaging tools are not available yet. Here we show that single-photon emission computed tomography (NanoSPECT/CT Plus) allows early visualization of blood brain barrier injury and inflammation in the brain after experimental stroke, well before signs of brain injury can be detected with magnetic resonance imaging (MRI). Penetration of 99mTc-DTPA into the brain parenchyma overlaps with areas of brain injury and perfusion deficits after cerebral ischemia. In addition, this approach allows for the detection of increased blood brain barrier injury induced by systemic inflammation after experimental stroke. Acute brain injury also leads to infectious complications in peripheral organs such as the lung, the urinary tract or the gut and early visualization of these changes would enable appropriate therapies to be initiated. Using specific radioligands such as 99mTc-DTPA, 99mTc-HMPAO or 125I-BSA, SPECT imaging revealed early changes in perfusion, barrier function and / or inflammation in the lungs and the gut after experimental stroke with good predictive value for outcome. Collectively, our results suggest that early inflammatory changes as detected by SPECT imaging precede injury in the brain and peripheral organs and these imaging tools could be developed further to support decision making in the clinic.

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Symp. IV. - Friday**The role of rapid microglial inflammatory responses in brain injury after cerebral ischemia**

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Inflammation plays a crucial role in the pathogenesis of cerebral ischemia. Inflammatory actions of microglia, the main inflammatory cells in the brain have been widely studied, but their functional contribution to brain injury is controversial. Here we have established a novel, two-photon microscopy-based approach to study early microglial responses in a remote filament model of experimental stroke in mice. In this experimental model, occlusion of the middle cerebral artery and induction of reperfusion are tightly controlled, allowing the assessment of vascular, neuronal and microglial responses in the brain at a millisecond time scale for several hours in vivo, without opening the skull up to 300 µm under the dura mater. By using genetically encoded calcium indicators and transgenic microglia reporter mice, we have assessed changes in neuronal network activity, Ca²⁺ levels in the tissue and microglia activation simultaneously, in real time. We show that excitotoxic neuronal injury in the ischemic cerebral cortex is delayed by several hours after the onset of ischemia and is closely linked with focal microglial activation. Microglia also react rapidly to early signs of vascular injury, preceding the breakdown of the blood brain barrier and isolate cells showing signs of oxidative stress in the injured brain. Selective depletion of microglia prior to experimental stroke facilitates the breakdown of the blood brain barrier and leads to markedly larger infarct size. Collectively, our investigations link early microglial inflammatory responses to brain injury and in spite of the known neurotoxic actions mediated by microglia, our data suggest that these cells could exert generally protective responses after cerebral ischemia. Understanding the cellular and molecular mechanisms through which microglia

contribute to stroke outcome could have important implications to the treatment of stroke and other forms of brain injury.

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P1/9

PACAP acts as an apoptotic and anti-mitogenic factor in a stage dependent manner during the second postnatal week of retinal development

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Pituitary adenylate cyclase-activating polypeptide (PACAP), a member of the secretin/glucagon/vasoactive intestinal peptide family, exerts various effects on neuronal development mediated by the differential expression of PAC1 receptor isoforms. Previously, we reported that Null, Hip and Hop1 receptor isoforms were detected in the rat retina. Furthermore, due to a dramatic drop of the Hip isoform expression, Hop1 isoform became predominant in various retinal cell types during the second postnatal week of retinal maturation. In the current study, our aims were (1) to determine the role of PACAP at this stage of development (2) to identify miRNA sequences that may contribute to the regulation of isoform expression.

To achieve these goals, PACAP (100 pmol) was administered intravitally into one eye of Wistar rats whereas other eye was treated with the same volume of saline. After retinas were removed, we employed TUNEL assay to detect apoptosis, western blot to measure protein levels (PCNA, Wnt1) and immunocytochemistry to localize protein expression (PCNA, glutamine synthetase, Wnt1). The miRNA sequences were identified using mirBASE database and we found that miR137 and miR147 are associated with Hip and Hop1, respectively. Their expression was followed by SYBRGreen-based Q-PCR on a developmental time scale between P5 and P10.

We measured a marked decrease to an undetectable level in Hip mRNA expression from P6 to P9 that was accompanied by the elevation of a miRNA miR137. Neither Hop1 nor miR147 levels changed from P6 to P9. Furthermore, PACAP injection at P5 had no effect on apoptosis, on PCNA levels but increased Wnt expression in the ganglion cell layer (GCL). However, an injection at P7 increased the number of TUNEL positive cells in the inner nuclear layer (INL), decreased PCNA in Muller cells and decreased Wnt1 levels in the GCL.

We identified for the first time that miRNA sequences regulate PAC1 isoforms. The data revealed that Hip expression could be ceased due to miR137 in the second postnatal week of retinal development. Furthermore, we report that PACAP exerts a complex effect on the developing retina: it can induce apoptosis in the INL, inhibits Muller glia proliferation and also regulates the level of Wnt1, a critical secreted protein in ganglion cells. Altogether, we show for the first time that PACAP operates in a stage dependent manner, either directly through differential expression of its receptors or indirectly by downstream secreted factors.

P1/11

Pharmacological and histological dissection of PACAP1-38 induced gene expression in newborn rat retina

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Pituitary adenylate cyclase activated polipeptide (PACAP) has been reported to be involved in a disparate array of developmental processes in the central nervous system (e.g. proliferation, differentiation, migration, neurite outgrowth) via three major receptors: PAC1, VPAC1, VPAC2. Although there is abundant evidence that PACAP has neuroprotective potential in degenerative malfunctions of the retina, the lack of data on its function in normal developmental processes is striking. Previously, we proved that PACAP affected five secreted proteins (Fgf1, Fgf4, Bmp4, Gddf3, Wnt1) in rat retina at postnatal day 1 (P1) and we also found that all three PACAP receptors are expressed at this age. Therefore, we aimed to (1) identify the PACAP receptor that regulates these proteins and (2) define the precise retinal layer where the targeted proteins are expressed.

To achieve these goals, we injected either PACAP (100 pmol) or PAC1 receptor antagonist (PACAP6-38, 5 nmol) or the combination of those intravitreally at P1. Control animals were injected with equal volume of saline. Six hours

post injection, retinas were removed and processed for RNA extraction. Changes in mRNA level were assessed using SYBRGreen-based Q-PCR. Laser captured microdissection allowed us to isolate the ganglion and neuroblast layers (GCL, NBL) independently. Collected tissues were then processed for end-point PCR.

We found that PACAP6-38 could inhibit PACAP effects on Fgf1, Bmp4 and Wnt1 expression. However, it was ineffective in terms of regulating Fgf4 and Gdf3 transcript levels. We detected Bmp4, Wnt1, Fgf4 and Gdf3 mRNAs in both GCL and NBL of the P0 retina. However, Fgf1 expression appeared to be restricted to GCL. In conclusion, PAC1-linked pathways can increase expression of Fgf1, Bmp4 and Wnt1 whereas downregulation of Fgf4 or upregulation of Gdf3 are mediated through VPAC receptors. In case of Fgf1, the induction may occur in the GCL, meanwhile the other proteins could be induced throughout the retina. Our results suggest pivotal roles for both PAC1 and VPAC receptors through regulation of key proteins expressed in the newborn retina. Furthermore, our data shed the first light on multiple roles of PACAP as a secretagogue in a variety of developmental processes in the retina.

P2/99

No acute and placebo effect of creatine monohydrate on anaerobic sport performance

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Background: Use of ergogenic substrates is widespread nowadays. It is likely that non-specific or placebo effects also contribute to the overall effects of these substances. The aim of our study was to examine specific and non-specific effects of a single dose of creatine monohydrate.

Method: 82 undergraduate university students participated in a double-blind, placebo controlled experiment with a no-treatment group (62.2% women, age: 19.95 years (SD=1.885)). They were randomly assigned to one of three groups: control group (N=27, 4 dl pure water), placebo group (N=26, administration of 80 mg/kg dissolved corn-starch which was believed to contain creatine) and creatine group (N=29, 80 mg/kg creatine in 4 dl water). Physical performance was assessed using one-minute standard anaerobic step tests (baseline and post-treatment, 40 minutes apart). Motivation state, personal experiences with creatine, and expectancies concerning its effectiveness were assessed as well as biological- (gender, height, body weight) and certain personality characteristics (Spirituality (SCQ-14), Belief in Sport Superstitions (BSSS), Optimism (LOT-R), Somatosensory Amplification (SSAS)). Perceived change in performance was also estimated by participants.

Results: There were no differences between the three groups (one-way ANOVAs) with respect to expectancies ($p=0.106$; $F=2.311$; $df=76$), objective change in performance ($p=0.951$; $F=0.05$; $df=81$), and perceived change in performance ($p=0.163$; $F=1.859$; $df=81$). Regular consumers of creatine had higher expectations than non-users (one-way ANOVA; $p=0.026$; $F=3.860$; $df=73$). Within the placebo group objective improvement in performance was not predicted by expectancies, neither by motivation nor by personality- or biological characteristics (multiple linear regression analysis; $R^2=0.439$; $p=0.075$). However, a significant expectancy effect was found on perceived increase of performance (ENTER method; $\beta=0.312$; $p=0.41$) which was replaced by the contribution of personality characteristics in the subsequent step of analysis (Optimism ($\beta=0.351$; $p=0.004$); Beliefs in Sport Superstitions ($\beta=0.241$; $p=0.045$); Spirituality ($\beta=0.256$; $p=0.043$); Somatosensory Amplification ($\beta=0.289$; $p=0.017$)).

Conclusions: A single dose of creatine monohydrate and its placebo did not increase objective and perceived performance in an anaerobic step test. Within the placebo group, personality traits were better predictors of perceived increase in performance than expectancies.

P2/73

Expression of interleukin-1 β in the superficial spinal dorsal horn of rats suffering from Freund adjuvant-induced chronic inflammatory pain

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Chronic inflammation evoked by long-term noxious stimulation lead to central sensitization of neural circuit within the superficial spinal dorsal horn. There is general agreement that interleukins play a crucial role in these mechanisms. It has been documented that due to the activation of nociceptive primary afferents interleukin-1 β (IL-1 β) is released from glial cells and act on neural interleukin-1 receptors. Although the role of interleukins in the

development of chronic pain is generally accepted, our present knowledge about the cellular distribution of IL-1 β in the spinal dorsal horn is insufficient. Thus, in the present experiment we investigated the expression and cellular distribution of IL-1 β in the superficial spinal dorsal horn in adult male rats suffering in chronic inflammatory pain evoked by unilateral plantar injection of complete Freund adjuvant (CFA). With the aid of Western blot analysis we showed that IL-1 β was substantially increased in the spinal dorsal horn following CFA injection. Double immunostainings followed by quantitative analysis showed that IL-1 β was abundantly expressed by astrocytes even in naive animals. The astrocytic expression of the protein was substantially increased after the administration of CFA. Neurons and microglial cells also expressed interleukin 1 β although in a much lesser extent. Our results suggest that IL-1 β released by astrocytes may play a role in the generation of spinal central sensitization and consecutive inflammatory pain.

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P2/81

Altered dopaminergic and opioid signaling behind increased hedonic food intake of intrauterine undernourished rats

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Intrauterine undernutrition (IU) may cause several health problems in adulthood. Individuals run to fat, have cardiovascular and metabolic problems. Alterations in homeostatic control mechanisms have been widely examined developing this phenotype. However, non-homeostatic mechanisms (reward) represent a prominent component of food intake regulation, and there is little information about this respect. The accumbens nucleus (Acc) is a key reward centre in the CNS. Dopaminergic and μ -opioid signaling within the Acc play an essential role in generating hedonic impact of food reward. We assumed that non-homeostatic aspect of food intake may be related to the phenotype disposed to obesity after intrauterine undernutrition. To investigate this, we set up a model by keeping the dams on 50 % reduced protein-containing rat food from the conception to delivery. Experiments were performed on 13- week old offsprings. In the first experiment we examined hedonic food intake by presenting sweetened condensed milk solution to control and IU rats after a prior training. Drinking was allowed for 10 min, milk consumption was measured. IU rats consumed significantly more sweetened milk than controls. This result indicated an intensified hedonic impact on food intake in case of IU rats. In the second experiment we wanted to get information about the possible differences in distribution of neuropeptide-receptors. Animals were killed by decapitation, and quantitative in situ hybridizations for enkephalin as well as for D1 and D2 dopamine receptors were performed on coronal brain sections from the area of interest. There was a decrease in D1 receptor mRNA expression in the Acc shell and a tendency for decrease in the core region of IU animals, while D2 receptor mRNA showed a tendency for increase, but only in the core region. Enk mRNA showed also a tendency for decrease in the Acc core, but not in the shell. Altered dopaminergic and probably opioid signaling in the Acc may contribute to the changed reward processing in IU animals.

P1/111

Exaggerated appearance of fear in vesicular glutamate transporter 3 knockout mice

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Glutamatergic neurones are the main excitatory component of the central nervous system characterized by vesicular glutamate transporters (VGLUT1-3) in their axonterminals. The recently discovered VGLUT3 follows a restricted pattern with appearance in the hippocampus, striatum, raphe and sensory system. To dissect its role in physiological processes a knockout (KO) strain was developed.

Our aim was the behavioral characterization of the VGLUT3 KO strain compared with wild type (WT); especially the role of VGLUT3 in fear processes. Fear is a defensive reaction of the body to strong, unpleasant stimuli, but it is often excessive or out of context. Footshock-induced fear is a widely used animal model of posttraumatic stress disorder.

To dissect basal physiological and behavioral differences body weight, food intake and water consumption were measured for a week, motor coordination was tested by rotarod and pain sensitivity by hot plate test. In conditioned fear test half of the animals were footshocked and mice were placed back into the same box 1 and 7 days later and freezing behavior was measured. Anxiety-like behavior was tested by elevated plus maze (EPM) and open field test. Corticosterone levels were measured at the end of open field test. To confirm the innate nature of enhanced fear 7 day old animals were maternally separated and ultrasound vocalisation (USV) was recorded.

There was no difference between the genotypes in the basal physiological and behavioral parameters except a reduced water consumption of KO animals. Without footshock the animals spend minimum time with freezing but KO mice were less active. One and 7 days after footshock the mice spent at least half of the time in freezing position with a greater enhancement in KO animals. KO mice spend less time in the open arms of the EPM than WT. Footshock-induced reduction in open arm time was more pronounced in KO. During open field test KO mice were slower, travelled smaller distance and spent less time in the centrum than WT. Footshock immobilized the animals independently from genotype. There was a tendency for higher resting corticosterone levels in KO mice, but their stress-reactivity was lower. The KO animals emitted more USV.

Taken together the VGluT3 KO mice were highly anxious and were very sensitive to threatening situations. They did not have motorcoordination problem, but in most case they moved less (fear from novelty?), which might confound the interpretation of many tests.

P1/75

Glucagon-like peptide-1 excites firing and increases GABA-ergic miniature postsynaptic currents in gonadotropin-releasing hormone (GnRH) neurons of the male mice

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Glucagon-like peptide-1 (GLP-1), produced in intestinal L-cells and the nucleus of the solitary tract in the hindbrain, regulates blood glucose and reduces feeding behavior, in part, by providing excitatory inputs to the parabrachial nucleus (Richard et al., *Endocrinology*, 2014, 155:4356). Beyond these roles, GLP-1 also influences arousal and interoceptive stress by exciting orexin neurons in the lateral hypothalamus (Acuna-Goycolea and van den Pol J. *Neurosci.*, 2004, 24:8141) and modulates reproduction centrally (Beak et al. *J. Clin. Invest.*, 1998, 101:1334). Accordingly, GLP-1 has been shown to produce a marked increase in the plasma luteinizing hormone (LH) concentration of male rats and enhance the release of GnRH from immortalized GnRH-producing neurons. The present study was undertaken to reveal the putative effects of GLP-1 on GnRH neurons and elucidate the mechanisms of the peptide actions. The effects of GLP-1 on firing and miniature postsynaptic currents (mPSCs) in GnRH neurons of acute brain slices obtained from adult male mice were studied. Loose-patch recordings demonstrated that a single bolus of the GLP-1 agonist, Exendin-4 boosted firing rate concentration-dependently being full effective at 1 μ M (434 $\pm$ 14 % of the control firing rate). The effect was abolished by pretreatment of the slices with the GLP-1 antagonist, Exendin-3(9-39) (1 μ M). Whole-cell patch clamp recordings revealed that GLP-1 (1 μ M) increased frequency of mPSCs related to GABAA-receptor in all neurons studied (199 $\pm$ 17 % of the control frequency). These results suggest that GLP-1 excites GnRH neurons, partially, by affecting their GABA-ergic synaptic input. Collectively, our electrophysiological data provide evidence for a direct regulatory role of GLP-1 in the hypothalamic control of reproduction.

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P2/43

Moderate functional improvement induced by transplanted human induced pluripotent stem cells into an injured rat spinal cord

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Spinal cord contusion injury leads to severe loss of gray and white matter and subsequent deficit of motor and sensory functions below the lesion. In this study we investigated whether application of undifferentiated human induced pluripotent stem cells (hiPS) is able to induce functional recovery in an injured rat cord. Human induced pluripotent stem cells were grafted intraspinally or injected intravenously one week after a thoracic (T11) spinal cord contusion injury performed in Fisher 344 rats. Control animals received physiological saline one week after injury via the same delivery routes. Locomotor analysis of the injured animals was performed by applying the BBB test and a detailed kinematic analysis system of the hind limb movement to ascertain improvements in locomotor function. The motion of the rats was videotaped with two fast video cameras recording 3-4 step cycles. The video sequences were analysed by using VirtualDub software and the selected frames where the animals were in a defined phase of motion were taken for measurement (ImageJ). Conversion of vector data to numerical ones was performed through the use of algorithms developed in our laboratory. We analyzed 10 parameters of locomotion (Tarsus-off-, Metatarsus-surface-, Tibia-surface angle, Spreading, Knee and Ankle flexion, Length of step cycle, Angle of lateral placing, Distribution of stance-swing phases, Footprint) for 9 weeks after the surgery. The retrograde tracer Fast Blue was injected into the intact spinal cord two segments caudally to the lesion to determine the extent of propriospinal axonal sparing/regeneration at 9 weeks after the injury.

HiPS cells applied either locally or intravenously induced moderate functional recovery after contusion injury. The contusion cavity at the epicenter was significantly smaller in grafted animals than in controls. Retrograde tracing studies showed statistically significant increase in the number of propriospinal neurons projecting to the distal spinal cord in both intraspinally and intravenously treated rats. The moderate morphological improvement was accompanied by significant functional improvement. These data suggest that human iPS cells are able to induce a moderate improvement in the outcome of spinal cord injuries and our kinematic analysis system is suitable to detect even minor positive functional changes after a spinal cord contusion injury.

P2/89

The role of microglial P2Y₁₂ in controlling neurotrophic virus infection in the brain

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Understanding immune mechanisms that are initiated against viral infections in the CNS is essential to develop appropriate therapies to diseases such as HSV encephalitis or viral meningitis. Microglial cells are known to be rapidly recruited to the sites of brain injury and we have demonstrated earlier that microglia form barriers around virus infected cells in the brain. However, the functional role of microglia in defense against neurotrophic viral infection and mechanisms controlling microglia recruitment to infected neurons are not understood. We have established in vitro cultures of microglia and pseudorabies virus (PRV)- infected neurons / astrocytes, and found that microglial recruitment to infected cells is associated with increased Ecto-ATPase activity, which was also confirmed in the infected mouse brain, in vivo. To study whether ATP could mediate the recruitment of microglial cells to sites of neurotrophic viral infection in vivo, we injected a highly attenuated, Bartha-derived PRV strain into the epididymal fat of wild type, P2X₇ and P2Y₁₂ knock out (KO) mice. In this experimental model viral particles are retrogradely transported to central autonomic neurons in the brain and the genetically modified pseudorabies virus strain used allows the assessment of early and late stages of neuronal infection. Microglia were rapidly recruited to infected neurons in the brain and phagocytosed cells showing signs of late infection. We found reduced microglia recruitment around infected neurons in P2Y₁₂ KO mice, which was associated with enhanced spread of viral infection in P2Y₁₂ KO, but not in P2X₇ KO mice. Our results suggest that ATP released from infected neurons and/or nearby cells could mediate rapid recruitment of microglia to the sides of neurotrophic viral infection in the brain. Therapeutic interventions facilitating the phagocytosis of virally infected neurons by microglia could be used to support the elimination of neurotrophic viruses and infected neurons from the CNS.

P2/98**Theta-gamma coupling and network dynamics in the hippocampus during sleep and behavior**Antonio Fernandez Ruiz^{1,2,3}, Azahara Gonzalez¹, György Buzsáki³, Antal Berényi^{1,3}¹University of Szeged, Physiology, Szeged, Hungary; ²Complutense University of Madrid, School of Physics, Madrid, Spain; ³University of New York, Neuroscience Institute., New York, United States

In the entorhino-hippocampal circuit theta rhythm organizes the activity of interconnected cellular populations during exploration and REM sleep. Sequential activation of cell assemblies mediates information flow and processing in the different nodes of the circuit and is reflected in the gamma oscillations of local field potentials (LFPs). We recorded LFPs and single-unit activity simultaneously in all hippocampal subfields during different behavioral task and sleep. Using advanced analytical techniques we characterize the spatial distribution and spectro-temporal properties of the different sources of hippocampal gamma oscillations to elucidate how they arise as a result of local circuit computations or reflect rhythmic inputs to the recorded region. Gamma patterns mediated by the entorhinal and intrahippocampal inputs are precisely organized in the theta cycle and coupled in characteristic frequency bands. We observe behavioral dependent changes in the theta-phase distribution and power of gamma oscillations. The present results shows that layer-specific gamma oscillations in the hippocampus reliably identify the temporal dynamics of the afferent inputs. Our results argue in favor of theta-gamma coupling as a functional mechanism to dynamically organize the processing of information accordingly to behavioral demands.

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P2/4**Validation of a novel, cylindrically shaped human depth probe in the rat brain**Fiath Richard^{1,2,3}, Kinga Tóth¹, Lucia Wittner¹, Domonkos Horváth^{1,2,3}, Frederick Pothof⁴, Patrick Ruther⁴, Guy A. Orban⁵, István Ulbert^{1,2}¹Research Centre for Natural Sciences of the Hungarian Academy of Sciences, Institute of Cognitive Neuroscience and Psychology, Budapest, Hungary; ²Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary; ³Semmelweis University, School of Ph.D. Studies, Budapest, Hungary; ⁴Albert-Ludwigs-Universität Freiburg, Institut für Mikrosystemtechnik (IMTEK), Freiburg, Germany; ⁵Università degli Studi di Parma, Department of Neurosciences, Parma, Italy

Commercial human depth probes provide only a limited number of contact sites and are thus restricted in their recording capabilities. The NeuroSeeker project funded by the European Commission aims to develop cylindrically shaped depth probes with an increased number of recording sites. Probe modules comprising microelectrode contacts will be integrated in-between macrocontacts of commercial depth probes. Prior to the application of these probe modules in human patients thorough biocompatibility tests and validation experiments in animal models are however required. This is performed with cylindrical probe modules (1.5 mm high, 800 µm in diameter) consisting of a polyimide foil with 16 integrated platinum contacts (35 µm in diameter, 300 µm vertical spacing). They are chronically implanted for several months into the neocortex of rats. First, we evaluated the histocompatibility of dummy modules without electrodes implanted for 3 months by investigating the neuronal cell loss and glial reaction next to the probe track. To evaluate the electrophysiological recording capabilities of the implanted devices with electrodes, we recorded local field potentials (LFP), as well as multiple- and single-unit activity (MUA, SUA) from the cortex on a daily basis and examined the stability of neuronal signals. The regions next to the probe track showed only a mild loss of neurons and a weak glial reaction. However, we found as well areas with a moderate cell loss and high numbers of astroglial cells in an about 100-µm-thick band starting from the probe track. These cell-deficient regions could be the result of bleeding or brain damage caused during the implantation of probes that are large compared to the applied animal model. With the implanted active probes we could record good quality LFP signals and MUA and SUA of moderate quality from most of the contact sites. Based on our results we can conclude that the investigated cylindrical probe could be a useful tool for the future. It could be used for a more accurate localization of the seizure focus in patients with focal epilepsy and help to understand the human

brain in more detail, for example exploring human cortical microcircuits by recording the activity of many single neurons.

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n°600925, OTKA K81354, ANR-TÉT Neurogen, ANR-TÉT Multisca, TÁMOP-4.2.1.B-11/2/KMR-2011-0002

P1/41

Functional network analysis of human intracranial EEG recordings

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Background

Drug-resistant epileptic patients are often treated by the surgical resection of the epileptic zone (EZ). Beside other diagnostic method, iEEG is considered as a „gold standard” for the localization of the EZ, however the exact localization of the injured brain region is frequently an unambiguous step of the successful surgical intervention. According to a recent theory, the generation and propagation of the seizure is rather the consequence of abnormal brain networks, than the effect of an isolated pacemaker region. Graph theoretical measurements could be applied on the network of functionally connected brain regions in order to identify the EZ. Functional hub regions (centers coordinating the information flow of a network) and extensive local connections presumably associated with the EZ.

Methods

7 epilepsy patients implanted with subdural grid and strip electrodes were involved in the present study. We analyzed ictal (total number of seizures: 47) and interictal events. Functional brain networks were investigated based on the measurement of phase synchronization (phase lag index) in five frequency bands (delta, theta, alpha, beta, and gamma). We applied several hub measurements (betweenness centrality, modular hub, high nodal strength) and the dynamical rewiring capacity of the functional modules as an indicator of the affected regions. The overlap of hub regions and clinically observed EZ were evaluated based on the surgery outcome.

Results

In case of the successful surgery hub regions showed a high degree of overlapping with the EZs identified by clinical experts. EZs in patients with structural lesion are clearly identified by most of the graph measurements. Moreover these parameters could determine the clinical seizure onset zone inside the resection area in interictal recordings.

Conclusions

Our results support the growing number of evidence, that functional network analysis could be an efficient clinical tool for EZ localization.

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P2/84

Dysregulation of energy balance in spontaneously hypertensive rats: the melanocortin and neuropeptide Y system

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Spontaneously hypertensive rats (SHR) develop hypertension by the 4-6th week of life, which rises further during aging. Their sympathetic tone is enhanced. Their hypertension may be prevented by chronic caloric restriction. Their food intake (FI) and body weight (BW) are lower than those of age-matched controls, suggesting a dysregulation of feeding and BW. Enhanced activity of the hypothalamic anorexigenic melanocortins (MC) is suggested in the background of their hypertension and metabolic dysregulation. Diminished activity of the major orexigenic mediator neuropeptide Y (NPY) was also hypothesized.

FI of adult male SHR rats and normotensive controls (NT) was recorded in a FeedScale system upon intracerebroventricular (ICV) injection of NPY (5 µg) or an MC agonist (5 µg alpha-melanocyte-stimulating hormone, alpha-MSH) or during a 7-day ICV infusion of an MC antagonist (HS024, 1 µg/h). The number of alpha-MSH producing cell was determined in the hypothalamic arcuate nucleus by immunofluorescent labeling. We also performed quantitation of alpha-MSH, MC4 receptor and NPY specific signal density (SSD).

Alpha-MSH-injection reduced spontaneous night-time FI more efficiently in SHR than in NT rats. HS024 started to increase daily FI and consequently BW in the NT group from the 1st, in SHR animals only from the 3rd day. In contrast, NPY-induced day-time FI was found to be suppressed in SHR. There was no difference between SHR and NT rats in terms of alpha-MSH-producing cell counts and NPY SSD, but the SSD of alpha-MSH immunosignal was significantly higher in SHR animals. Despite expected receptor down-regulation, SSD value for MC4 receptors did not differ.

Our in vivo and also in vitro results suggest higher MC-production and –responsiveness. The lack of MC4 receptor down-regulation and lower NPY-responsiveness in SHR rats may also contribute to the dysregulation of their BW. (TÁMOP-4.1.1.C-13/1/KONV-2014-0001)

P1/33

Unilateral labyrinthectomy resulted different modifications of versican levels in PNNs of functionally distinct areas of the rat's vestibular nuclear complex

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Unilateral lesion of the vestibular system in mammals results in disturbances of eye movements, coordination of skeletal muscle tone, and posture. Symptoms spontaneously restore 1-2-week-long period, during the vestibular compensation. Recent studies showed that the extracellular matrix (ECM) has an important role in the plastic modifications of the central nervous system (CNS). We earlier observed that unilateral labyrinthectomy (UL) is accompanied by the modification of hyaluronan and chondroitin sulfate proteoglycan staining pattern in the perineuronal net (PNN) and neuropil of the lateral vestibular nucleus (LVN) in the rat. The time course of PNN reestablishment was parallel with the restoration of functional deficits, suggesting the possible role of ECM in the restoration of the impaired function. In the present study the expression of versican was examined in each vestibular nucleus following UL.

The experiments were carried out on adult rats. The vestibular receptors were mechanically damaged and after a period of 1, 3, 7 and 14 days of survival, immunohistochemical reaction for versican was performed on brainstem sections.

In the control animals the versican reaction revealed heavily stained dots in the pericellular region and neuropil as well as continuous PNNs were also recognizable in the superior (SVN) and descending vestibular nuclei (DVN). Following UL, the most prominent changes were seen on 1st postoperative day in the ipsilateral SVN and DVN. The versican staining almost disappeared from the PNN and restored from 3 days after lesion. The staining intensity of neuropil weakened in each nucleus, mostly in the SVN, parallel to the decrease in the number of versican positive dots.

Our results suggest that the decreased expression of non-permissive versican in the PNN of the SVN and DVN may be involved in the restoration of vestibular functions within these nuclei.

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P1/95

Mapping of brain structures involved in response dependent reinforcement: application of manganese-enhanced magnetic resonance imaging (MEMRI) in rats

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MEMRI technique was applied to map rodent's brain areas involved in response dependent reinforcement used thirst motivated operant signal detection task with continuous reinforcement. MEMRI measurements were adapted to 3T clinical MR scanner. T1 recovery time was determined by turbo spin echo inversion recovery sequence. T1 maps were calculated and compared among groups of 1) response dependent reinforced animals, 2) yoked controls for response independent reinforcement, 3) control rats in experimental context without reinforcement 4) control with restricted access for water without participating in experimental context.

Results showed significant differences between T1 recovery times in brain areas of the response dependent reinforced animals compared to control group without participating in experimental context. The following areas were more active: visual, agranular insular, retrosplenial, piriform, ectorhinal, perirhinal and entorhinal cortices, cortical amygdaloid nuclei, hypothalamus, ventral tegmental area and periaqueductal gray area. In response dependent reinforced animals compared to control rats in experimental context without reinforcement, higher activity pattern of the following areas were observed: motor cortex, somatosensory cortex, temporal association cortex, auditory, visual, agranular insular and perirhinal and entorhinal cortices, centro-medial amygdala, amygdalo-hippocampal area, hippocampus, zona incerta, pedunculo-pontine tegmental nucleus. Compared to signal intensities between groups of response dependent and independent reinforced animals higher activity pattern of the following areas were observed: in somatosensory, auditory, visual cortices and the temporal association cortex, granular and dorsal insular cortex, centro-medial amygdala, the amygdalo-hippocampal region with the retrosplenial cortex.

In conclusion, the activity-induced MEMRI detected local brain activity changes related to thirst motivated operant behaviour. The differences in increased signal intensities in centro-medial amygdala, the amygdalo-hippocampal region with the retrosplenial, insular, sensory cortical areas and temporal association cortex could be related rather to discriminative stimulus - response than response – outcome contingency.

P2/48

Proteomic investigation of the prefrontal cortex in the clomipramine model of depression

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Clomipramine (CLI) is a tricyclic antidepressant, which increases the amount of norepinephrine and serotonin in the synapse. Neonates treated with CLI show depressive-like behavioral and neurological alterations in adulthood. In depression, there is evidence of the dysfunctionality of the monoaminergic, dopaminergic systems, as well as the importance of inflammatory cytokines, but the exact pathomechanism is still unknown at molecular level. Recent data shows strong relation between the disturbance of early ontogenetic development and the sensitivity to late life psychiatric diseases. The aim of the study was to explore the protein expression in the prefrontal cortex (PFC) in

adulthood after the neonatal treatment with CLI (between postnatal days 8-21), between the treated and control group of male Wistar rats. We performed a 2D-DIGE proteomic analysis of PFC. In the group treated with CLI, we found significant expression changes in 35 spots compared to the healthy group. The altered proteins have functions in inflammation, cell metabolism and structural changes in the cytoskeleton. We further investigated the distribution of macrophage migration inhibitory factor - a protein, which showed the largest difference in expr. between the groups -, in the PFC with Western blotting and immunohistochemistry. Thereby, we validated the increase in its level and also showed that this protein is predominantly expressed in astrocytes. Conclusions: changing the brain monoaminergic transmission in early brain development stage induces altered protein networks in adulthood giving a molecular basis for clinical observation that neonatal psychoactive drug treatment makes high incidence of psychiatric diseases in adults.

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P1/110

Neurons producing melanin-concentrating hormone: regulation of food intake and sleep / awake cycle - a study of the mouse hypothalamus

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One of the functions of the hypothalamus in mammals is the control of food and water intake. To serve this regulatory task a great number of neuropeptides are produced locally and distributed throughout the brain. Some of these hormones do increase food intake (i.e. are orexigenic), while others have the opposite effect, thus are anorexigenic.

One of the orexigenic hormones is the melanin-concentrating hormone (MCH). Somata of MCH-neurons are mainly located in the caudal part of the hypothalamus being predominant in the lateral hypothalamic area (LHA) and the zona incerta (ZI). The anatomy of the MCH-neurons is best known in the rat, interspecific differences of their somata and projections, however, were recorded (Crozier et al, 2013) even in two closely related rodents, like rats and mice. Some possible species-specific function are suggested e.g. due to the presence and lack of MCH-positive somata within the posterior periventricular nucleus (PVP) in the rat and mouse, respectively.

Besides acting as a neuromodulator on both hypothalamic and distant targets melanin-concentrating hormone is involved in regulating the sleep / awake cycle (Monti et al., 2013). We have initial recordings of temporal, diurnal differences of the MCH-immuno-positivity of neurons in the mouse hypothalamus.

Symp. III. - Friday

The impact of self relevance on the processing of social signals.

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Emotions are known to serve several intra- and interpersonal functions. They notably structure and organize ongoing social interactions. The emotional expressions displayed by others have a communicative function in the sense that they provide crucial information about the sender's current emotional state and potentially also about her/his. The ability to read these emotional expressions constitutes a basic process for social adaptation. By manipulating a number of dimensions that impact the self-relevance of perceived emotional displays (gaze and group membership of the emitter) and by using a multi-level methodological approach, we investigate how the reading of emotion varies as a function of self-relevant perceptual features as well as when and where the evaluation of self-relevance impacts the neural correlates of the reading of emotion.

P2/97

The effect of empathy of restrained stress on learning and spatial memory of rats

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Empathy defines as a fundamental mechanism for the expression of altruistic behavior, and it is the product of an integrated process of the brain. Empathy refers to our ability to share emotions and sensations of others and relies on several portions of the brain such as limbic, Para-limbic structures and hippocampus.

Stress can have profound effects on memory function, experienced both chronically and acutely. Acute stress or elevated stress hormones facilitate memory consolidation while impairing the retrieval of information. Restrained stress is a special kind of stress which enhances the release of Glucocorticoids into the blood such as corticosterone.

In the present study, we investigated the effect of emphatic restrained stress, a combination of restrained stress and empathy trait, in the rat model in three groups; in the first group, they experienced stress alone; and the second one confronted the restrained stress with cage mates and final group was determined as intact one. In both stress alone and empathy groups, the protocol was applied during 7 days (two hours per day). In order to create restrained stress (of motion confinement type), the animals were put in plastic confinners upside down. At the end of 7th day, we generated blood sampling from the heart following the remove of rat from the restrained box. After 24 hours of blood sampling, Morris water maze was performed for all groups. The results showed that empathy group had more impairment than stress alone group. We will report the result of hormone level in each group after analyzing by kitp. As our research results, we find significantly differences between empathy and stress alone group of the data resulting from Morris water maze.

As a consequence, the experimental protocol resembles many aspects of human empathy of acute stress in a rat model that is amenable neuronal alterations for empathy of acute restrained stress and its impairment in learning and memory, so based on these information, we may be able to clarify the concept of empathy stress as a psychological matter more detailed.

Keywords: empathy, restrained stress, corticosterone, learning, memory

P1/27

The effects of optical, electrical and chemical stimulation on serotonin release from median raphe and hippocampus of mice

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Serotonergic neurotransmission has been implicated in the etiology of disorders of emotion regulation such as anxiety disorders and depression, and it is a primary target for psychotherapeutic drugs. The present study has examined several characteristics of the release of [3H]5-HT and [3H]glutamate ([3H]Glu) from the median raphe nucleus (MRN) and hippocampus in terms of its dependence of nerve impulse. We used electrical stimulation and the sodium channel opener veratridine, which excite all of the neuronal processes in the stimulation field, and optogenetics to selectively stimulate those terminals which express channelrhodopsin-2 (ChR2) and compared 5-HT release evoked by electrical and chemical depolarization and by light.

We injected an adeno-associated virus containing DNA construct encoding ChR2 into the MRN of mice and investigated [3H]5-HT and [3H]Glu release from mouse MRN and hippocampal slices (300 micron thick) superfused with Krebs solution under quiescent conditions. Serotonergic nerve terminals was locally stimulated with 473 nm light (blue laser diode) and electrically by bipolar electrode (20, 50, 100 Hz) and by veratridine (20 uM) and transmitter release was monitored by collecting the effluent in a fraction collector. Electrical field stimulation and veratridine resulted in a significantly increase in the efflux of 5-HT, whereas optical stimulation of ChR2 expressing nerve terminals at various frequencies (10, 20, 50, 100 Hz) elicited only a negligible increase in 5-HT release either from the hippocampus or from the MRN itself.

The electrically induced release of radioactive neurotransmitter was completely inhibited by perfusion with tetrodotoxin (10 uM). We have also applied the 5-HT transporter inhibitor, fluoxetine (10 uM) and the GABAA blocker bicuculline (10 uM) to relieve released 5-HT from re-uptake and any endogenous inhibition. Nevertheless, the effect of optical stimulation remained closed to the detection limit under these conditions.

In conclusion, whereas our method is suitable to detect [3H]5-HT efflux in response to ongoing neuronal sodium channel activity its sensitivity is too low to detect transmitter efflux evoked by focal optogenetic stimulation. The most likely reason for the failure of detection of 5-HT efflux in response to light stimulation is that ChR2 is expressed only by a small subpopulation of nerve terminals.

P1/60**Place cells properties in CA1 depend on different inputs.**

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Although strong evidence has been provided describing the hippocampal formation as a key component in the navigational system of the rats, the mechanisms connecting the single cell physiology with the functional properties of the network still remain unclear. We

used large-scale high-density silicon probes and recorded both, population activity (LFP) and single unit activity from the subiculum-fimbrial (transversal) axis of the hippocampus in behaving rats. First, we found that notably different spatial properties can be associated to the proximal and distal poles of the recorded axis. Those more proximal place cells have mostly express single place fields and their firing patterns carry more space related information. Conversely, more distal (subicular) located place cells most frequently have several and sparse place fields and are less informative regarding the spatial location. Secondly, investigating the synchrony of the population during hippocampal theta oscillations, we found that cells corresponding to different sides of the recorded axis coordinate to fire preferentially at different theta phases. At the proximal region, cells fire following the ascending phase of the theta cycle in a precisely monotonic manner while for more distal located cells the phase distribution appears to be bimodal, suggesting an additional theta-coupled generator at this side. Finally, proximal CA1 cells are strongly locked to slow gamma (30-50Hz), which has been reported as characteristic of the CA3 evoked activity, while distal cells showed more synchrony with fast gamma (80-100Hz), which is associated with entorhinal input. Taking together these results and comparing with the anatomy, we concluded that the spatial computations that take place in CA1 region of the hippocampus are modulated by a continuum of the two complementary main inputs, dominated mainly by the activity of the CA3 at the fimbrial side and by entorhinal activity on the subicular side. These inputs are putatively related with different functional modes of the whole network during spatial navigation. Supported by the EU-FP7-ERC-2013-Starting grant (No. 337075), the 'Momentum' program of the Hungarian Academy of Sciences (LP2013-62/2013), and the Hungarian Academy of Sciences (KEP-1.2/2014)

P2/72**Prenatal immune activation induced alterations in the synaptic proteome of adolescent rats**

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Pre- and perinatally experienced stress, like infection can adversely affect proper neuronal development, leading to long-term malfunction in the central nervous system. Epidemiological studies, and immunological examinations on human maternal sera suggested the association of maternal infection and the higher incidence of neurodevelopmental disorders as autism and schizophrenia in the offspring. Investigations on animal models have also strengthened this phenomenon, and described a large set of behavioural and anatomical alterations in the offsprings which are correlated to the human disorders. In order to get a better insight in the molecular consequences of in utero immune activation we examined the pre- and postsynaptic proteome of cerebral cortices of adolescent rats. Lipopolysaccharide (20 µg/kg, i.p.) was administered to pregnant rats on gestational day 13.5. At

postnatal day 35 cerebral cortices of male offsprings' were subjected to subcellular fractionation. We have prepared and extensively validated presynaptic membrane and postsynaptic density fractions and analyzed the effect of treatment with high-throughput proteomics methodology based on different gel electrophoretic techniques coupled with mass spectrometry and further validations. Our results revealed extensive proteomic changes in both synaptic sites, which suggests prominent cortical synaptic dysfunction in the pathomechanism of these neurodevelopmental diseases. According to the altered protein sets, divergent cellular functions are affected, while the quality and quantity of proteins with changed amounts between the two synaptic regions are distinct. Furthermore, candidate signaling mechanisms implicated in the proteomic alterations can be proposed and likely different in the pre- and postsynapse, as suggested by pathway analyses.

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P2/3

Complement protein label at the synaptic region: Is there a chance to separate labeled synapses?

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High-throughput gene expression studies in the last decade focusing on the central nervous system have revealed that several classically immune system related molecules are expressed in neurons and glial cells. The complement system has attracted particular attention because functional examinations demonstrated their participation in brain development and synaptic pruning. A role of brain resident complement proteins is perhaps the activity-dependent modification of the pattern of synaptic connections via induction of microglial phagocytosis of specific synapses. Because the molecular mechanisms underlying complement triggered synaptic pruning is fairly unknown, we aimed to uncover neuronal complement proteins and their interacting partners, moreover, the molecular composition of the complement labeled synapses. First, we prepared highly purified synaptosome fractions using subcellular fractionation supplemented with synaptosome sorting technique by FACS. We demonstrated the appearance of members of the classical complement cascade as the cascade triggering C1q, and C1q binding neuronal pentraxins on some of the synaptosomes. Immunolabeling of C1q containing synaptosomes enabled us to separate C1q-tagged and untagged fractions of the sample, with fluorescent activated synaptosome sorting. Since the selective phagocytosis of C1q tagged synapses has been described in diseased and normal brains, sorting the synapses to tagged and untagged fractions allows discovering protein differences between them with gel electrophoresis and mass spectrometry based proteomics.

P2/104

The role of local feature, holistic shape and color information in object categorization

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In the present electroencephalographic (EEG) study two experiments were carried out with the participation of 38 healthy subjects to test the assumption that color removal influences object categorization and related neural processes more robustly when global image information is disrupted. In the experiments a car vs. bird classification of colorful and grayscale photos was required, which were presented either in their original, easily identifiable version or in three scrambled versions with reducing recognizability. In support of the negligible role of colors when shapes are easily recognized, similar classification performance and event-related potentials (ERPs) were obtained

for chromatic and achromatic pictures. Notably, the recognition of moderately scrambled images was more difficult when colors were absent, and in accordance with this, significant color-related neural effects appeared from approximately 150 ms after stimulus onset above posterior brain regions only. In addition, neural responses were relatively independent of target-detection/motor response and not entirely related to feature-specific attention to colors.

P2/105**The development of executive functions in secondary school students participating in bilingual education**

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The term of executive functions (EFs) refers to a group of inter-related processes (e.g. monitoring, mental flexibility, inhibition, planning, organization and anticipation) responsible for purposeful, goal-directed behavior, playing an important role in cognitive processes, behavior, social interactions and emotional regulation. Since the operation of EFs is regulated by the frontal cortex, these processes are thought to have a prolonged maturation period ending only in early adulthood, ensuring a longer time for development. There are many factors influencing the changes in EFs, one of which is bilingualism. Recent studies have concluded that the brain development of bilinguals differ from that of monolingual individuals, bilinguals having certain advantages on tasks requiring the use of monitoring, inhibition and mental shifting processes. In their investigation Prior and Macwhinney (2010) explained these results by bilinguals having to use certain EFs more often in order to be able to successfully switch between two languages (monitoring, mental shifting) and inhibiting the currently unused language during this process to prevent interference (inhibition). Based on the authors' assumption, the more frequent use of these mechanisms in bilinguals leads to the more efficient development of these functions. Several current experiments have also found that these characteristics might not only be present in "early" bilinguals but also exist in subjects acquiring the second language in a high level during later years of life. To investigate this hypothesis the present study aims to explore the effects of bilingual education on the development of the EFs of Hungarian high school students. The results have high educational and cognitive psychological relevance.

P2/106**The correlation of mood, anxiety, coping mechanisms and personality to bilingual education**

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These days many people use more than one language during their course of daily life. A special group among these individuals are the so-called bilinguals, who acquire and use multiple languages in a very high level. Nowadays bilingual education being widely popular, current researches investigating the subject of dual language use also extended their focus on the effects of dual-language education having on children. Based on the growing number of studies many authors have concluded that the neural development of monolingual and bilingual children are different, both groups having certain advantages and disadvantages themselves. For example, while monolinguals perform better on tasks requiring lexical knowledge, bilinguals achieve better when the use of executive functions (i.e. inhibition, mental shifting, monitoring) is needed. However, there are no differences between the two groups regarding working memory. Furthermore, many researchers have found that individuals having some advantages in executive functions can also be characterized with better mood, lower level of anxiety, more adaptive coping mechanisms, more openness and emotional stability. A plausible explanation to this might be that both executive functions, emotion regulation and personality are regulated by the frontal lobe, moreover executive processes themselves have an important contribution to regulation and control mechanisms. Thus, the goal of the present study is to investigate the possible correlations of mood, anxiety, coping strategies and personality with bilingual education. The administration of the Beck Depression Index, the Spielberger State-Trait Anxiety Inventory, the Coping Inventory for Stressful Situations and the Eysenck Personality Questionnaire to 14-, 16- and 18-year-old bilingual, high-level English, and high-level Hungarian-History students enables us to examine differences both related to age and form of education. The present research has both educational and psychological relevance,

aiming to explore the complex effects of dual-language education on mood, anxiety, coping and personality. The results can not only be of high importance to further scientific investigations, but can also be helpful to students when deciding about high school applications.

P1/57**Specialized cortical feed-back in the cortex-basal ganglia-thalamus loop**

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Complex cognitive functions (e.g. perception, planning and organization of movements) depend on intact functional connectivity between the neocortex and the higher order (HO) thalamus. HO thalamic nuclei receive large, cortical terminals arising in layer 5 (L5) in addition to small, layer 6 (L6) cortical terminals. Whereas L6 inputs can only modulate thalamic activity (modulatory terminals), L5 afferents (similarly to the subcortical driver inputs in the first order thalamic nuclei) reliably drive their thalamic targets in the HO thalamus (driver terminals). Basal ganglia (BG) recipient thalamic nuclei are considered as HO but contain no large corticothalamic terminals (Rovo et al., 2012). Since the BG recipient thalamic region is pivotal in motor execution and planning and is involved in Parkinson's disease, its cortical control is of significant interest. We tested if L5 input to BG recipient thalamic nucleus of the mouse (ventromedial n. VM) display any nucleus specific structural or functional properties, using L5 specific transgenic lines (Thy1-Chr2-EYFP and Rbp4-Cre).

Selective labeling of L5 axons revealed small diameter (1-2µm) terminals in the VM compared to the giant L5 driver terminals in the somatosensory HO nucleus (posterior nucleus, Po, 4-8µm). The size range of L5 boutons in VM was close to that of modulatory L6 inputs. At the ultrastructural level, however, L5 and L6 terminals displayed significant differences in VM. Compared to L6 boutons, L5 boutons established much larger often multiple perforated or branching synapses, always contained mitochondria and had significantly larger volume. L5 terminals innervated specialized large diameter (>300nm) dendritic appendages (often reminiscent of spines) whereas L6 terminals targeted thin (≤0,5 µm) dendritic shafts. Photoactivation of L5 input reliably drove VM neurons and the response did not or only mildly depressed up to 20 Hz, unlike in Po, where L5 response displayed significant depression above 5 Hz. Unexpectedly, we found that VM cells can be driven by L5 input arising from the contralateral cortex. According to our preliminary data, L5 terminals from the contralateral cortex have larger volume and postsynaptic density area compared to the L5 terminals from the ipsilateral cortex. Our results demonstrate that neocortex can exert strong, top-down influence on the information transfer at the thalamic stage of the basal ganglia circuit via morphologically specialized synaptic connections.

P2/51**Evaluation of the components of the cortico-cortical evoked potentials with single and paired pulse subdural electrical stimulation in epilepsy patients**

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Cortical electrical, and transcranial magnetic stimulation (CES and TMS) can be reliably used in the investigation of the cortical excitability, the localization of the seizure focus and the mapping of the cortico-cortical networks. Moreover they can be effective in the therapy of drug-resistant epilepsy. Paired-pulse TMS study delivering preconditioning and test stimulus with various ISIs showed short interval inhibition (1-6ms), a short interval excitation (8-30ms) and a long interval inhibition (50-200 ms). Recently our group found that CES applied on subdural electrodes resulted in a single wave of slow oscillation (SO) regardless of the vigilance state of the patient. Physiological mechanisms underlying electrophysiological changes observed during cortical electrical stimulation is barely known in humans, although there are presumptions regarding to the excitatory and inhibitory neuronal mechanisms during paroxysmal discharges.

We studied single (n=8) and paired-pulse (n=1) electrical cortical stimulation on drug-resistant epilepsy patients implanted with subdural grid and strip electrodes, and laminar multielectrodes (24 contact, 200µm). We applied a

brief single (10mA, 0.2ms, 0.5Hz) and pair (ISI: 6.6, 10, 20, 30, 40, 50, 100, 200, 500, 1000ms, on the best single response electrode-pair) current pulses on adjacent contacts of the grid and strip electrodes. Various phases of cortico-cortical evoked potentials (CCEP) were analysed using custom scripts in Matlab.

We found P1, N1, P2, N2, P3 components with average latencies of 7, 30, 60, 120, 350 ms respectively. The larger was the distance between stimulating and recording electrodes on the same gyrus, the longer latency components were evoked only. The laminar profile showed surface sink and layer IV source for N1, surface source and layer IV sink for P2, and cortical wide source for N2 in the middle layers. Amplitude difference of P1-N1, N1-P2, P2-N2 was correlated with ISI. Values measured at 2000ms ISI was used as baseline. P1-N1 amplitude was increased at ISI 500 and 1000ms, and decreased at 100 and 200ms.

We were able to describe the laminar profile of different phases of the CCEPs, and succeeded to identify excitatory (P1, N1) and inhibitory (P2, N2) components. Previously reported evoked SO was identical to N2 in the present paper. In our paired pulse stimulation setting we demonstrated these inhibitory and excitatory effects on the second CCEP response.

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P1/38

Significance of different bilateral pathological electrophysiological signs in medial temporal lobe epilepsy-Correlation of foramen ovale data with surgical outcome, MRI, PET, and neuropsychological findings

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Temporal lobe epilepsy (TLE) is the most common form of therapy resistant focal epilepsy. Temporal lobectomy provides seizure freedom in 60-80% of cases where unilateral seizure onset was observed. Unilateral pathology and seizure onset does not necessarily mean that the seizure remains unilateral. Several patients who underwent surgical intervention show bilateral neuropsychological, fMRI, PET and interictal EEG abnormalities. Despite the fact that bilateral involvement is associated with poor surgical outcome, no thorough evaluation of the question has yet been published. We assume that the examination of the varying degree of involvement of the contralateral side is not only an important factor in the prediction of postoperative seizure outcome but also brings us closer to the understanding of the progression of temporal lobe epilepsy.

In the current study, we analyzed data from 40 mesial temporal lobe epilepsy (mTLE) patients with foramen ovale (FO) electrodes. 24 of the 40 patients underwent temporal lobectomy. We analyzed patterns of bilaterality in interictal FO spiking, side of seizure onset in FO electrodes, latency of seizure propagation to the contralateral FO electrode, frequency of the seizure propagation pattern, side of seizure termination, the involvement of the scalp electrodes, surgical outcome (Wieser test registered yearly), MRI, PET and neuropsychological data.

We identified specific bilateral electric phenomena during interictal, seizure onset, ictal and postictal periods, such as contralateral electric inhibition, the sudden increase of frequency on the same or the contralateral side (induction), and the alternating involvement of the two sides, called flip-flop mechanism. Average follow up time after surgery was 7.3 (1-15) years. The rate of seizure freedom (Wieser 1 and 2) based on the last 3 years of follow-up was 62.5% (15/24). Data analysis was focused on the relationship of the speed of contralateral seizure propagation (<10sec), the frequency of contralateral seizure propagation (<10Hz), the existence of ipsilateral or contralateral induction, contralateral electric inhibition, flip-flop mechanism and seizure termination with surgical outcome, neuropsychological, PET and MRI data. We believe that unequivocal seizure onset is not a sufficient determinant of surgery outcome. Data suggests that seizure propagation differences may signify different outcome variations.

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P1/36

Validation of the error of CT to MRI registration in deep brain stimulation patients

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Background

Fusion of the postoperative CT to the preoperative MRI images is a frequently used method to define the location of the DBS electrodes. However, fusion error can produce unreliable results. The CT to MRI fusion hasn't been systematically verified yet. In this study we wanted to provide a method which can define the fusion error of the CT to MRI registration algorithm and provide data if it is reliable on a clinically acceptable level.

Methods

6 patients, who underwent bilateral STN DBS implantation, were enrolled in the study. All of them had postoperative CT (axial 1 mm slices, no gap, no gentry tilt) and MRI (axial 3D T1, 1x1x1 mm voxel size, 1.5 T scanner) images. The FSL's (Oxford University) FLIRT tool was used to register the CT images to the MRI space (nonlinear registration, 6 degrees of freedom, mutual information). The registration was done 10 times in each patients. To exclude the metal artifact both on the CT and MRI images, one voxel was marked by using an intensity based center of gravity method in each axial slice at the contacts levels. Finally we compared the overlap and distance of the electrodes on the CT and MRI images.

Results

We found overlapping of the electrodes location on the CT and MRI images 61.19% on the left and 46.88% on the right side of the axial slices. In the remaining slices, the electrodes were in the neighbouring voxels on the CT and MRI images.

Conclusion

According to our results, the error of CT to MRI fusion is between 0-1 mm. Therefore we believe that this method can be reliably used to define the DBS electrodes position. However different CT and MRI imaging parameters can cause different fusion error but the above described method can be used to define the fusion error of the center's individual imaging parameters.

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P2/37

N-acetyl cysteine treatment rescues cellular deficits in the animal model of schizophrenia

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Schizophrenia is a mental disorder, which affects nearly 1% of the population. Polymorphisms at G72 locus are considered to show one of the most robust associations with the disease. Transgenic (G72Tg) mice that express the human LG72 protein (translated from gene G72), show behavioural changes that are expected in an animal model of schizophrenia. Physiological data suggest changes in the plasticity of perforant path synapses on granule cells of the dentate gyrus, where LG72 is strongly expressed in G72Tg mice. Therefore, we investigated the underlying cellular or molecular alterations in dentate gyrus. Using stereological methods, we found that transgenic mice had about 10% fewer granule cells and the molecular layer of the dentate gyrus received about 36% fewer excitatory synapses in the termination zone of the perforant path, compared to wild type littermates. On the other hand, results obtained with pre- and post-embedding methods showed that these excitatory synapses are about 32% larger in G72Tg mice and therefore contain more NMDA receptors per synapse, compared to wild type littermates. Previous data suggest that LG72 protein can induce mitochondrial alterations and can elevate oxidative stress level, which could be responsible for the observed changes. Oxidative stress lowers the level of the reduced form of glutathione (GSH) in the brain. Because, previously, per os N-acetyl-cysteine treatment (NAC is a precursor molecule of GSH) ameliorated behavioural alterations in G72Tg mice, we tested whether it can reverse the observed cellular changes as well. We found that NAC treatment could almost completely restore the number of

granule cells and the number of excitatory synapses to the wild type level, while the size of the examined NMDA-receptor-containing synapses were also restored by about 22%. These indicate that NAC treatment rescues this type of cellular deficits in this schizophrenia model, which suggest that NAC treatment can be a real causal treatment for G72 gene related schizophrenia.

P2/30**Endocannabinoid-induced endocannabinoid release from spinal astrocytes**

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Accumulating evidence supports the role of astrocytes in endocannabinoid modulation of neural activities. It has been reported that astrocytes express CB1 receptors (CB1-R), and have the potential to produce the endogenous cannabinoid ligand 2-arachidonoylglycerol (2-AG). However, any relationship between astroglial CB1-R expression and 2-AG production has never been demonstrated. Here we show that rat spinal astrocytes both in vivo and in culture co-express CB1-Rs and diacylglycerol lipase alpha (DGL-α), synthesizing enzymes of 2-AG, in close vicinity to each other. We also demonstrate that due to the activation of their CB1-Rs astrocytes show a substantial elevation in intracellular calcium concentration. Finally, we provide evidence that the evoked calcium transients lead to the release of 2-AG from cultured astrocytes. Our results support the notion that there is neuron-astrocyte-neuron bidirectional communication pathway which is mediated by endocannabinoids in both directions, and suggest that 2-AG released by astrocytes must be considered in the interpretation of the effects of endocannabinoids on neural activities.

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P1/24**Astrocytic synchronization is coupled to recurrent seizure-like events**

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Excessive synchronization of neurons is a hallmark of epileptic conditions. Accordingly, neuronal activation patterns are extensively studied during seizures. However, much less is known about the role of astrocytes in the generation, maintenance and termination of seizures. Since increasing evidence suggest that astrocytes are able to respond to and modulate neuronal function both in physiological and pathophysiological conditions, we investigated whether astrocytic networks also contribute to epileptogenesis. We observed that seizure-like events in the low-[Mg²⁺] in vitro epilepsy model provoke astrocytes to respond by massive, long-range synchronization which is spatiotemporally coupled to the synchronized neuronal activity reaching its maximum at the tonic/clonic transition. Cross-correlation analysis of neuronal and astroglial Ca²⁺ signalling demonstrates that high degree of synchronization arises in the population of both astrocytes and neurons. Importantly, astrocytes also synchronize their activity to neurons, manifesting in astroglial seizure-like events. The delay of astrocytic to neuronal synchronization suggests causal relationship between the two emergent processes. We further show that gap junction proteins contribute to astroglial synchronization since their inhibition by carbenoxolone increased the interictal interval or completely prevented recurrent seizure-like activity. In addition, carbenoxolone also induced unsynchronized Ca²⁺ transients associated with decreasing incidence of epileptiform discharges afterwards. We may anticipate therefore that local, unsynchronized astroglial Ca²⁺ transients inhibit, while long-range, synchronized Ca²⁺ signalling promotes recurrent seizure-like events.

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P1/87

Hemokinin-1 regulates chronic restraint stress-induced behavioural alterations in mice

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The Tac1 gene-encoded Substance P acting predominantly at the tachykinin NK1 receptor has been described to be involved in psychiatric disorders, but NK1 antagonists as antidepressants failed in clinical trials. The Tac4 gene-derived hemokinin-1 (HK-1) is present in brain regions related to anxiety, stress and pain. It also activates the NK1 receptor with different binding site and signaling pathways, but several other mechanisms have also been proposed for its actions. Therefore, we investigated the involvement of HK-1 in chronic stress-evoked behavioural alterations and pain reactions.

Chronic restraintment was performed in 25 mm diameter plastic tubes for 6 h/day during 4 weeks in C57Bl/6 wildtype (WT) and Tac4 gene-deleted (Tac4^{-/-}) mice. Touch sensitivity was assessed with aesthesiometry, cold tolerance by paw withdrawal latency at 0°C, anxiety and locomotor activity in the light-dark box (LDB) and tail suspension tests (TST). Adrenal gland and thymus weights were measured at the end of the study.

Non-stressed Tac4^{-/-} mice spent significantly less time in the lit compartment of LDB and showed longer immobility in TST. Their thymus weighed significantly less and adrenal gland more compared to the WT as characteristic non-behavioural responses to chronic stress. In WT stress induced a 20% mechanical and a 75% cold hyperalgesia related to primarily central and peripheral sensitization processes, respectively. Stressed WT mice spent more time in the light with exploration showing increased anxiety, thymus weight decreased and adrenal gland increased. Significantly smaller mechanical, but not cold hyperalgesia developed in stressed Tac4^{-/-} animals than in WT. They spent remarkably more time in light part of LDB and showed increased mobility in the TST. The stress-induced thymus and adrenal gland weight changes were absent in the Tac4^{-/-} group as compared both to respective non-stressed controls and stressed WT.

We provide the first evidence for HK-1-mediated potent anti-anxiety, anti-depressant and central pro-nociceptive mechanisms under normal and chronic stress conditions. Identification of its targets and signaling can open new directions in anxiety and depression research.

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P2/44

Hemokinin-1 is an important mediator of hyperalgesia in a chronic traumatic neuropathy model of the mouse

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Traumatic neuropathy is a chronic persistent pain condition caused by mechanical injury of peripheral nerves. The pathophysiological mechanisms are not understood, and there is no satisfactory therapy. Although the regulatory role of tachykinins have earlier been proposed, our results have not supported the involvement of substance P and its NK-1 receptor in neuropathic pain. The Tac4 gene-encoded hemokinin-1 (HK-1) has similar immunological and pharmacological properties to substance P: it also activates the NK1 receptor with different binding site and signaling pathways, but several other mechanisms have been proposed. Our goal was to examine the role of HK-1 in neuropathic hyperalgesia.

Traumatic mononeuropathy was induced by tight ligation of 1/2-1/3 of the right sciatic nerve in male C57Bl/6 (wildtype) and HK-1 gene-deficient (Tac4^{-/-}) mice. On the 3rd, 5th and 7th postoperative days the mechanonociceptive threshold was measured by dynamic plantar aesthesiometry, cold tolerance with paw

withdrawal latency from 0°C water and motor coordination on the accelerating RotaRod wheel. Nerve growth factor (NGF) was determined with ELISA from homogenates of the hindpaws excised on day 7 after the operation. The mechanonociceptive threshold of wildtype mice decreased by 35% (mechanical hyperalgesia), the cold tolerance by 70% (cold hyperalgesia) by the 5th postoperative day, but motor function did not change. In contrast, in the Tac4^{-/-} group nerve ligation-induced both mechanical and cold hyperalgesia were significantly smaller compared to the wildtypes. Although motor function did not change in neuropathic mice, motor coordination was basically significantly worse in HK-1-deficient animals throughout the whole experiment. The control NGF-level in Tac4^{-/-} mice was lower compared to the wildtypes. The NGF level did not change seven days after nerve ligation in the paw of wildtype mice, but significantly increased in Tac4^{-/-} ones as compared to their respective intact controls.

We provide the first evidence for an important role of HK-1 in the development of mechanical and cold hyperalgesia in traumatic mononeuropathy, in which the inhibition of peripheral NGF production can be an important factor.

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P2/45

Development and characterization of a novel murine passive-transfer-trauma model for Complex Regional Pain Syndrome (CRPS)

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The etiology and pathophysiological mechanisms of Complex Regional Pain Syndrome (CRPS) usually developing after a small limb injury are unclear. Immune response against antigens released from sensory nerves and complex neuro-immune interactions are suggested to be responsible for the severe, persistent pain, hyperalgesia, edema and autonomic symptoms. Since an appropriate animal model is needed for experimental investigation of the pathophysiological processes, we set up a murine passive transfer-trauma model of CRPS.

Limb injury was evoked by plantar skin-muscle incision in female C57Bl/6 mice (day 0), and they were treated with purified serum-IgG of CRPS patients and healthy volunteers daily during 10-day-experimental periods. The mechanonociceptive threshold was measured with aesthesiometry, paw volume with plethysmometry, motor coordination with RotaRod, spontaneous locomotor activity in the open field, paw temperature with a contact thermometer, plasma leakage, reactive oxygen species (ROS) generation and myeloperoxidase activity (MPO) with fluorescent and luminescent in vivo imaging. Sensory neuropeptides and inflammatory cytokines were measured with radioimmunoassay and ELISA/Luminex, respectively, in the paw homogenates.

Similarly to the clinical signs, paw oedema and mechanical hyperalgesia of the CRPS-IgG-treated mice were significantly increased and longer lasting compared to the healthy-IgG treated group. These difference were much greater, when the animals were treated every day. However, CRPS-IgG did not influence motor coordination, locomotor activity and paw temperature. Plasma leakage did not occur in this model, but both ROS and MPO activities were significantly increased by the CRPS-IgG even in the non-injured paws. Substance P (SP)-like immunoreactivity in the paw significantly elevated, which was remarkably increased by CRPS-IgG. Concentrations of calcitonin gene-related peptide and several inflammatory cytokines did not change in the paw on day 10.

We provide the first evidence that the characteristic clinical signs of CRPS can be elicited in mice by passive transfer of the patients's IgG fraction, strongly suggesting the pathophysiological role of autoantibodies. Our model is suitable for precise identification of mechanisms and key mediators of CRPS. Selective increase of SP originating from sensory nerves and immune cells in the paw suggests, that it may play a role in these processes.

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P1/105

Resting-state functional connectivity predicts the repetition suppression of fMRI responses in the fusiform gyrusPetra Hermann^{1,2}, Mareike Grotheer^{3,4}, Gyula Kovács^{3,4,5}, Zoltán Vidnyánszky^{1,5}¹Research Centre for Natural Sciences, Hungarian Academy of Sciences, Brain Imaging Centre, Budapest, Hungary;²Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest, Hungary; ³Friedrich-Schiller-University of Jena, Institute of Psychology, Jena, Germany; ⁴Friedrich-Schiller-University of Jena, DFG Research Unit Person Perception, Jena, Germany; ⁵Budapest University of Technology and Economics, Department of Cognitive Science, Budapest, Hungary

Repetition suppression (RS) of fMRI responses is a well-established phenomenon reflected in reduced blood oxygen level dependent (BOLD) signal to repeated presentation of a specific stimulus. It has been shown that the repetition of identical face stimuli leads to fMRI response attenuation in the face-selective region of the fusiform gyrus, namely the fusiform face area (FFA). However, the neural source of the RS observed in the FFA is unclear as of today. Therefore, here we aimed at identifying regions contributing to the FFA RS by measuring both the repetition-related reduction of the fMRI responses and the resting-state functional connectivity of the right FFA in the same human participants. Our results revealed that the strength of the intrinsic functional connectivity between the FFA and an extended network of brain areas including the striatum, thalamus, insula, supplementary motor area, middle frontal and precentral gyrus predicts the size of the RS effect in the FFA: the stronger the functional connectivity between these regions during rest, the higher the magnitude of the RS for repeated face stimuli in the FFA. Thus, here we identified a cortical network underlying repetition suppression in the fusiform gyrus and showed that the intrinsic functional connectivity within this network predicts the inter-individual differences in the magnitude of the FFA response decrement in the case of repeated face images.

P2/53**The electrical threshold to trigger cortical spreading depolarization is altered by brain maturation and ischemia**Péter Hertelendy¹, Ákos Menyhárt¹, Tamás Kiss¹, Gergely Tóth¹, Ferenc Bari¹, Eszter Farkas¹¹University of Szeged, Department of Medical Physics and Informatics, Szeged, Hungary

Spreading depolarization (SD) is a wave of synchronized depolarization of neurons and glia cells, which propagates across the cerebral gray matter at a rate of 2–6 mm/min. SD is closely followed by a local hemodynamic response characteristic of the metabolic state of the tissue. SD is suggested to be the cause of aura in classical migraine, and also occurs in the vicinity of ischemic brain lesions, where it is considered to aggravate the initial damage. Even though age is a highly significant predictor of both migraine and stroke, very few studies investigated whether aging has an impact on SD evolution.

The major goal of this study was to determine the electric threshold of SD elicitation before and during incomplete forebrain ischemia in rats of various age groups.

Male Sprague-Dawley rats (n=33, age groups: 7, 8, 9, 10, 12 and 16 weeks-old) were anesthetized with isoflurane in N₂O:O₂. Both common carotid arteries were dissected for the latter induction of incomplete forebrain ischemia by occlusion of the vessels (2VO). Two, separate craniotomies were created on the right parietal bone for SD elicitation and data acquisition. SDs were triggered by cathodal direct current stimulation of the dura mater, allowing the calculation of the exact amount of current delivered. To determine the threshold of an SD, the current was elevated stepwise, until SD was detected. SDs were identified using DC- and local-field potentials. In order to discriminate between the non-ischemic and ischemic threshold of SD elicitation, 3 SDs were elicited prior ischemia induction. Next, 2VO was implemented. Three additional SDs were elicited during ischemia.

The threshold of SD elicitation was higher ($2142 \pm 318 \mu\text{C}$) during ischemia as compared with the non-ischemic condition ($3451 \pm 687 \mu\text{C}$), in all age groups studied. The threshold proved to be the lowest in the 9-week-old group, both for non-ischemic ($1045 \pm 330 \mu\text{C}$) and ischemic SDs ($1827 \pm 712 \mu\text{C}$), and the highest in the 12-16-week-old groups (non-ischemic: $3076 \pm 301 \mu\text{C}$; ischemic: $5343 \pm 65 \mu\text{C}$).

Our data demonstrate that the threshold to trigger SDs increases with progressing life time. The susceptibility of the brain to SD may be determined by the gray matter's biochemistry and cytoarchitecture that undergo adaptational changes with maturation. The threshold of SD elicitation also increased during ischemia. Since decreasing pH has been known to hinder SD evolution, acidosis created by ischemia is suggested to inhibit SD elicitation.

P1/42

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

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Epilepsy is a neurological disorder associated with neuronal hyperactivity. When pharmacological treatment proves to be ineffective, surgical tissue removal is considered as an alternative. Cortical brain tissue obtained this way shows spontaneous population activity (SPA), which has been associated with epileptic processes. Here, neocortical tissue obtained via brain tumor surgery has been used as a non-epileptic control. SPA could be detected in tissue slices obtained from both epileptic and non-epileptic patients. This study investigates similarities and differences in neocortical synchronous population activity in epileptic and non-epileptic tissue.

The local field potential gradient (LFPg) was recorded from brain tissue slices using a 24 channel laminar microelectrode. SPA consists of high frequency oscillations and elevated cell firing superimposed on a LFPg transient. Cell clustering analysis shows, that while some neurons increase, some decrease and some do not change their firing during SPAs, the extent to which neurons change their firing and the ratios of the neurons behaving in different ways significantly differ between epileptic tissue and non-epileptic tissue. Moreover, both principal cells and interneurons participate in the generation of SPAs, but they show differences in their cell firing. The involvement of a neuron also depends on its location relative to the site of the SPA.

In summary, even though slices from both epileptic and non-epileptic tissue generate spontaneous population activity, the involvement of different groups of neurons differs significantly.

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Role of the Transient Receptor Potential Ankyrin 1 (TRPA1) ion channel in the acute and chronic inflammatory pain models of the mouse

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Introduction: Transient Receptor Potential Ankyrin 1 (TRPA1) is calcium-permeable non-selective cation channel, which is predominantly expressed on capsaicin-sensitive sensory nerve endings. Beside cold temperature (<17 °C) TRPA1 is stimulated by many endogenous noxious agents and exogenous irritants like mustard oil, cinnamaldehyde, H₂S and allicin. Since few contradictory data are available on the involvement of this receptor in inflammation and nociception, we aimed to investigate the role of TRPA1 in acute and chronic inflammatory pain models.

Methods: Experiments were performed with male TRPA1 gene-deficient mice (TRPA1^{-/-}) in comparison with their wildtypes (TRPA1^{+/+}). The acute inflammation was evoked by intraplantar injection of 3% carrageenan, while the chronic inflammation was induced by injected Complete Freund's adjuvant (CFA) in the right hindpaw and the root of the tail. The noxious heat and the mechanonociceptive thresholds were measured with increasing-temperature hot plate or dynamic plantar aesthesiometer and the paw volume with plethysmometry. Cold allodynia was determined by the latency decrease of the nocifensive behaviours in icy water. The activity of neutrophil myeloperoxidase was investigated with luminol based luminescent in vivo imaging in the CFA-model.

Results: In the TRPA1^{-/-} animals there were no significant difference between the carrageenan-induced inflammatory hyperalgesia as well as the paw volume compared to their wildtypes. In contrast, the mechanical hyperalgesia and paw edema were significantly lower in TRPA1^{-/-} mice in chronic inflammation model. The myeloperoxidase activity indicating neutrophil activation was not influenced by deficiency of TRPA1.

Conclusion: The chronic paw edema and mechanical hyperalgesia caused by central sensitization can be mediated by TRPA1. However it is not involved in the neutrophil activation and production of reactive oxygen species. This receptor may not play an important role in acute inflammation.

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P1/65

Interactions of auditory evoked responses during the sleep-wakefulness cycle in the cat

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Changes of auditory cortical evoked potentials depending on vigilance state are well known. However, underlying cellular and circuit level mechanisms are still unclear. In the present study, we have implanted chronically metal linear array multielectrodes into the medial geniculate body (MGB) and auditory cortex (AC) of cats. We recorded local field potential (LFP), multiunit (MUA) and single unit activity (SUA) evoked by paired clicks or short snippets of wideband noise from the MGB or AC of freely moving and behaving animals. We analyzed and compared the properties of evoked responses during the different phases of the sleep-wakefulness cycle. We have found in the LFP that response to the second stimulus of the pair is modulated by the phase of evoked potential elicited by the first stimulus both in MGB and AC. During slow-wave sleep (SWS), late positive phase of the first evoked potential effectively attenuates the amplitude of evoked potential elicited by the second stimulus. The amount of attenuation depends on the phase of potential oscillation evoked by the first stimulus. Similar changes were observed in the unit responses.

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P2/34

Poincaré plot analysis of temperature variability in control and schizophrenic rats

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Introduction: Besides the frequency domain analysis, a valuable tool to determine the variability of physiological parameters (e.g. heart rate) is the Poincaré plot analysis (PPA). This time domain analysis can determine short and long term variabilities (SD1 and SD2) and the asymmetry in these values. Body temperature (BT) variabilities have not yet been investigated by this method; therefore, the goal was to reveal the effects of day phases (active/dark and passive/light) on the Poincaré parameters in control rats and to disclose the potential disturbances of a new substrain showing several signs of schizophrenia.

Methods: Naive male Wistar rats without any treatment and the 18th generation of selectively bred animals with both isolation and ketamine treatment were implanted intraabdominally with E-Mitter transmitters at the age of 6 month, and the BT was monitored for 8 days without any disturbances. The PPA was performed separately for light and dark periods, i.e. SD1 and SD2 values and their asymmetries (BTA) were determined.

Results: BT showed circadian rhythm in both groups, but BT of the new substrain was higher during the active phase compared to the control group. The analysis of SD1 and SD2 values showed that the animals in both groups have significantly higher values during the active phase compared to the passive period. The new substrain had significantly higher short-lasting variability during the active phase, and these animals had higher long-lasting variability during both phases than control rats. Regarding BTA, both short- and long-term asymmetries were present, thus in the case of short-term asymmetry the decreases, while in the case of long-term asymmetry the increases of the body temperature variabilities were dominated. The active phase accompanied with decreased asymmetry compared to the passive one in the control group, but not in the new substrain. The comparison of the long-term asymmetry of dark vs. light phases did not show significant differences in either group, but the new substrain had lower level of asymmetry in both day periods.

Conclusion: Our results showed disturbed thermoregulation in a new substrain related to schizophrenia. We firstly characterized the temperature variability and asymmetry with PPA showing that this kind of analysis might be a useful technique for detecting changes in thermoregulation.

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P1/78

Pubertal changes in neuropeptide gene expression of the hypothalamic arcuate nucleus in male miceErik Hrabovszky¹, Csilla S. Molnár¹, Miklós Sárvári¹, Csaba Vastagh¹, Csilla Murnyi¹, Csaba Fekete², Zsolt Liposits¹¹*Institute of Experimental Medicine, Laboratory of Endocrine Neurobiology, Budapest, Hungary;* ²*Institute of Experimental Medicine, Laboratory of Integrative Neurobiology, Budapest, Hungary*

Neuropeptides of the hypothalamic arcuate nucleus (ARC) regulate important homeostatic and endocrine functions and also play a critical role in pubertal development. Altered peptidergic and amino acidergic neurotransmission underlying the pubertal maturation of the ARC are poorly understood. Here we studied the pubertal shift in the gene expression profile of the ARC of male mice. RNA samples for quantitative RT-PCR studies were isolated from the ARC of day-14 prepubertal and day-60 adult male mice with laser-capture microdissection. The expression of 18 neuropeptide-, 15 neuropeptide receptor-, 4 sex steroid receptor and 6 classic neurotransmitter marker mRNAs were compared between the two groups. Adult animals showed increased mRNA levels encoding cocaine- and amphetamine-regulated transcript, galanin-like peptide, dynorphin, kisspeptin, proopiomelanocortin, proenkephalin and galanin and reduced expression of mRNAs for pituitary adenyl cyclase activating peptide, calcitonin gene-related peptide, neuropeptide Y, substance P, agouti-related protein, neurotensin and growth hormone-releasing hormone. From the tested neuropeptide receptors, melanocortin receptor-4 showed the most intriguing (5-fold) increase. Melanocortin receptor-3 and the Y1 and Y5 neuropeptide Y receptors increased 1.5-1.8-fold, whereas δ -opioid receptor and neurotensin receptor-1 transcripts were reduced by 27 and 21%, respectively. Androgen- progesterone- and α -estrogen receptor transcripts increased by 54-72%. The mRNAs of glutamic acid decarboxylase 65, and 67, vesicular GABA transporter and choline acetyltransferase remained unchanged. Tyrosine hydroxylase mRNA increased by 44%, whereas type-2 vesicular glutamate transporter mRNA decreased by 43% by adulthood. In ongoing studies, the functional significance and causal relationship to pubertal development of these changes are addressed with transgenic methodologies and the neuronal phenotypes showing altered receptor and glutamate marker coexpression are investigated with in situ approaches.

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P2/18**Expression of hyaluronan and chondroitin sulfate proteoglycans in the olfactory bulb of the rat**Andrea Hunyadi¹, Botond Gaál¹, Szilvia Kecskes¹, Ibolya van der Wijk¹, Klára Matesz^{1,2}¹*University of Debrecen, Department of Anatomy, Debrecen, Hungary;* ²*Hungarian Academy of Sciences, MTA DE - Neuroscience Research Group, Debrecen, Hungary*

The extracellular matrix (ECM) molecules fill the extracellular space. They may aggregate around the neurons and form the perineuronal net (PNN) or they are distributed throughout the neuropil. Hyaluronan (HA) is the first molecule synthesized, and is the backbone of PNNs, and anchors lecticans of the chondroitin sulfate proteoglycan (CSPG) family. The ECM molecules are involved in levels of plasticity, engaged in signal transduction pathways, and its expression is determined by neuronal activity.

In vertebrates smells are sensed by olfactory sensory neurons in the olfactory epithelium. After binding an odorant, receptors send signals into the olfactory bulb, where information is encoded during a complicated signal processing. How olfactory information is coded in the bulb and transmitted toward to the brain is still being researched.

The aim of our work was to map the distribution of HA and CSPG in the olfactory bulb, by histochemical reactions.

Examinations were performed on female adult Wistar rats. Olfactory bulbs were removed and fixed in St. Marie's fixative. On transverse sections reactions of HA and WFA were made and visualized by DAB. Areas of the glomeruli, external plexiform-, tufted cell-, and mitral cell layers were recorded.

HA showed diffuse expression throughout the olfactory bulb. Further accumulation formed sporadic PNNs around mitral cells. Severe difference occurred among glomeruli in their HA patterns suggesting differing presence. WFA staining was experienced in the internal plexiform layer and surrounding mitral cells, even forming PNNs. There was great difference between CSPG expression of glomeruli.

The continuous replacement of the olfactory epithelium suggests constant synaptic reorganization in the olfactory bulb. Variable WFA labeling in glomeruli suggests ongoing reorganization in synaptic units. The diffuse and high hyaluronan expression corresponds with this argument.

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P1/109

Long term behavioral and cognitive effects of PARP inhibitor (L-2286) in spontaneously hypertensive (SHR) and normotensive (WKY)

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Chronic hypertension induces oxidative stress and known to activate apoptosis and necrosis factors, affecting the brain and often leading to various cognitive disorders such as dementia. In hypertension, the inhibition of poly ADP-ribose polymerase (PARP) enzyme has been proven to be protective, anti-apoptotic and anti-necrotic. However, it is not known how long term inhibition of PARP enzyme activity may influence cognitive function of spontaneously hypertensive rats and their normal counterparts. Here we tested the putative long term behavioral effects of chronic intraoral administration of PARP-inhibitor (L-2286) compound on general locomotor activity and memory performance in spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats. Experimental animals were divided to four experimental groups: PARP-inhibitor treated WKY (n=12), control WKY (n=12); PARP-inhibitor treated SHR (n=15), control SHR (n=14). Treated animals received PARP-inhibitor (10 mg/kg b.w.) in their drinking water for 32 weeks. Behavioral tests were conducted at the beginning of PARP-inhibitor treatment, and at weeks 16 and 32. Open field test (OF) was used to measure general locomotor activity and exploration. Spontaneous alternation in T-maze was used for testing spatial working memory. Novel object recognition task (NOR) was used for testing long-term memory. In the OF, SHR rats were generally more active. Locomotor activity gradually decreased in both PARP-inhibitor treated groups, but not in hyperactive SHR controls. In T-maze alternation behavior, no effect of treatment (PARP-inhibitor vs. control) was observed within the strains. However, in the end of PARP-inhibitor treatment (in week 32), performance of treated animals in the NOR test was impaired in both strains compared to control groups.

In sum, treatment with PARP-inhibitor (L-2286) decreased locomotor activity of SHR rats, and impaired long-term memory performance with preserved spatial working memory function. Taken together, long term treatment with anti-apoptotic agents may not be fully beneficial for the reorganization of brain networks in physiological ageing and may interfere with cognitive function.

P1/14

Effects of optogenetic stimulation on motility of differentiating neural stem cells

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Neural stem/progenitor cells (NSCs/NPCs) are exposed to bioelectric stimuli from their birth to tissue integration (Flavell, Greenberg 2008). Spontaneous Ca-oscillations are spreading in the early neural tube (Donovan 1999), and giant depolarizing potentials are travelling in the developing brain (Ben-Ari 1989) before and during the formation of synaptically coupled neuronal networks. The responsiveness of various NSC/NPC populations to these stimuli or the necessity of such stimulation for survival, migration or adopting the right phenotype, however have not been explored in details. Novel optogenetic methods (Zhang et al., 2007) opened ways for controlled long-term, non-invasive stimulation of in vitro-maintained stem cell populations, and enabled us to follow the fate of stimulated cells in their later development. For non-invasive stimulation, channelrhodopsin- (ChR2)-expressing radial glia-like

(RGI) NSC populations (Marko et al., 2011) were established from CAGloxpxStoploxpxChr2(H134)-EYFP transgenic mouse embryos. The RGIloxpxChr2 cells were either transfected with Cre-recombinase resulting in RGIChr2+ cells or were used as Chr2-non-expressing (RGIChr2-) controls. Chr2-expressing NSCs were obtained also from existing RGI cell lines (Jelitai et al., 2007; Marko et al., 2011) by transfecting with Chr2-coding constructs. Radial glia-like NSCs display elongated cell shape, vivid nuclear translocation along the entire cell-axis, and migrate by sliding along neighbours. In contrast, differentiating progenies decrease motility with the appearance of neuronal features. Both, RGIChr2+ and RGIChr2- cells generated neurons in response to EGF withdrawal. RGIChr2+ cells responded to illumination (488nm) with inward cation currents. Illumination modified the motility of differentiating RGIChr2+ cells by decreasing both the distance and rate of nuclear translocations and migration. The findings demonstrated that optogenetic stimulation modifies the in vitro behaviour of differentiating radial glia-like neural stem cells and suggest that bioelectric activation influences the fate of developing neural cells from early stages of neuron formation.

Plenary lecture III.

Molecular steps involved in the exocytotic fusion of synaptic vesicles

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Neurotransmitter release from presynaptic nerve endings is mediated by Ca^{2+} -dependent exocytosis of synaptic vesicles. During the past years the molecular steps mediating exocytotic membrane fusion have been unraveled although a lot of open question remain. Fusion is carried out by the SNARE proteins synaptobrevin/VAMP, syntaxin 1, and SNAP-25. Upon membrane contact, the vesicular SNARE synaptobrevin forms complexes with the plasma membrane-resident SNAREs SNAP-25 and syntaxin 1. Complex formation proceeds from the N-terminal end towards the C-terminal membrane anchors, thus pulling the membranes together and initiating fusion ("zipper" hypothesis of SNARE function). The steps of SNARE assembly are controlled both by members of conserved protein families such as the SM- and CATCHR-proteins, and they are tightly controlled by specialist proteins responsible for calcium regulation such as the calcium sensor synaptotagmin and complexins.

In our own work, we have focused on understanding the mechanisms of SNARE assembly and SNARE-induced fusion using structural and biochemical approaches and in-vitro fusion reactions with native and artificial membranes. Our recent results lend strong support to the zipper hypothesis, showing that during SNARE complex formation the helical bundle extends into the membrane and that only few SNARE complexes may suffice for effective fusion of bilayers. Furthermore, we have studied intermediate states of the SNARE-dependent fusion pathway involving techniques such as cryo-electron microscopy, resulting in novel insights into the structure of fusion intermediates.

In addition, we have investigated the interactions of the calcium sensor synaptotagmin 1 with SNAREs and with membranes. Our results lend support to the view that the interactions with membranes that are primarily of electrostatic nature are indeed crucial for synaptotagmins function in regulating exocytosis. Currently we believe that the calcium-dependent formation of a cross-link between the vesicle and the plasma membrane may play a central role in activating the SNARE proteins

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Ligand-dependent estrogen and thyroid hormone receptor mRNA expression is modulated by the glia

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Estrogen and thyroid hormones regulate cerebellar development through their cognate receptors ER α,β and TR α,β . Glial cells are also involved in cerebellar tissue development in many ways. A growing body of evidence indicates that endocrine disrupting chemicals (EDs) can interfere with the regulatory effects of these hormones, however, their role in the potential modulation of glia-neuron interaction during nervous tissue development is little known.

Here we demonstrate in an in vitro experimental model that four EDs (bisphenol A, zearalenone, camphor and arsenic) administered in environmentally relevant concentrations alone and in combination significantly alter the expression of these nuclear receptors in primary cerebellar cell cultures. The effects of the listed EDs were not necessarily identical and depended on the presence or absence of either or both of the natural hormone ligands estrogen and T3. The results confirm previous observations that these EDs could act on multiple nuclear receptors simultaneously, and that the signaling modalities used by distinct EDs may be different. Therefore, our present findings may explain the synergistic and/or versatile influence of environmentally co-occurring EDs on CNS development.

P2/38**Does enriched environment have a neuroprotective effect on Parkinson's disease?**

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Introduction: Environmental enrichment is a popular strategy of neuroprotection. The protective effects of enriched environment have already been shown in traumatic, ischemic and toxic nervous system lesions. The aim of our study was to investigate the effects of early postnatal environmental enrichment in a rat model of Parkinson's disease in adulthood.

Methods: We used adult Wistar rats in our experiment. The animals of the standard group were placed under regular conditions. For environmental enrichment, we placed rats in larger cages, supplemented with toys of different shape and material during the first five postnatal weeks. Three months later the rats were treated with unilateral injections of 2 µl 6-OHDA (5 µg/ 1 µl) into the left substantia nigra, control animals received 2 µl physiological saline. Behavioral experiments were done before the injury, and 1, 10 days after the operation. After the behavioral testing tyrosine-hydroxylase immunohistochemistry was performed to label dopaminergic cells of the substantia nigra.

Results We observed, that animals kept under regular conditions showed more severe acute neurological symptoms 1 day after the injury compared to enriched animals. The 6-OHDA treatment made significant cell loss in the standard group: 40% of dopaminergic cells died, while in rats which were kept in enriched environment the cell loss was significantly lower, only 23%.

Conclusion: Our experiments provided evidence for the protective effect of early postnatal environmental enrichment in adulthood, because rats under regular circumstances showed more severe acute neurological signs and dopaminergic cell loss after 6-OHDA lesion of the substantia nigra compared to animals grew up in environmental enrichment.

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P2/56**Structured interactions established through learning determine the stability, global dynamics and the representation of activity sequences in a hippocampal network model**

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Sharp wave-ripples (SWRs) are transient bursts of population activity generated in “off-line” states of the hippocampus. During SWRs, neuronal populations “replay” activity patterns observed during theta-gamma activity in the exploring animal. Our novel hippocampal slice preparation generates SWRs spontaneously, and this has allowed the characterization of the firing behavior and synaptic inputs of various cell types during SWR activity, as

well as the direct measurement of the underlying cellular and synaptic parameters. We applied our recently developed software tool to systematically fit simplified models to the data, and built a large-scale model of the CA3 network. Simulated networks displayed spontaneous bursts of activity, but models in which the efficacies of recurrent excitatory connections were uniform or varied randomly could not reproduce the experimentally measured characteristics of SWRs such as the low average firing rates of pyramidal cells. When we used spike-timing-dependent plasticity with simulated place cell activity during exploration to set up the strength of recurrent synapses, the model network produced spontaneous SWRs during which learned place cell sequences were replayed on a faster time scale, and the level of activity was also physiological. We then manipulated the synaptic weight matrix in various ways to reveal which aspects of its structure are critical in determining the stability of global activity levels, the emergence of coherent fast oscillations, and the generation of activity sequences. When we shuffled the synaptic weights in ways that preserved their local statistics but changed the global structure, replay was eliminated as expected, but steady-state firing rates and the properties of oscillations were also radically altered. More sophisticated manipulations of the weight matrix allowed us to selectively probe the effects of different aspects of its structure on the network dynamics. We concluded that the detailed structure of weights, as established during learning, is not only essential for the expression of meaningful neural representations, but is also a major determinant of population-level dynamics, and is, in particular, critical in producing the experimentally measured characteristics of SWRs.

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P2/54

The stratified organization and disorganization of the inner plexiform layer revealed by TNAP activity in healthy and diabetic rat retina

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Tissue non-specific alkaline phosphatase (TNAP), an abundant ectophosphatase, is present in different organs including the brain and the retina of several vertebrate species. Evidence is emerging that TNAP might influence neural functions in multiple ways. In rat, in addition to retinal vessels, strong TNAP activity was found in photoreceptors and both synaptic layers. In the present study we identified eleven strata of the inner plexiform layer (IPL) by using TNAP histochemistry alone. The TNAP strata exactly corresponded to the strata seen after combined immunohistochemistry with four canonical IPL-markers (TH-ChAT-CR-PKCα). Therefore, as already described in other mammalian species, our data support the existence of multiple morphologically and functionally discernible IPL strata in rats as well. Remarkably, the stratification pattern of the IPL was severely disrupted in a diabetic rat model, even before changes in the canonical IPL markers were detectable. These findings indicate that TNAP histochemistry offers a more straightforward, but also more sensitive, method to investigate retinal strata and their diabetes-induced degeneration

P2/98

Age-dependent changes of theta oscillatory response to reward and punishment

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Processing of feedback stimuli is a core mechanism in the evaluation of the functionally significant aspects of outcome, guiding our behavior towards avoidance or approach. Rapid evaluation of the motivational impact of outcome events is reflected by specific stimulus-locked increase in the ongoing theta (4-7 Hz) oscillatory power of the EEG at the fronto-central sites.

The present study aimed to investigate the event-related oscillatory background of age-dependent changes in feedback evaluation related to reward or punishment. 64-channel EEG was recorded from 20 young and 17 old participants while they performed a monetary gambling task in which they had to choose between high and low monetary stakes trial-by-trial. Time-frequency analysis using Morlet-wavelet method was performed in the time window corresponding to the feedback about the outcome (gain or loss) of the previous choice.

Whole scalp analysis of the event-related spectral perturbation showed that lateralized theta response characterizes the feedback evaluation process mostly dependent on the type of the outcome event. This theta response was found to be sensitive to distinct properties of feedback stimuli in the young; however, it was attenuated and less reactive to these features in the elderly.

Substantial changes of the event-related oscillatory response in the theta range suggest altered processing of feedback stimuli in elderly which is possibly related to the altered behavioral adjustment strategies in ambiguous situations characterizing the elderly.

P2/16

Precerebellar nucleus without perineuronal net: The inferior olive

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A very complex sensorimotor integration is required for the maintenance of the equilibrium. The most important part of the underlying neural network is the vestibular nuclear complex of the brainstem which is sending direct or indirect signals via climbing and mossy fibers of the precerebellar nuclei to the cerebellum. Previous results on the morphologically and functionally different subdivisions of the vestibular nuclei showed an area-dependent molecular composition and distribution pattern of an aggrecan-based extracellular matrix (ECM). The aim of this study was to examine whether the only climbing fiber generating precerebellar nucleus, the inferior olive (IO) is associated with a specific ECM pattern.

We have observed positive reactions by using histochemical and immunohistochemical methods for the hyaluronan, tenascin-R, HAPLN1 link protein and various chondroitin sulfate proteoglycans in the IO of the rat. In the ECM positive neuropil, the small, ring-like structures might indicate the accumulation of ECM around the axon terminals of synaptic glomeruli established by the gap junction-connected spines of olivary projection neurons and extraolivary GABAergic and glutamatergic axon terminals. The most characteristic finding was the lack of perineuronal nets (PNN) presumably due to the absence of synapses on the perikarya and proximal dendrites. The staining intensity and distribution of ECM molecules revealed a number of differences between the somatosensory, visuomotor-vestibular and integrative nuclei of IO. The most striking result was the absence of aggrecan in the visuomotor dorsal cap nucleus, where the aggrecan-free neuropil may allow the rapid modulation of synaptic events during the oculomotor activity.

Our results showed that the heterogeneous distribution of the ECM is associated with the various cytoarchitecture, afferent and efferent connections, and physiological properties of functionally different subnuclei of the IO.

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P2/36

Decreased pain sensitivity induced by a three hit animal model of schizophrenia

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Introduction: Patients with schizophrenia are less sensitive to pain related to their reduced experience of painful sensation and specific deficits of pain processing as well. It may have significant consequences including the

potential for misdiagnosis, as well as delays in treatment, which can ultimately lead to worsened morbidity and increased mortality.

For translational researches it would be important to develop an appropriate chronic animal model that mimics this alteration in pain sensitivity as well. Thus we induced schizophrenia-related behavioral alterations by selective breeding after subchronic ketamine treatment with social isolation in rats. The aim of the present report was to assess the pain sensitivity changes of the individuals of the subsequent generations (V-XXIII.).

Methods: Twenty three generations of male Wistar rats after weaning (at 3 weeks of age) were both housed individually for 4 weeks and treated with ketamine (30 mg/kg intraperitoneally) for 3 weeks followed by resocialization. The control group did not receive any treatments. The body weight of the animals was measured weekly. The tail-flick (TF) test was applied to determine the acute heat pain sensitivity at 48 °C at the age of 3 and 9 weeks. The relative TF latency was also calculated as $[TF \text{ latency (s)} \times 100] / \text{body weight (g)}$.

Results: Regarding the body weight, it was significantly lower in the new rat line at the age of 9 weeks from the XVth generation, while this tendency could only be observed in certain generations at younger age. The TF latency was permanently and significantly longer in the new rat line compared to the naive control counterparts from the XXIst generation, both at the age of 3 and 9 weeks. Regarding the relative TF latency, it was permanently higher from the XVth at weaning and from the Xth generation at the age of 9 weeks.

Conclusion: Our study proved that selective breeding after a double hit treatment led to a permanently decreased pain sensitivity in an acute heat pain model, suggesting that this new rat line can simulate the hypoalgesic phenomenon related to schizophrenia. Since the increase in relative TF latency appeared at weaning before any kind of treatment from the XVth through 9 generations, it suggests an inheritable phenotypic alteration in pain sensitivity induced by selective breeding.

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Symp II. - Thursday

Attentional and reinforcement signals in nucleus basalis

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The nucleus basalis gives rise to the central cholinergic neuromodulatory system that innervates the entire neocortex and is thought to regulate cortical states, attention, and plasticity. However, it is not known when cholinergic neurons are recruited during behavior and how their activity might support different aspects of cognition. To address this we used optogenetic identification to record cholinergic neurons in an auditory detection task in mice. I will discuss how the responses of cholinergic and other nucleus basalis neurons are correlated with brain states, measures of sustained attention and reinforcement feedback.

P2/8

A method to analyze the human cortical spontaneous synchronous population activity in vitro with multimodal experiments

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From the cortical slices of epileptic and non-epileptic tumor patients maintained in physiological medium in vitro spontaneous synchronous population activity (SPA) emerges. 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. We introduced the two-photon line scan Ca-imaging technique on human in vitro slice preparations to gain additional information about the network mechanisms involved in the SPA generation. The excellent spatial coverage and resolution of this technique supplements the laminar extracellular, sharp intracellular and whole cell patch 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 and extracellular patch electrodes. Bulk loading of OGB-1-AM and SR101 was applied on the tissue. This way we were able to image the SPA related Ca-transients in pyramidal or interneuron cells with two-photon technique, simultaneously with extracellular and whole cell patch measurements. The patched cells were loaded with biocytin as well for later anatomical analysis. The filled cells were 3D reconstructed, and observed with light microscope, and transmission electron microscope.

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.

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P1/104

Reward enhances alerting and orienting through the modulation of EEG alpha oscillations

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A well-established neural mechanism underlying visual attention is the adaptive modulation of oscillatory EEG activity in the alpha band, which is related to cortical excitability. Visuospatial orienting is known to be reflected in suppressed contralateral and enhanced ipsilateral alpha oscillations over the visual cortex. The perspective of being rewarded on correct performance in a perceptual task boosts phasic alertness, which is, again, manifested in attenuated visual cortical alpha oscillations and enhanced perceptual sensitivity. However, the effects of reward during orienting have not yet been investigated.

To address this question, we conducted an experiment in which human subjects performed a delayed match-to-sample task with a visuospatial orienting and an alerting cue. The alerting cue, presented in the beginning of trials, informed participants about the (monetary) reward they would receive upon correct response. The orienting cue was 100% valid, and it was presented shortly before the sample stimulus. The critical question was that whether reward modulations during the orienting phase (following the orienting cue) would be a simple carryover of the global gain from the alerting phase, or would it amplify the lateralization instead, reflecting an adaptive gain control mechanism.

Our data show that prospective reward led to widespread suppression of alpha activity during the alerting phase. We also found that the orienting cue caused robust alpha lateralization: posterior alpha power was larger ipsilaterally than contralaterally to the cued hemifield. In the orienting phase, importantly, reward did not cause an independent global suppression in alpha power (as during alerting), but further strengthened the attention-related alpha lateralization as compared to the no-reward condition. Thus, these results support the hypothesis that reward actively amplifies adaptive gain control during orienting, as indexed by the strengthening of EEG alpha lateralization.

P2/110

Sleep spindle frequency is modulated by body temperature

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Body core temperature has been known to vary across states of consciousness as well as different stages of sleep, and correlate with certain properties, such as sleep spindle frequency. It is not established, however, if there is a direct effect of temperature on spindling, or both are influenced by some common regulator.

Here we investigated the properties of sleep spindles in urethane anesthetized mice at different body temperatures. We recorded multiunit spindles from the ventrobasal nucleus of the thalamus with multisite silicon

probes while varying the temperature between 34 and 39 °C.

At 34 and 39 °C no spontaneous spindling was observed, only in the range of 35-38 °C. No clear relationship was found between spindle prevalence or duration and temperature. The frequency of individual spindles, however, showed a positive correlation with actual body temperature. This data suggests that body temperature has indeed a direct effect on sleep oscillation, and may a possible means in the therapy of sleep disorders.

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P1/56

Direct control of the arousal by midline thalamic networks

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Electrical stimulation of medial thalamus has been shown to increase wakefulness both in rodents and humans. However, the site of action is unclear in case of electrical stimulation in the brain. It may not only excite but inhibit local neurons, and antidromically activate cells which project to the site of stimulation (both brainstem and cortex, in case of midline thalamus). As a consequence, presently no direct evidence supports the role midline thalamus (MT) in arousal. Here, by using cell-specific optogenetic manipulation (for details, see the poster by Mátyás et al.) which allows selective activation of MT cells, we quantitatively assessed the role of MT in arousal. We postulated that if MT is part of the arousal system it should fulfill at least three criteria. 1) Brief activation of MT should result in prolonged wakefulness together with the accompanying EEG changes. 2) The effect of MT activation should depend on the sleep state. 3) The magnitude of arousal response should depend on the intensity and frequency of stimulation.

Sleep-wake states were monitored by a combination of EEG signals recorded from bilaterally placed frontal and parietal screws, EMG signals and video tracking of the animal's movement. The results verified all three predictions. Brief (10 sec) optogenetic stimulation of MT at 10 Hz resulted in persistent arousal accompanied by intense locomotion, lasting up to tens of minutes. Shorter (1 sec) stimulations induced brief microarousals, including transient EEG desynchronization and muscle activation without overt sign of behavioral arousal. The effect on EEG always preceded muscle activation and lasted longer. The evoked effects were state dependent, as in NREM-sleep the cortex was more susceptible to midline activation than in REM-sleep. Varying the strength of laser pulse demonstrated tight correlation between the laser intensity and the duration of evoked effects with little variability among the animals. Varying the frequency of activation (1-to-20Hz) indicated stronger effect of higher frequencies. Our results provide direct evidence that selective activation of MT could effectively initiate arousal in naturally sleeping mice. Furthermore, the present findings show this mice line can be used as a powerful tool to investigate questions which require precise regulation of sleep states and arousal as well as to study the role of the midline thalamus in various behavioral context at the cellular and network levels.

P2/80

Nesfatin-1/NUCB2 in the supraoptic nucleus participates in osmotic regulation

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Nesfatin-1/NUCB2 (nesfatin) is an anorexigenic protein expressed in several areas in the CNS. Beside reducing food intake nesfatin also decreases water intake, and a large amount of nesfatin is produced in the magnocellular cells of the supraoptic (SON) and paraventricular nuclei where nesfatin is highly coexpressed with vasopressin (VP). VP is a main regulator of fluid and osmotic balance; it promotes water reabsorption by the kidneys and also causes vasoconstriction. Since food and water intake are in close relationship, it is a question whether the antidiuretic effect of nesfatin is independent of its anorectic action. Two different research groups reported opposite results on nesfatin mRNA expression changes in the hypothalamus after subjecting rats to water deprivation or hypertonic challenge. Both research groups used ad libitum fed rats as controls. To clarify this question, we measured nesfatin mRNA expressions in the SON after water deprivation (WD) and hypertonic challenge (2% NaCl) and used ad libitum

fed (Co) and also pair fed (WD-Co and 2% NaCl-Co) controls. To separate better the effect of food intake on fluid homeostasis another group of rats was fasted for 48h. Food and water consumption as well as the body weights of animals were monitored. Additionally, we investigated whether VP deficiency (Brattleboro rats) affects nesfatin mRNA expression in the SON. Nesfatin mRNA levels increased significantly in the WD and 2% NaCl groups independently of food intake. WD and 2% NaCl animals consumed less than ad libitum fed control rats. Decreased food intake of WD-Co and 2% NaCl-Co rats pair fed with WD and 2% NaCl groups, respectively, did not change the water intake and nesfatin mRNA expressions of these animals. Animals of 2% NaCl group drank much more fluid than controls. Body weights of treated animals decreased dramatically and showed significant difference compared to the appropriate controls. However, negative energy balance itself did not influence nesfatin mRNA expression in the SON, as we did not find any alterations in the 48h fasted rats. Yet, VP deficiency resulted in an elevated nesfatin mRNA expression in the SON compared to the wild-type rats. Our data show that nesfatin participates in maintaining the osmotic balance, and this action is independent of its role in food intake regulation.

P2/39**Phase interference in the 6-OHDA model of Parkinson's disease.**

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Parkinson's disease (PD) is a debilitating neurodegenerative disorder characterised by substantial loss of dopaminergic cells in the basal ganglia. The loss of dopaminergic neurons gives rise to abnormal synchronization in the beta frequency range (around 20Hz) across the cortico-basal ganglia network in parkinsonian patients and animals. There is mounting evidence that the amount of beta synchronization correlates with the severity of motor symptoms. It has been recently demonstrated that using the power of ongoing beta oscillations to determine the timing of high frequency (130Hz) deep brain stimulation can provide greater symptom relief than continuous stimulation. Studies of patients with essential tremor suggest that utilising the phase of ongoing tremor (around 5Hz) oscillations (phase interference) has the potential to provide an even more effective damping of pathological neuronal activity and resulting symptoms. However, this strategy has not yet been attempted for faster oscillations, such as the exaggerated beta oscillations observed in PD. The principal aim of this study was to test if the amplitude of healthy or pathological oscillations in the cortex and basal ganglia are modulated according to the specific phase of ongoing activity at which electrical stimulation is delivered. We stimulated the cortex at approximately the same frequency as ongoing oscillations while recording the electrocorticogram (ECoG), basal ganglia local field potential (LFP) and single unit activity in healthy and 6-OHDA lesioned rats. In healthy animals, we found that stimulating on a certain phase of the spindle oscillation (10Hz) resulted in an amplitude-modulation of the cortical ECoG and the STN LFP. The same effect could be observed with pathological beta (20Hz) in 6-OHDA lesioned animals: stimulation on the peak of the oscillation robustly damped the ECoG beta amplitude, whilst in the basal ganglia LFPs, this modulation occurred with the opposite polarity. We propose that these results provide preliminary evidence for the efficacy of the phase interference for reducing pathophysiological activity in PD.

Symp III. - Friday**Action Representations of self and other during joint action planning**

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Successful human interaction benefits and often depends on the formation of a joint action plan, which may include the representation of one's own action, but also the representation of the action of others, who are involved in the interaction. Previous behavioural and neurophysiological research suggests that representation of another person's action do not just take place at a cognitive level, but also at a "lower" sensorimotor level. I will discuss findings from EEG studies, which investigated representations of self and other during the planning phase of simple everyday tasks, such as passing an object and clinking glasses with another person. The results showed that people employ their sensorimotor system to represent in advance the action of an interaction partner. Interestingly, such representations depend on the specific task of each person who is involved in the interaction and they only concern the aspects of the action that are necessary for the successful performance of the

interaction. These findings point towards a more efficient and task-specific sensorimotor representation of another person's upcoming action, which may take place concurrently with the planning of one's own action and may facilitate the performance of a joint task.

P1/100

Right versus left index finger tapping in right, and left handed healthy young adults. A high power EEG based brain imaging study of the sensory-motor cortex.

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Cortical organisation of voluntary motor activity is still subject of intensive research. Different imaging technologies and data processing methods provide new insight into organisation of oscillations of cortical and sub-cortical neuronal assemblies. A high power EEG with 128 channels and 0.5 ms temporal resolution seems to be an appropriate tool for functional brain imaging, however its technical limitations are well known.

The aim of this study was – utilising an automatic data processing flow on MatLab environment – to visualize handedness related differences in the bioelectric activities of the sensory-motor cortex during right or left index finger tapings.

Ten left handed and ten right handed young female study subjects (19-26 yrs) were involved in this study. Visually cued index finger tapings with 4 s time period were recorded by the BioSemi 128 channels EEG. After different artefacts removing methods based on channel statistics and independent component analysis, 100-120 tapings of both left and right index fingers were remained. Laplace transformation eliminated the effect of volume conduction and produced a sort of source localisation on the scalp. Different oscillations were separated by the time-frequency resolution. Visualisation of separate frequency bands yielded cleaner imaging of the neural activities around the sensory-motor cortex. The high amount of data in the channel-time-frequency space was statistically by cluster-based permutation testing.

Our results indicate that the high power EEG is an appropriate tool to reveal the differences in the activity of motor and sensory cortices related to the handedness. Dominant finger movement was induced by the significantly smaller areas of sensory-motor cortex. Differences in the post-processing beta activity of right and left handed subjects has been observed.

P2/29

Depolarization-induced suppression and potentiation of excitation are present in the pedunculo-pontine nucleus of mice

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The pedunculo-pontine nucleus (PPN) is a member of the reticular activating system, which is thought to modulate sleep and wakefulness. Besides several other homeostatic and neuromodulatory influences, the endocannabinoid signalling also has a significant role in modulating this nucleus. In our previous study, we revealed that certain PPN neurons are depolarized, others are depolarized by CB1 receptor agonists. These effects were unaffected by blockade of the fast synaptic neurotransmission. Rather, CB1-receptor dependent activation of astrocytes leads to glutamate release and tonic modulation of neurons via different sets of mGluR.

In the present work, we sought evidence whether the depolarization-induced suppression of excitation exists in this nucleus as well. In order to test it, whole-cell patch clamp recordings were performed on PPN neurons in coronal midbrain slices. A stimulatory electrode was placed close to the patched soma, and paired pulses were delivered with a frequency of 50 Hz, in the presence of blockers of inhibitory neurotransmission. Besides functional methods, immunohistochemical labelling of glutamatergic axon terminals and CB1 receptors was also performed.

We revealed that, besides the previously shown astrocytic location, CB1 receptors are located on the glutamatergic axon terminals terminating on PPN neurons. Paired pulse stimulation of presynaptic glutamatergic fibers elicited facilitating excitatory postsynaptic currents (EPSCs). Either depolarization of the postsynaptic neuron or application of CB1 receptor agonists lead to the decrease of EPSC amplitude and an increase of paired pulse ratio. However, in 20% of all cases, postsynaptic depolarization or CB1 receptor agonists lead to the increase of the EPSC amplitude. Both changes were absent in CB1 receptor knockout animals.

Taken together, we demonstrated that CB1 receptor dependent depolarization-induced suppression of excitation exists in the pedunculopontine nucleus, as well. In a few cases, postsynaptic depolarization induced potentiation of EPSCs, to which astrocytic activity might contribute. Summarizing our previous and present findings, endocannabinoid signalling has a compound effect in the pedunculopontine nucleus, affecting neuronal excitability in a tonic manner via astrocytic activation, and regulating strength of excitatory inputs, thus contributing to the actions of the cannabinoids on sleep and wakefulness.

P2/41

PACAP transgenic mice in the three hit model of depression: The involvement of BNST - CRF, cpEW - Urocortin1 and DR - serotonin

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According to the three hit theory of depression genetic predisposition, epigenetic factors and stress may lead to manifest major depression. Based on this, we aimed to set up a new mouse model for depression: forced swim test (FST) and corticosterone (CORT) measurement were used to validate the model at behavioral and hypothalamus pituitary adrenal axis (HPA) level. The contribution of corticotropin releasing factor (CRF) producing neurons in the oval bed nucleus of the stria terminalis (BNSTov), central nucleus of the amygdala (CeA), in addition the role of the urocortin1 (Ucn1) peptide containing neurons of the central projecting Edinger-Westphal nucleus (cpEW) and serotonergic (5HT) neurons in the dorsal raphe nucleus (DR) were studied. For genetic predisposition we used offspring of mice heterozygous for the gene of pituitary adenylate cyclase-activating polypeptide (PACAP). Litters were exposed to severe maternal separation to induce epigenetic changes vs. non-deprived or briefly separated controls. Half of adult offspring was exposed to chronic variable mild stress (CVMS) paradigm. We hypothesized that mice carrying all three risk factors will show maladaptive alterations supporting the validity of the model. We put forward that CRF, Ucn1 and 5HT systems will also be affected in their peptide content and/or neuronal activity. Our results showed stress paradigm was effective as in stressed groups the adrenal gland weights significantly increased, which rise was the greatest in mice with maternal separation history. CORT measurements supported this, indicating the maladaptation of the HPA. Further, mice carrying all risk factors showed increased immobility in FST. Histological results revealed that maternally deprived mice exposed to CVMS reacted with an increase in CRF immunoreactive cell counts and specific signal density (SSD) in the BNSTov. In the CeA, CVMS induced increase in the CRF SSD only in maternally non-deprived mice. The CVMS induced rise of neuronal FosB and peptide content in Ucn1 neurons was abolished in maternally deprived mice. In line with this, DR-5HT neurons failed to react with FosB expression to CVMS. The elevated CRF contents in BNSTov neurons, and decreased Ucn1 and 5HT neuronal activity suggest that multiple systems are affected in this model of mood disorders. The model of the three hit theory of depression in PACAP heterozygote mice provides an applicable model to study the pathophysiology of stress-related mood disorders.

P1/103

Face inversion effect is invariant to the size, eccentricity, and phase coherence of face images.

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In previous studies it has been established that stimulus inversion deteriorates performance in face recognition tasks, a phenomenon known as the face inversion effect (FIE). While the neural basis underlying this effect is still under debate, it was suggested that the fusiform face area (FFA) is the primary neural source of the FIE. In the present study, we investigated whether stimulus manipulations to which the FFA has been shown to be sensitive affect the behavioural FIE. Subjects performed a three-alternative forced-choice face identity discrimination task during which we manipulated eccentricity, size and phase coherence in separate experimental blocks. We found a robust face inversion effect in face identity discrimination, irrespective of stimulus manipulations. Our results suggest that besides the FFA, other face-sensitive cortical regions may contribute to the behavioural face inversion effect. This assumption is in line with recent results supporting the conception that configural face processing is mediated by a distributed cortical network.

P1/43**Inosine, guanosine and uridine change the absence epileptic activity in Wistar Albino Glaxo/Rijswijk rats**

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Non-adenosine nucleosides such as inosine (Ino), guanosine (Guo) and uridine (Urd) may have regionally different roles not only in the regulation of physiological processes but also under pathophysiological circumstances in the central nervous system such as epilepsy: Ino and Guo decreased quinolinic acid-induced seizures and Urd reduced penicillin-, bicuculline- and pentylenetetrazole-induced seizures via GABAergic system (for Ino and Urd) and glutamatergic system (for Guo). GABAergic and glutamatergic system are involved in pathomechanism(s) of absence epilepsy, thus, we hypothesized that Ino, Guo and Urd might change absence epileptic activity. We investigated whether intraperitoneal (i.p.) application of Ino, Guo, Urd, GABAA receptor agonist muscimol, GABAA receptor antagonist bicuculline, non-selective Ado receptor antagonist theophylline and non-competitive N-methyl-D-aspartate receptor antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo (a,d) cyclohepten-5,10-imine maleate (MK-801) alone and in combination have modulatory effects on absence epileptic activity in Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats. We found that Guo and Urd decreased the number of spike-wave discharges (SWDs) whereas Ino increased it dose-dependently. Combined application of compounds revealed that Ino, Guo and Urd may exert their effects on absence epileptic activity via GABAA and adenosine (Ado) receptors for Ino, as well as putative Guo and Urd receptors. Our results suggest that Guo, Urd and their analogues may be potentially effective drugs for treatment of human absence epilepsy.

P2/66**Distribution of primary trigeminal afferents on facial motoneurons responsible for mouth opening**

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The prey-catching behavior of the frog is a complex, well-timed sequence of stimulus response chain of movements. This process is based on the coordinated activity of muscles corresponding to different stages of feeding. After visual analysis of the prey, the size dependent feeding program is selected in the motor pattern generator of the brainstem. Although the feeding program is predetermined, the various direct and indirect sensory inputs provide flexible adjustment for the optimal contraction of the executive muscles. The aim of our study was to investigate whether trigeminal primary afferents establish direct contacts with facial motoneurons in the frog.

The experiments were carried out on adult common water frog (*Rana esculenta*). The facial and trigeminal nerve of the anaesthetized animals were prepared and sharply transected. Crystals of neurobiotin was put on the proximal

stump of trigeminal nerve and dextran conjugated Alexa Fluor 555 was applied to the proximal cut end of facial nerve in the same animal. Transverse serial sections of the brainstem were cut with a freezing microtome at a thickness of 50 µm. For visualizing the neurobiotin, the sections were treated with streptavidin-Alexa Fluor 488. In order to visualize the location of labeled neurons and afferents in the brainstem, low magnification images were photographed using Olympus camera DP 72. Images were recorded using Olympus FV1000 confocal laser scanning microscope. The spatial distribution of trigeminal afferents and dendritic arborization of the facial motoneurons were illustrated in three-dimensional reconstruction of the frog's brainstem with Neurolucida 8.0 program. Direct connections were identified between the labeled trigeminal afferent terminals and the facial motoneurons. The results suggest that the possible monosynaptic connections may serve as one of the morpho-functional constituent responsible for the very quick motor activity during the pray-catching behavior of the frog.

P2/75**Effect of yeast cell wall extract-prebiotic (YCWP) on intestinal microbiota and behavior in C57/Bl6 mice exposed to chronic stress (CVS)**

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There is a bidirectional communication between the central nervous system and gut microbiota. Chronic stress and certain psychopathologies, like anxiety and depression alters the composition of gut microbiome and conversely, the gut bacteria affect autonomic and neuroendocrine functions and have an impact on cognition and affective behavior. For instance, increased hypothalamo-pituitary-adrenocortical (HPA) activity during stress results in increased gut permeability through which gut bacteria or their cell wall components might reach the systemic circulation and induce local and global inflammation which further activate HPA axis and induce overt changes in behavior. Prebiotics are non-digestible food ingredients that are specifically affect the growth and metabolism of commensal bacteria. The aim of our experiment was to investigate the effects of a prebiotic on the composition of gut flora, HPA activity and behavior in mice exposed to chronic stress.

C57/Bl6 mice were exposed to chronic variable stress (CVS) for 3 weeks during which one subgroup of the animals were treated with yeast cell wall prebiotic (YCWP) via oral gavage. The other CVS subgroup and unstressed controls received vehicle. Animals were tested in open field and elevated plus maze and then were sacrificed, blood, coecum and colon fecal samples and the brain was collected. The composition of gut microbiome was characterized by 16S rRNA based qPCR measurement of five major bacterial filia: Bacteroides, Firmicutes, Coliforms, Lactobacilli and Bifidobacteria. CVS resulted in a tendency to increase of Bacteroides, while Lactobacilli and Bifidobacteria decreased in colon contents. Although YCWP treatment itself had no significant effect on number of investigated bacteria, it restored the microbiome composition of chronically stressed animals to that seen in nonstressed controls. CVS results in anxiety-like behavioral changes such as increased stereotypic locomotor activity in open field and increased time spent in the closed arm of the elevated plus maze. Following prebiotic treatment the behavior in anxiety tests of chronically stressed mice were comparable to the measures of non-stressed animals. Our results indicate that CVS alters the gut microbiota with preferential increase of putative pathogens and decrease of „good” bacteria and results in anxiety. The prebiotic made from yeast cell wall (YCWP) was able to prevent chronic stress-induced changes in gut microbiome and behavior.

P2/12**Closed loop real-time epileptic seizure detection**

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Many neurological and psychiatric diseases are associated with clinically detectable, altered brain dynamics. The aberrant brain activity, in principle, can be restored through electrical stimulation. In epilepsies, abnormal patterns emerge intermittently, and therefore, a closed-loop feedback brain control that leaves other aspects of brain functions unaffected is desirable. Formerly, it has been shown by our research group that seizure-triggered, feedback transcranial electrical stimulation (TES) can dramatically reduce the duration of spike-and-wave (SW) episodes in a rodent model of generalized epilepsy. We hereby present a non-supervised algorithm to reliably

detect the onset of seizures, and to study the long term effects of TES on the incidence of seizures. The developed algorithm has much less computational cost compared to the currently used time-frequency spectrum-based methods and provides a shorter seizure detection delay time. In contrast to the seizure detection based on amplitude thresholding, our method has higher specificity and sensitivity. These features capacitate the algorithm for reliable, fast and precise on-line detection of SW episodes triggering the feedback TES of the epileptic brain. After adaptive miniaturization, closed-loop TES can be an effective clinical tool to reduce pathological brain patterns in drug-resistant patients.

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P2/65

Localization of CD26/DPP4 enzyme and effects of its inhibitors in the rat spinal cord

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CD26/DPP4 is a moonlighting protein existing within and in the membrane of several cell types and acts as a proteolytic enzyme, a receptor and a costimulatory protein. It has a role in immune response and is involved in adhesion and apoptosis. We have shown that DPP4 inhibitors (vildagliptin and the tripeptide isoleucine-proline-isoleucine; IPI) had opioid mediated spinal antihyperalgesic effect in inflammatory conditions. In this study we (1) looked for evidence for the existence of DPP4 enzyme in the spinal cord and (2) examined which opioid receptors are involved in the antihyperalgesic effects of DPP4 inhibitors in carrageenan-induced hyperalgesia.

Dot-like immunolabelling of the DPP4 appeared in the spinal dorsal horn. DPP4-positivity was found on cell bodies and axon terminals of neurons as well as on the surface of glial cells. DPP4- positive dots were frequently surrounded by mu-opioid receptor immunoreactivity. Antihyperalgesic effect of subcutaneous vildagliptin was completely eliminated by the highly delta-selective antagonist TIPP(ψ), while the mu-selective CTAP and kappa-selective gNTI abolished only the 35% and 45% of this effect, respectively. However, IPI-induced antihyperalgesia was fully blocked by CTAP, while TIPP(ψ) and gNTI did not alter the nociceptive threshold, suggesting the exclusive involvement of mu-receptors.

These data suggest that DPP4 enzyme exists in the spinal cord and interacts with the endogenous opioid system in a very complex manner.

Plenary lecture IV. - Friday

Gamma oscillations from the bottom up

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Gamma-band oscillations have been implicated in many functions, including mediating selective functional connectivity in the brain. The role of fast-spiking parvalbumin-positive interneurons in hippocampal and neocortical gamma is well established, but it is less clear what role pyramidal neurons play in modulating the frequency, amplitude and phase of oscillations. By combining electrophysiology, optogenetics and computer simulations, we have found that manipulating pyramidal cell excitability is able to alter all three features of hippocampal gamma oscillations, in accordance with predictions from a simple mean-field model. The malleable phasic and spectral properties of hippocampal gamma are suited for flexible coupling with other oscillators and for multiplexed information transmission among anatomically connected brain areas

P2/76

Thermoregulation and glucose homeostasis in mice acclimatized to different temperatures .

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Brown adipose tissue (BAT) plays an essential role in whole body energy balance and fuel metabolism. In cold environment, adaptive thermogenesis via induction of uncoupling protein-1 (UCP-1) in BAT contributes to thermo(homeo)stasis. Activated BAT uses lipids and glucose for thermogenesis, however, the relationship between glucose homeostasis and BAT function remains to be established.

C57Bl6 mice were acclimatized to 21oC, 30oC and 4oC, fasted overnight and challenged with 1U/kg insulin. Animals were sacrificed 1 hour after ip. insulin and blood glucose, corticosterone was measured, epididymal WAT and intrascapular BAT dissected and processed for histology and real time qPCR measurements.

During fasting, mice kept at high temperature lost significantly less weight than animals at lower temperatures. At 21oC and 4oC mice became hypoglycaemic following insulin challenge, however, blood glucose levels remain above 3 mM in animals kept at 30oC. BAT UCP-1 and NPY mRNA levels were reduced in mice kept in warm environment. These thermosensitive changes were accompanied by overt alterations of adipocyte morphology and macrophage recruitment in BAT and WAT.

Fractalkine signalling plays a pivotal role in the recruitment of monocytes into adipose tissues and previous studies from our laboratory revealed significant differences in blood glucose homeostasis and thermoregulation in fractalkine receptor (CX3CR1) knockout mice. In a second series of experiments we have investigated the dependence of insulin-induced hypoglycaemia on environmental temperature in CX3CR1 +/- and -/- mice. In contrast to C57Bl6 controls, mice with impaired fractalkine signalling do not become hypoglycaemic at 21oC in response to insulin. These changes in glycaemic control correlate with expression in NPY in the BAT. Furthermore, CX3CR1 knockout animals maintain their core body temperature during cold challenge.

These results highlight the importance a brown adipose tissue in blood glucose homeostasis that is related to macrophage recruitment and NPY expression in adipose tissues.

P1/74

Thyroid hormone regulates mitochondrial activity in the hypothalamic median eminence

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Neurosecretory neurons of the hypothalamus are master players of the endocrine brain and regulate metabolism, growth, stress and reproduction. Their cell bodies are distributed in different regions of the hypothalamus but all parvocellular secretory neurons send their axons to the external zone of median eminence, where the hypophysiotropic hormones are secreted into the hypophyseal portal system. Neurosecretory neurons represent a major target of hypothalamic thyroid hormone signalling but the way how T3 can access these neurons was not clear for a long time. We have earlier demonstrated that the monocarboxylate-transporter-8 thyroid hormone transporter is present in the axon varicosities of neurosecretory neurons in the median eminence that suggests direct access of thyroid hormone into the axonal compartment, that also contain numerous mitochondria. Thyroid hormone is a potent regulator of energy expenditure by modulating mitochondrial function. Therefore we hypothesized that thyroid hormone might affect intracellular energy homeostasis of neurosecretory neurons by regulating mitochondrial activity in the axonal compartment of these cells. Male Wistar on aluminium rats were made hypothyroid using 0.1% methimazole for 2 weeks. Thyroid status was confirmed by the measurement of free thyroid hormone levels. Median eminence of five hypothyroid and control rats were microdissected and pooled, experiment were performed in triplicates. Measurement of mitochondrial oxygen consumption with Oxygraph (Oroboros Instr.) demonstrated a ~3 fold decrease of oxygen consumption in hypothyroid animals vs. controls (~5 vs. 15 pmol/(s*ml)/ug protein). In contrast, we could not evoke changes in oxygen consumption using acute application of thyroid hormone (T3 (200nM), T2 (200nM)) to median eminence samples. The obtained data indicate that chronic but not acute changes in thyroid hormone levels regulate mitochondrial function in the axons of neurosecretory neurons suggesting that genomic events are involved. Since neurosecretion is an energy dependent process, local regulation of mitochondrial function in axon varicosities adjacent to release sites of trophic hormones might represent a novel way to regulate neurosecretion. Additional studies are required to prove this concept.

P1/3

Collateral sprouting of nociceptive afferents is inhibited by spared axons in the chemodenervated rat skin

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Sprouting of axon collaterals of intact cutaneous chemosensitive afferent nerves into denervated skin areas was studied following neurotomy or selective sensory chemodenervation by capsaicin. Serial measurements of topographical changes in the spatial distribution of cutaneous sensory neurogenic vasodilatation were used to identify denervated skin areas and to evaluate the progress of functional cutaneous reinnervation.

Adult male Wistar rats (n=12, 250-300g) were used in this study. The right saphenous nerve was either surgically transected or treated locally with capsaicin (1%, 0.1ml) under chloral hydrate anaesthesia. Three to sixty days after surgery the animals were re-anaesthetised and blood flow of the dorsal skin of the right hindpaw (SBF) was measured by capturing consecutive perfusion images with a laser Doppler perfusion imager (LDI, Perimed, Sweden) before and after the application of mustard oil (5% in liquid paraffin) onto the skin. To compare the spatial distribution of the vasodilatory responses, perfusion maps depicting relative increases in SBF were generated.

The transection of the saphenous nerve resulted in a rapid substantial decrease of mustard oil-induced vasodilatation in the medial part of the dorsal hindpaw skin already 3 days postoperatively (control: 71.0±21.9%, transected: 24.6±13.8%). The vasodilatory response showed a marked progressive recovery towards control values in the saphenous skin area bordering the innervation territory of the sciatic nerve after 12-20 days after surgery. Perineural treatment with capsaicin produced changes in SBF similar to nerve transection 3-5 days after treatment but, importantly, a tendency for recovery of the vasodilatory response was not observed. However, 12-30 days after transection of the previously capsaicin treated saphenous nerve on the 50th postoperative day a substantial restitution of the vasodilatory response was observed in the previously chemodenervated skin area.

By using a novel experimental approach, the present findings confirm the partial reinnervation of surgically denervated skin areas via collateral sprouting. In the chemodenervated skin, collateral reinnervation by intact sciatic afferent axons developed only after prior transection of the capsaicin-treated (saphenous) nerve. These findings suggest a hitherto unrecognized inhibitory effect of capsaicin-insensitive, most probably myelinated sensory nerves on the collateral sprouting of chemosensitive afferent axons.

P1/94

The role of oxytocin in positive reinforcement in the rat central nucleus of amygdala

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Neuropeptide oxytocin (OT) is involved in social and non-social behavior and stress. The central nucleus of the amygdala (CeA), part of the limbic system, plays an important role in learning, memory, anxiety and reinforcing mechanisms. CeA was shown to be rich in OT-receptors (OTR). The aim of our study was to examine the possible effects of OT and OTR antagonist on reinforcement in the CeA in conditioned place preference test and on anxiety in elevated plus maze test.

Male Wistar rats were microinjected bilaterally with 10 ng OT or 100 ng OT (Sigma: O6379, injected in volume of 0.4 µl) or 10 ng OTR antagonist (Sigma: L-2440) alone, or OTR antagonist 15 min prior 10 ng OT treatment or vehicle solution into the CeA.

Rats receiving 10 ng OT spent significantly longer time in the treatment quadrant during the test session, while 100 ng OT treatment did not influence the place preference. Prior treatment with the non-peptide OTR antagonist blocked the effects of OT. The antagonist in itself did not influence the place preference. The elevated plus maze test revealed that 10 ng OT significantly increased the time spent in the open arms.

Our results show that in the rat CeA OT has (a dose dependent) positive reinforcing effect via OTR, because selective OTR antagonist can block this action. OT -in a dose of 10 ng- has anxiolytic effect when microinjected in the rat CeA.

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P1/4

Migrating postmitotic neuroblasts override apoptosis via inactivation of alpha-beta hydrolase domain-containing protein 4 (ABHD4)

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Epithelial-mesenchymal transition (EMT) of epithelial cells and delamination of the non-radial glia-fated daughter cells (nRGFCs) during embryonic cortex development have several common features. It is presently unclear however whether a protective apoptosis which eliminates epithelial cells undergoing unwanted EMT is also present in the developing cortex and if it is, then how nRGFCs escape it. Here, we demonstrate that disruption of adherens junction between radial glia progenitors induces a caspase-dependent apoptotic process. Moreover, we provide evidence that Abhd4, a serine hydrolase implicated in the metabolism of N-acyl phospholipids, including a potential lipid precursor for anandamide is expressed in the embryonic forebrain. We found that Abhd4 mRNA is expressed strongly and specifically (validated on Abhd4^{-/-} samples) by radial glial cells (RGCs) in the ventricular zone throughout prenatal development. Despite this restricted pattern, we could not detect any defects in processes regulated by RGCs including proliferation, neuroblast migration or adult cortical lamination in Abhd4^{-/-} cortices. On the other hand, the disappearance of Abhd4 expression from the non-radial glia-fated daughter cells after asymmetric division suggests that elimination of Abhd4 is essential for proper neuroblast migration. Indeed, ectopic expression of wild-type Abhd4, but not of a hydrolase-dead mutant in postmitotic neuroblasts arrested their migration and induced cell death. Since lack of Abhd4 alone did not cause any defects in all processes examined including developmental cell death, we asked whether this serine hydrolase could be involved in the cell death process evoked by disrupting adherens junctions between radial glia cells. Indeed, disassembly of adherens junctions via in utero electroporation of Δ nCdh2 induced robust cell death in wild-type, but not in Abhd4^{-/-} embryos.

Taken together, these findings suggest that Abhd4 plays a stand-by, „guardian” function in radial glial cells by controlling adherens-junction-dependent cell survival during cortical development.

P2/92**Altered expression of insulin-like growth factor binding protein 3 in the maternal brain**

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The postpartum physiological and behavioral changes are important parts of reproduction regulated by a complex neuronal network, which includes parts of the hypothalamus. Lesions of the preoptic area abolish maternal behaviors while the arcuate nucleus plays a role in the regulation of lactation.

In our previous microarray study, gene expression alterations were described in the anterior hypothalamic areas of mother rat. The highest elevation was shown by the amylin, which we identified as a novel neuropeptide. The expression of insulin-like growth factor binding protein-3 (IGFBP-3) also increased significantly in lactating mothers as compared to mothers whose litter was taken away immediately after birth. In the present study, this finding was also validated by RT-PCR. In the blood, IGFBP-3 is the major carrier molecule of insulin-like growth factors (IGFs) in the blood. IGFBP-3 forms a ternary complex with an acid-labile subunit of either IGF-1 or 2. The distribution of the IGF-system including IGFBP-3 has not been described in the brain. Therefore, we developed in situ hybridization probes to map IGFBP-3. In the hypothalamus, IGFBP-3 mRNA was abundant in the anteroventral periventricular nucleus and the mediodorsal subdivision of the arcuate nuclei. In addition, we found significant IGFBP-3 mRNA levels in the choroid plexus, too. We could also confirm the elevation of IGFBP-3 expression in the hypothalamic sites in mother rats. However, a change was not seen in the choroid plexus suggesting specific roles of hypothalamic IGFBP-3 in the maternal brain. Using a combination of in situ hybridization and immunohistochemistry, we also discovered co-localization of IGFBP-3 with tyrosine-hydroxylase (TH), a marker of tuberoinfundibular dopaminergic cells in the arcuate nucleus. The time course of alterations in the IGFBP-3 mRNA

levels during the reproductive cycle was also determined: IGFBP-3 mRNA level is low in control female rats, does not elevate by the end of pregnancy but is markedly induced by the first postpartum day. These data suggest specific roles of IGFBP-3 in lactation as well as other adaptations of the female brain to maternal responsiveness. Support was provided by OTKA K100319 and KTIA_NAP_B_13-2-2014-0004 Program.

P1/21

Distinct morphological and functional properties of dendritic and perisomatic axon terminals of CCK+ interneurons in the hippocampus

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Cholecystokinin (CCK)-containing interneurons (INs) in the hippocampus form a morphologically and functionally diverse cell population. Here we compare the properties of axon terminals of basket and dendritic layer innervating (DLI) CCK+ INs by performing Ca²⁺ imaging experiments and electron microscopy (EM). We have found that basket cells have ~30% smaller [Ca²⁺] transients compared to DLI cells ($p=0.0008$, G/G_{max} basket = 0.17 ± 0.06 , $n_{basket}=18$; G/G_{max} DLI = 0.24 ± 0.06 , $n_{DLI}=19$). This difference can be the consequence of distinct 1) calcium buffering; 2) active zone / bouton volume ratio; or 3) type and/or number of voltage-gated Ca²⁺ channels. Analyses of the time course of the decay of the [Ca²⁺] transients showed no significant difference between the two cell types ($p=0.72$) arguing against a difference in the endogenous buffer capacity. Three-dimensional EM reconstruction of CCK+ axon terminals in strata pyramidale and radiatum revealed that basket cells have on average 70% larger bouton volumes and similar active zone (AZ) areas, resulting in a 40% smaller AZ / bouton volume ratio. SDS-digested freeze-fracture replica immunolabeling for Cav2.1 (P/Q type) and Cav2.2 (N type) Ca²⁺ channel subunits revealed the presence of Cav2.2 subunit in both types of terminals, which was confirmed by a similar, almost complete block (88% on basket and 95% on DLIs) of [Ca²⁺] transients by 1 μ M ω -Conotoxin-GVIA. Assuming that the calcium channels are restricted to the AZ and have similar densities in both cell types, our results demonstrate that the difference in the bouton volume alone can account for the detected differences in the [Ca²⁺].

Symp IV. - Friday

Modality-general categorization in parietal cortex

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Perceptual decision making is the cognitive process of converting stimuli into an abstract format that allows further cognitive or motor processes to engage appropriately. In categorization tasks, perceptual decisions result in the classification of sensory information into discrete, abstract categories.

Various studies in the field of perceptual decision making have demonstrated some level of category-specificity within parietal regions of both humans and monkey. However, nearly all of these studies have investigated the matter through the visual domain, leaving the question of domain-general categorization in parietal cortex open to speculation.

Recent work into non-visual perceptual decisions has employed paradigms containing predictable motor responses. These studies often implicated regions of the frontal lobe in perceptual decision making. However, due to the confounding of motor acts with perceptual decisions, one cannot reliably interpret which mechanisms of the perceptual decision making framework are reflected by such results.

We sought to disentangle overt responses from stimulus categorization by employing a delayed match-to-category paradigm, in which participants categorized the direction of low-level auditory and tactile frequency-modulated (FM) sweeps while we measured their blood-oxygenation-level dependent signal using functional magnetic resonance imaging. To detect cortical representations of FM sweep directions, irrespective of the sensory domain, we used whole-brain multivariate pattern analysis implemented via support vector machine (SVM) classification.

By removing confounds related to higher-level semantics and predictable motor outputs, the SVM was able to decode categorical representations for both sensory modalities within only the right intraparietal sulcus. These findings offer a new view that the parietal lobes, rather than frontal regions, play a domain-general role in abstractly representing task-relevant categories.

P1/25**Signal conduction -related parameters of myelinated axons established by inhibitory large basket cells in the cat primary visual cortex**

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Myelin thickness is one of the key parameters for determining signal propagation speed in axons of nerve cells. It can predict the conduction velocity of axons, set the rate of information transmission to the target structures and their temporal activation. In the cerebral cortex, fast spiking, long-range projecting large basket cells are assumed to inhibit the target cells synchronously through their myelinated axons which run up to several mm parallel to the cortical surface. However, it is not known whether myelin coverage has a uniform thickness along basket cell axons or changes systematically with distance from the soma origin.

Here we investigated axons of biocytin filled large basket cells using correlated light- and electron microscopy. Axons were reconstructed from serial sections (60 µm thick) using x100 objective and the Neurolucida system. Light microscopy allowed measuring the thickness of labelled axons point-by point at 0.18 µm accuracy. Thereafter, the same axons undergone correlated light- and electron microscopic (EM) examination. To this electron microscopic images were analysed with a public domain program and Electron Microscopy Imaging Platform (ITEM). The following parameters were determined: (i) total diameter of axon including myelin, diameter of axoplasm, (ii) thickness of myelin, number of myelin lamellae. Our correlated LM and EM observation showed that LM measures underestimate the total thickness of myelinated axons by about 20%. The number of membrane lamellae of the myelin varied 4-13 resulting a g-ratio of 0.8-0.9.

Our findings provide parameters on the myelination of inhibitory large basket cells, which can be utilised in biologically realistic simulation of their temporal effects including axonal conduction time and neuronal integration delay.

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Symp. IV. - Friday**Serotonergic modulation of central olfactory information processing**

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Released from neurons located in the brainstem dorsal raphe nucleus (DRN), serotonin (5-hydroxytryptamine, 5-HT), has been implicated in a wide range of sensory, motor and reward functions. In addition to its slow modulatory effects some studies suggest that 5-HT might also exert its effects on faster timescales as many DRN neurons express transient changes in their firing rates, locked to specific events, such as sensory stimuli, rewards and reward predicting cues suggesting that 5-HT could influence sensory information processing on a fast timescale. In the rodent olfactory system, 5-HT neurons of the DRN richly innervate the olfactory bulb (OB) and pharmacological studies have shown that activation of 5-HT receptors suppresses odor-evoked activity in olfactory receptor neuron input to the OB on a time scale of minutes via activation of GABAergic interneurons. The DRN also densely innervates the olfactory "piriform" cortex (PC), but relatively little is known about the action of 5-HT on central sensory processing. Here, using optogenetic activation of RN 5-HT neurons in vivo we show that 5-HT can rapidly reduce the spontaneous firing of the majority of PC neurons while leaving their odor responses unaffected. Simultaneously recorded neurons in the OB were less affected by DRN photostimulation and the activity of

modulated neurons was increased suggesting that the PC itself, rather than its OB derived afferent inputs are the primary locus of action of DRN-evoked serotonergic modulation. Photostimulation of ChR2 expressing DRN derived 5-HT axons in the PC in vitro confirmed the local nature of the effects. Moreover the effects of 5-HT were pathway specific only decreasing associational and not afferent responses. Taken together, these results provide novel evidence for the involvement of the 5-HT system in the rapid modulation of central olfactory information processing.

P1/51**Histological evaluation of early diabetic neurodegeneration in Zucker Diabetic Fatty (ZDF) Rats**

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Purpose: Literature data indicates that in diabetes retinal dysfunctions related to neural retinal alterations exist prior to clinically detectable vasculopathy, however, the histological background behind these functional deficits is largely unknown. Previously, a detailed description about the histology of the outer and inner retina was given in streptozocin (STZ) induced (type 1) diabetic rats, where neural retinal degeneration preceded apoptotic loss of cells. Here, we investigated the histopathology of the retina in early diabetes using Zucker Diabetic Fatty (ZDF) rats, a well-know model of type 2 diabetes.

Methods: The morphology, density and staining characteristics of the labeled cells were analyzed by immunohistochemistry after six months of diabetes, using cell-type specific antibodies.

Results: Glial reactivity was observed in all diabetic retinal specimens, manifested by strong GFAP upregulation. Marked outer segment degeneration was detected in rods and in the majority of M/L-cones, without an apparent decrease in the number of stained elements and without any major change in the expression of other photoreceptor specific markers like arrestin and transducin. An increase in the number of dual cones, coexpressing two photopigments was also visible. The immunoreactivity of parvalbumin (staining mostly AII amacrine cells) was markedly decreased and we detected a change in the number of PKC- α labeled amacrine and displaced amacrine cells. In contrast to these, most other markers used (calretinin, recoverin, tyrosin hydroxylase anti-Brn-3a and also calbindin) did not show any major alterations in the intensity of immunoreactivity or in the number of stained elements.

Conclusion: Overall, the retina of ZDF rats shows a surprising similarity to STZ induced type 1 diabetic rats, indicating that most of the changes are consequences of the high glucose levels while the lack of insulin does not play a decisive role in the development of histological alterations. The presence of early retinal degeneration raises the possibility that neuroprotective therapy in diabetic patients should be beneficial even prior to vasculopathy or overt loss of cells.

P2/40**Evolution conserved neuroprotective function of pituitary adenylate cyclase-activating polypeptide (PACAP) in dopamine-based neurodegeneration**

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In this presentation, using snail and rat model systems, we show the evolutionarily conserved function of PACAP in models of Parkinson-disease (PD).

PACAP is a widespread neuropeptide with diversephysiological functions. The structure of PACAP is conserved during phylogeny. Our earlier findings showed that a homolog of the vertebrate PACAP and its receptors exist in annelids (*Lumbricus terrestris*, *Lumbricus polyphemus*, *Eisenia fetida*) and snails (*Lymnaea stagnalis*, *Helix pomatia*), similarly to what was found in the fruit fly (*Drosophila*). In invertebrate species PACAP has been shown to play

important roles in several biological processes, such as feeding, active-inactive state, learning and memory, as well as aging.

Here, our aim was to examine the protective effect of PACAP in models of Parkinson's disease: in rotenone (commercially organic pesticide)-treated snails and 6-hydroxy -dopamine (6-OHDA) -treated rats, measuring the dopamine and serotonin levels in the brain. A further aim was to map the proteomic profile in the substantia nigra of control, 6-OHDA and 6-OHDA+PACAP injected rats. Finally, our goal was to investigate changes in dopamine metabolic enzymes (S-COMT, MB-COMT and MAO-B) after treatments in both the invertebrate and vertebrate models.

The Parkinson's disease inducing effect of rotenone and 6-OHDA was attenuated by ~50% in the PACAP injected invertebrate and vertebrate samples. Qualitative and quantitative differences were not found between the control, 6-OHDA and 6-OHDA+PACAP injected groups except in case of DJ-1 (PARK7) protein. The DJ-1 protein was only identified in the control group. The possible changes in the metabolic enzymes was also observed. S-COMT was reduced in the rotenone and 6-OHDA and rotenone+PACAP or 6-OH DA+PACAP treated model animals contrast the control groups. However, the highest intensity of MB-COMT was in the rotenone and 6-OHDA treated groups. MAO-B decreased in 6-OHDA treated samples, while in the PACAP injected group it had an intensity similar to that of untreated controls. The MAO-B was not present in the snail central nervous system.

Based on our results we conclude that PACAP is a neuroprotective agent in dopamine-based neurodegenerative disorders, like PD and the rotenone is also suitable for developing and testing the neurodegenerative disorders.

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P2/15

Caged glutamate and GABA compounds for measurements of dendritic integration

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Two-photon uncaging is a potent tool for investigation of dendritic integration. The rapid and focal release of different neurotransmitters allows the evocation of neuronal activity in an arbitrary spatio-temporal pattern. However, currently available caged neurotransmitters have limited availability, due to high excitation energy needed for optimal photolysis and high rate of spontaneous hydrolysis. Side-effects, such as blockade of GABAA receptor activities also interfere with the measurements. Here, I introduce several novel caged glutamate and GABA compounds, which were tested in the course of acute physiological in vitro measurements. Based on preliminary results, the two new GABA cage compound, DMAiPO-DNI-GABA and DMAiBO-DNI-GABA, are good candidates for two-photon GABA uncaging. These novel glutamate cage compounds are photochemically more effective than previously described ones, as less excitation energy is required to release the same amount of neurotransmitters. Moreover, we have developed a special enzymatic method to eliminate the accumulation of free glutamate due to spontaneous hydrolysis of the caged glutamate compounds. One promising candidate described here for glutamate uncaging experiments have several times more efficient photochemical release and a smaller GABAA receptor blocking effect, than currently available caged glutamate compounds.

P1/48

Characterization of normal „up” states and epileptic seizures in entorhinal cortex of rat brain slices using MEA (multi electrode array) system.

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Although epileptic activity and normal slow wave oscillations are both intensely investigated, little is known about their accurate mechanisms either in the network or in the cellular level.

MEA (multi electrode array) system is a very good approach for studying the spread of activation, specifying clusters and mapping the connections of different areas and clusters.

Our aim was to compare the slow wave oscillation, and induced epileptic events in the entorhinal cortex of horizontal rat brain slices. For this, the initiation foci were determined and horizontal and laminar spread of slow wave oscillations and epileptic seizures in the neocortex were characterised by MEA measurements. The pharmacological sensitivity to kainate receptor (KARs) antagonist was also tested by using UBP296.

We used a two dimensional MEA chip, which covered the whole entorhinal area laterally and all of its layers. From multi unit activity recordings single cell firing pattern was determined and correlation between firing pattern and field events was also investigated. Spike sorting was made by a Matlab based program developed in our lab. We extracted the time stamp datas of the identified clusters to get information about the firing pattern of the clusters and to map the probable connections, and this way we could make conclusions for the network mechanisms. We compared burst lengths, and prepared two-dimensional CSD (current-source density) analysis to compare the distribution and the initiation foci of normal and epileptic activity.

We found, that burst lengths were longer in epileptic conditions, than in UP states in untreated slices and frequency was higher in epileptic slices. Latency of appearing epileptic activity in Mg²⁺ free solution was shorter than appearing of UP-states during ACSF incubation. CSD analyses showed, that the main activity was initiated in layer V. in UP states, but in layer II/III. in epileptic activity.

Investigating KARs, functions showed, that KARs have no effect on the frequency or the number of clusters, but influence the burst shaping and the distribution of activity. Burst length and number of firings in each burst were also affected by the presence or absence of functioning KARs. UBP296 reduced both the burst lengths and the number of firings in bursts.

P1/19

Compartment-dependent synaptic cooperativity and plasticity in dendrites of hippocampal CA1 pyramidal neurons

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The vast majority of excitatory synapses arriving onto pyramidal neurons contact dendritic spines on the elaborate dendritic tree. Spines and dendrites are equipped with voltage dependent ion conductances, allowing nonlinear interactions between spatiotemporally correlated inputs. While the ability of CA1 pyramidal cell dendrites to produce regenerative dendritic spikes by massive synaptic activity is well established, little is known about the cooperativity of synaptic function in the subthreshold regime. The electrical interactions between coactive synapses may be location dependent due to dendritic cable properties and locally regulated active conductances. Here we investigated cooperativity of synaptic signals and plasticity among neighboring spines in thin dendrites of hippocampal CA1 pyramidal neurons using two-photon imaging and multisite two-photon glutamate uncaging combined with electrophysiology in acute slices from adult rats.

Physiological-like stimulation of up to four nearby spines by glutamate uncaging (subthreshold to generation of dendritic spikes) induced cooperative nonlinear amplification of NMDA receptor mediated spine Ca²⁺ signals, an effect that steeply increased from proximal to distal sites along individual dendrites, while summation of synaptic EPSPs remained largely homogenous. Cooperativity of spine Ca²⁺ signals was closely related to the local excitability of the particular dendritic branch and was regulated by transient voltage-gated K⁺ currents. Consistent with the location-dependent nonlinearity of spine Ca²⁺ signaling, cooperative long term plasticity could be evoked by a limited number of coactive synapses in distal but not in proximal dendritic segments. Thus, high impedance compartments created in distal parts of long dendritic branches may be a preferred domain to promote the induction of spatially clustered forms of synaptic plasticity even among small numbers of functionally related inputs. The resulting structured connectivity, together with locally plastic dendritic excitability, may enhance the ability of neurons to detect, store and respond to specific patterns of correlated presynaptic neuronal activity.

P1/7

Vascular scaffolding for neuroblast migration in the rostral migratory stream of the rat

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New neurons, that are continuously generated throughout the life span of the rodents in the subventricular zone (SVZ), migrate for a long distances via rostral migratory stream (RMS) to the olfactory bulb (OB). In the OB they differentiate into the interneurons. The mechanism of neuronal precursor migration in the postnatal period differs from those in the development. Contrary to the embryonic development, new neurons arising postnatally could not use radial glia processes as a scaffold for their migration, because radial glia disappears in the early postnatal period. The widely expected concept of neuroblast migration in the postnatal RMS, explains it as a movement of neuroblasts along each other, ensheathed by astroglial tubes, without additional scaffolding. The recent findings, that blood vessels could guide and influence neuroblast migration in the SVZ/RMS/OB, have changed this former concept. Nevertheless, all experiments dealing with an arrangement and a role of vasculature in connection with the postnatal neuroblast migration have been exerted in mice. Since there are some morphological and developmental differences between mice and rats we decided to examine an arrangement of blood vessels in the rat RMS as well as a mutual organization of blood vessels, neuroblasts and astrocytes. In this study juvenile rats of different age stages of the first postnatal month, and adult rats were used. Brain sections were processed for triple immunofluorescent methods using anti RECA-1, anti Dcx and anti GFAP antibodies to label blood vessels, neuroblasts and astrocytes, respectively. We have found that the arrangement of blood vessels in the RMS of adult rats is of a typical pattern. For the most of the RMS extent, blood vessels are oriented parallel to the axis of migratory pathway, but contrary to this in the caudal part of the RMS, blood vessels are arranged in spiral-like configuration. We assume that the arrangement of blood vessels in the RMS is species specific, because it differs from this demonstrated in mice. Moreover, the organization of the blood vessels, astrocytes and neuroblasts in the RMS of adult rats suggests that the scaffold for migrating neuroblasts is established by contribution of both blood vessels and astrocytes. In juvenile rats, we found, that vasculature and cellular composition of the RMS undergoes significant reorganization during first postnatal month.

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P2/79

Starvation induced neuronal plasticity in the arcuate nucleus

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The arcuate nucleus (ARC) of the hypothalamus is the primary regulatory center of food intake receiving a bulk of humoral and neuronal information about the state of satiety and the energy balance. It is located at the ventral part of the third ventricle adjacent to the median eminence (ME). The ME belongs to the circumventricular organs in the CNS, devoiding of the blood-brain barrier (BBB) this way it allows humoral inputs toward the ARC. To prevent diffusion of the molecules via the cerebrospinal (CSF), the CSF is separated from the ME by the tight junctions between specialized glia cells called tanycytes around the floor of the third ventricle. The ARC houses two different populations of food intake regulatory neurons; there are anorexigenic neurons in the lateral subdivision (alpha-melanocyte stimulating hormone (α MSH) colocalized with cocaine- and amphetamine-regulated transcript), and orexigenic neurons (neuropeptide Y colocalized with agouti-related peptide) in the medial subdivision. During starvation the BBB opens toward the medial ARC, while the tight junctions between the tanycytes close more strictly by expanding dorsally in the ventricle. Opening of BBB in the medial ARC induces gene expression changes in the ARC and the tanycytes promoting adaptation to the starvation. Nesfatin-1/NUCB2 (nesfatin) is an anorexigenic neuropeptide expressed in the lateral ARC, and coexpressed highly with α -MSH. We investigated the expression of nesfatin and activity of the cells (c-Fos positivity) in control and 1, 3 and 5 day-long fasted rats by immunohistochemistry. Additionally, the effect of intra-cerebroventricularly (icv) injected nesfatin on Fos activity was studied in normal fed rats. In controls nesfatin cells located in the lateral ARC, and many of them was double labeled also for α -MSH. After 5 days of starvation, nesfatin-positive cells appeared also in the orexigenic area of the medial ARC. Nesfatin injected icv activated the tanycytes medially to the ARC. Additionally, similar Fos reaction was seen after 5 days of starvation. We suggest that opening of the BBB during starvation induces nesfatin expression in the medial ARC. Nesfatin expression increase may contribute to the activation of the tanycytes. Further studies are needed to elucidate how all this is related to the adaptation process during starvation.

P1/55**Anatomical and functional mapping of the midline thalamic arousal circuitry**Ferenc Matyas¹, Péter Barthó¹, Ákos Babiczky¹, Csaba Dávid¹, Boglárka Barsy¹, László Acsády¹¹*IEM-HAS, Dept. Cellular and Network Neurobiology, Budapest, Hungary*

Setting the right state of alertness is a key step for shaping a behavioural action. Midline thalamus is regarded to be the rostral most member of the ascending reticular activation system and is believed to play a crucial role in the regulation of arousal. However, no direct experimental evidence is available to support this assumption. This is largely due to the lack of selective tools to examine and manipulate a small, irregularly shaped brain region, like the midline thalamus, embedded in functional heterogeneous networks.

Here, we describe a midline thalamus selective marker (MSM) which allowed us to perform specific anatomical and electrophysiological investigation of the midline thalamic nuclei and explore their role in arousal. MSM was expressed in over 98% of midline thalamic cells expressing cFos as a result of elevated arousal states. MSM also specifically labelled almost all thalamic cells (92-98%) which project to one of the main forebrain targets of midline thalamus (prefrontal cortex, amygdala and nucleus accumbens). Injection of floxed AAV virus into the midline thalamus of MSM-Cre-recombinase mouse line revealed strong axonal labelling in these limbic regions confirming the eligibility of this marker for the midline thalamus. The use of the same virus in a retrograde manner demonstrated that many midline thalamic cells have multiple forebrain targets allowing them to synchronously alter distinct limbic regions. This observation was also strengthened by single-cell Sindbis-palm-GFP electroporation which visualized branching axons in distinct target areas. Optogenetic activation of midline thalamic cells in the MSM-Cre transgenic mice under urethane anaesthesia induced short latency, frequency-locked multi-unit activation in the prefrontal, amygdalar and accumbal regions together with cortical desynchronization. In vivo activity of midline cells was strongly modulated by the fluctuating state of anaesthesia. This was confirmed in drug-free, freely-moving condition: 10-20 Hz but not 1 Hz stimulation of the midline thalamus caused cortical desynchronization and awakening (see details in the poster of Komlósi, Mátyás et al.).

In summary, anatomical and physiological analysis of MSM labelled cells in the thalamus demonstrate that the presently identified marker opens a new area to investigate neuronal mechanisms underlying control of consciousness and arousal.

P1/1**Migratory pathways of late born neurons populating the superficial spinal dorsal horn in mice**Zoltán Mészár¹, Rita Varga¹, Anita Balázs¹, Fujio Murakami², Miklós Antal¹¹*University of Debrecen, Department of Anatomy, Histology and Embryology, Debrecen, Hungary;* ²*Osaka University, Laboratory of Neuroscience, Graduate School of Frontier Biosciences, Osaka, Japan*

Neurons of the superficial spinal dorsal horn, forming neural circuits underlying nociceptive information and pain processing, are derived from a single progenitor cell group during neurogenesis. During their embryonic maturation the progenitor cells differentiate into an excitatory (dILB) and an inhibitory (dILA) postmitotic neuron populations due to the expression of a series of interacting transcription factors. Although their neurogenic origin is well described, we still do not know how these neurons migrate from the ventricular zone into the most superficial laminae of the spinal dorsal and how they find their final location there. Thus, after labeling the progenitor cells with in utero electroporation of GFP at embryonic day 12.5 (E12.5), we studied the migration of the neurons by time lapse microscopy. Incorporation of BrdU, which was intraperitoneally injected at the time of electroporation, into the labeled neurons verified that they became postmitotic immediately after the electroporation, at day E12.5 - E13. Two days after the labelling, GFP labeled cells showed prominent immunoreactivity for the neural marker, beta III tubulin. The labeled neurons were also immunostained for either Pax2 or Brn3a in a non-overlapping manner indicating that their differentiation into inhibitory (dILA, Pax2 positive) and excitatory (dILB, Brn3a positive) neurons had been completed by E14 - E15. Track analysis of confocal time-lapse microscopic images of the labeled neurons in organotypic spinal cord cultures revealed that first they follow an inside-out radial migratory pathway. After reaching the most superficial compartment of the dorsal horn, they continue their migration parallel to the pial surface either into the medial or lateral directions till they presumably find their final destination within the future superficial spinal dorsal horn.

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P1/13

Identification of the interaction partners of the formin dDAAM required for axon growth

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In the developing nervous system growth cones have an essential role in guiding axons to their correct target sites. Directed growth cone motility in response to extracellular cues is produced by the coordinated regulation of peripheral F-actin and central microtubule networks. Key regulators of actin dynamics are the so called nucleation factors, such as the Arp2/3 complex and formins, that promote the formation of new actin filaments. We have previously shown that the Drosophila formin dDAAM plays a major role in the regulation of axonal growth by promoting filopodia formation in the growth cone. In order to better understand how dDAAM functions during neuronal development, an LC-MS/MS analysis was carried out to identify potential partners that work together with dDAAM during axonal growth regulation. Interestingly, the list of the potential binding partners includes several microtubule binding proteins. Currently, we are investigating the mechanism as to how dDAAM induced actin assembly might be coupled to the regulation of microtubule dynamics in axonal growth cones.

P1/8

The Formin DAAM Functions as Molecular Effector of the Planar Cell Polarity Pathway during Axonal Development in Drosophila

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Recent studies established that the planar cell polarity (PCP) pathway is critical for various aspects of nervous system development and function, including axonal guidance. Although it seems clear that PCP signaling regulates actin dynamics, the mechanisms through which this occurs remain elusive. Here, we establish a functional link between the PCP system and one specific actin regulator, the formin DAAM, which has previously been shown to be required for embryonic axonal morphogenesis and filopodia formation in the growth cone. We show that dDAAM also plays a pivotal role during axonal growth and guidance in the adult mushroom body (MB), a brain center for learning and memory. By using a combination of genetic and biochemical assays we demonstrate that the PCP signaling proteins Frizzled, Strabismus, Wnt5 and Dishevelled act in concert with the small GTPase Rac1 to activate the actin assembly functions of dDAAM essential for correct targeting of MB axons. Collectively, these data suggest that dDAAM is used as a major molecular effector of the PCP guidance pathway. By uncovering an entire signaling system from the Wnt5 guidance cue to an actin assembly factor, we propose that the Wnt5/PCP navigation system is linked to the regulation of the growth cone actin cytoskeleton, and thereby growth cone behavior, in a direct way.

P1/30

The chemical basis of distinct mechanisms of actions for sodium channel inhibitors

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There is increasing evidence for multiple binding sites and multiple mechanisms of action for sodium channel inhibitors. Distinct mechanisms of action alter the activity patterns of excitable cells differently, which may underlie therapeutic multiplicity of sodium channel inhibitors. For this reason, understanding the exact mechanism of action is important in drug development. In this study we used special voltage and drug application protocols in whole-cell patch clamp electrophysiology experiments, and identified four distinct mechanisms of action for five sodium channel inhibitors: lidocaine, bupivacaine, flecainide, phenytoin, and riluzole.

The protocols address the question of conformational state-dependent affinity vs. accessibility, i.e., does the drug prefer specific conformations of the channel because it has higher affinity to them, or because it can access its

binding site more easily in them. These two possibilities have been formularized in the modulated receptor hypothesis (MRH) and the guarded receptor hypothesis (GRH), which have been the two major theories that explain the properties of drug-induced inhibition of sodium channels.

We argue that the two mechanisms may co-exist, and developed a method for quantifying their respective contribution in the case of specific sodium channel inhibitor drugs.

In a simple HH-type phenomenological model of channel gating (activation and inactivation) we interpreted and quantified the mechanisms by expressing it in free energy differences in binding and gating transitions. We observed that the chemical properties of individual sodium channel inhibitors determine their affinity and accessibility values to different channel conformations, and therefore we argue that it will be possible to predict the mechanism of action from chemical structure.

Symp. I. - Thursday

Estrogen membrane-initiated signaling: reproduction and beyond

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Estradiol signaling in the central nervous system involves direct nuclear actions that mediate transcription and membrane-initiated signaling (EMS) that activate second messenger cascades, which affect a host of physiological processes including transcriptional regulation. Over the years, we have characterized such EMS in the regulation of reproduction, morphological plasticity, and nociception. An underlying principle that has emerged from these studies is that estrogen receptor- α (ER α) at the membrane signals through a transactivation of metabotropic glutamate receptors (mGluR1a), which regulate both positive and negative actions of estradiol. In terms of reproduction, EMS modulates sexual receptivity through control of β -endorphin neurons that innervate the medial preoptic area. Both classical estradiol action and EMS are needed for estrogen positive feedback of the luteinizing hormone (LH) surge that triggers ovulation. In kisspeptin neurons, nuclear estradiol action induces the expression of progesterone receptors, while EMS induces kisspeptin expression. In astrocytes, peaking levels of estradiol rapidly facilitate progesterone synthesis. This neuroprogesterone activates membrane progesterone receptors on kisspeptin neurons, triggering the release of intracellular calcium and the secretion of kisspeptin, which stimulates the surge release of gonadotropin releasing hormone (GnRH) – the signal for ovulation through the release of LH. In the hippocampus and the arcuate nucleus (ARH), estradiol induces dendritic spines, which in the ARH are critical for sexual receptivity. Spinogenesis requires EMS to deactivate cofilin allowing the formation of new spines. EMS has implication in non-reproductive pathways as well. In dorsal root ganglion (DRG) neurons, estradiol attenuates ATP-activation associated with nociception. ATP signals through the activation of voltage-gated calcium channels (VGCC). EMS attenuates calcium flux through the L-type VGCC by activating ER α -mGluR_{2/3}. These studies demonstrate the importance of rapid, EMS through the nervous system and the role of estradiol as a signaling molecule.

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Plenary lecture I.

Memory Consolidation: Synaptic Tagging and Schemas

Richard G M Morris

University of Edinburgh Centre for Cognitive and Neural System, UK

Memory is fundamental to human life. Qualitatively distinct types of memory enable us to change our behaviour in response to experience, to acquire and use a repository of knowledge, to recollect events from the past, and to plan for the future. The use of memory is changing, with an increasing amount of human knowledge now externalised and then sought on-demand via the internet. Language is replete with aphorisms about knowledge. “Knowledge is power” - “A little bit of knowledge is a dangerous thing” - “Europe needs a knowledge-based society”. These and other assertions reflect the apparently central place that ‘knowledge’ plays in contemporary life. But how much do we understand about the brain mechanisms responsible for the acquisition and use of knowledge?

I shall argue that the accumulation of knowledge rests, in part, on the assimilation of newly acquired episodic information via the separate but interdependent processes of *cellular* and *systems* consolidation. The latter is a relatively slow, network-level process, widely thought to involve the interaction of brain structures in the medial temporal lobe, such as the hippocampus, with the prefrontal cortex and midline regions such as cingulate and retrosplenial cortex. Cellular consolidation is a kind of filter into systems consolidation that enables only a subset of new information, automatically encoded during the day, to be retained and so to persist.

Two contributions that my group have made to understanding these complex processes are: (1) To offer evidence that cellular consolidation requires the automatic setting of local *synaptic tags* in a post-translational manner that can then capture the products of plasticity-related protein synthesis and so stabilise synaptic change; and (2) To draw attention to the role of *schemas* (activated frameworks of prior knowledge) embedded in neocortical networks in systems consolidation. Drawing upon electrophysiological, biochemical, behavioural and optogenetic data, I shall endeavour to explain how we are attempting to learn more about the process of assimilating new information with what we already know

P1/99**Relations between the brain serotonergic system and abnormal aggression stemming from social adversities in childhood.**

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Human studies have demonstrated that early-life social adversities (such as abuse and neglect) may lead to the development of psychopathologies in adulthood that involve abnormal manifestations of aggression. To further study this phenomenon, we have previously developed the model of post-weaning social isolation, in which isolated rats show exacerbated stress-responses and a marked increase in abnormal forms of aggression. Here we proposed to investigate the links between certain key components of the serotonergic system and abnormal aggression induced by post-weaning social isolation. We have examined the gene expression levels of the enzyme monoamine oxidase-A (MAOA), 5-HT1a and 5-HT1b receptors in two brain regions which play a fundamental role in the control of aggression: the medial amygdala (MeA) and the medial prefrontal cortex, using qRT-PCR. Our results show that under resting conditions, MAOA mRNA levels in both the MeA and mPFC were significantly higher in socially isolated rats. Aggressive interaction significantly increased MeA MAOA levels in socially housed rats, in contrast, isolated rats showed a significant decrease in both brain regions. No significant differences were found in either 5-HT1a or 5-HT1b mRNA levels between groups, although a significant negative correlation was found between mPFC and MeA 5-HT1a mRNA levels. We have also investigated whether adult re-socialization of isolated rats coupled with chronic treatment of the selective serotonin reuptake inhibitor fluoxetine would allow isolated rats to re-learn normal social behavior. The results show that fluoxetine, coupled with adult re-socialization, significantly decreased abnormal aggressive behavior in previously isolated rats. Our findings may help in mapping out the complex relationship between childhood neglect, adult abnormal aggression and the brain serotonergic system in humans and may aid in the development of novel treatment strategies.

P1/22**Distinct properties of excitatory synaptic transmission from pyramidal cells to fast spiking basket and axo-axonic cells in the human and rat cerebral cortex**

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Identified human pyramidal cells are capable of triggering stereotyped series of monosynaptic and polysynaptic postsynaptic potentials in simultaneously recorded target cells. Induction of these event series requires spike-to-

spike coupling from pyramidal cells to interneurons that elicit downstream hyperpolarizing and depolarizing GABAergic responses.

We explored the properties of excitatory connections made by pyramidal cells on basket and axo-axonic cells by performing simultaneous whole-cell patch-clamp recordings in acute slices derived from human and rat association cortices. We found that the amplitude of AMPA receptor-mediated excitatory currents was an order of magnitude larger in the human cortex compared to the rat. However, light microscopic estimates of the number of synapses linking the coupled cells showed a similar number of potential contacts. To reveal quantal parameters of unitary connections and the time course of synaptic glutamate release, we used multiple probability fluctuation analysis and deconvolution of evoked EPSCs. Human connections are characterized by increased quantal size and multiple release of quanta with successive waves of asynchronous release.

Our results suggest that prevalent feed-forward spike transmission in human neural networks is driven by quantal properties and asynchronous synaptic currents unprecedented in rodents leading to robust depolarization of interneurons.

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P2/68

Assessment of motor capabilities of FL-PGC-1 α -deficient mice - fine details on motor coordination

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OBJECTIVE: To revisit the contrary results about motor features of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α)-deficient mice, with special focus on coordinative motor skills and their age-related progression.

BACKGROUND: Dysfunction of mitochondria in several neurodegenerative diseases is attended by a deficient function of PGC-1 α master regulator gene, involved in proper mitochondrial functioning. PGC-1 α -deficient mice have been proposed as a morphological model for mitochondrial diseases. Prior results found with behavioral examination of the two different PGC-1 α -deficient murine strains are conflicting. The complete knockout strain of PGC-1 α was shown to be hyperactive with normal muscle strength, contrasting with the strain deficient in full-length protein (FLKO) but expressing a functional N-terminal fragment, which exerted decreased motor performance and muscle strength.

METHODS: Examinations were performed on 15-18 age-matched male FLKO and C57Bl/6 (wild-type, WT) mice per group. Animals of a wide spectrum of age were investigated (median 47.1 w and 48.2 w). Spontaneous locomotor activity was measured with an open-field motimeter for 30 min. Muscle strength was tested by 3-trial-assessment of the latency to fall from an inverted screen. Motor coordination ability was defined by a 3-trial rotarod paradigm. Gait pattern was marked by a video-assisted catwalk system.

RESULTS: Motimetric examination showed that FLKO mice exert a modestly decreased motor performance and fall from both the inverted screen and the rotarod after notably shorter time durations compared to WT. Gait analysis revealed a moderate widening of the base of support relative to a well-preserved stride length. None of the parameters declined with the age of life in FLKO mice.

CONCLUSIONS: The lack of PGC-1 α protein in mice results in a behavior defined by modest hypomotility and ataxia, and plainly decreased rotarod performance and muscle strength. These changes appear at young ages and did not worsen with the age of life. Subtle changes in gait pattern compared to the robustly decreased rotarod and inverted screen performances suggest that observed motor alterations in FLKO mice might be mostly attributable to muscle weakness rather than a movement disorder due to the known pathologies in the cerebellum and brainstem. Applied paradigms and FLKO mice may serve as valuable tools to screen therapeutic candidates aiming to treat mitochondrial myopathies.

P1/46

Increase of Syntaxin 1B staining intensity in the cortex and hippocampus of Genetic Absence Epilepsy Rats from Strasbourg (GAERS)

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Recent investigations have shown that the absence seizures in the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) could originate from somatosensory regions of the cerebral cortex. In addition, a question arose, whether epileptic reorganization is present in the hippocampus in idiopathic epilepsy. In light of this we have investigated the expression level of Syntaxin 1B (STX1b), a synaptic protein known for its role in vesicle fusion and synaptic plasticity. Quantitative analyses was carried out in the somatosensory cortex and hippocampus by measuring the staining intensity profile of immunostained sections. Because recurrent epileptic seizures arise in 3 months old GAERS rats, we used 4 months old GAERS animals in the phase of chronic epilepsy for our experiments. STX1b intensity has been measured in layer V of the somatosensory cortex and in the CA1, CA3 regions of the hippocampus. We found that Syntaxin 1B intensity level has increased significantly in GAERS in the hippocampal CA3a,b regions in comparison with non-epileptic controls. However, the increase measured in the somatosensory cortex and other hippocampal regions was not statistically significant. This leads to the conclusion that absence seizures may cause alterations in the expression levels of vesicular fusion proteins in the synapse, presumably due to the hyperactivity of hippocampal networks. On the other hand, it may also suggest the presence of mossy fiber sprouting in GAERS. Further studies are needed to clarify which mechanism is responsible for our results. Our findings were achieved using fluorescent immunocytochemistry and confocal microscopy at the Nikon Microscopy Center at the IEM-HAS. Intensity levels were measured using the NIS Advanced Research software.

P2/64**The modulatory effect of N-Arachidonylethanolamide on nitroglycerin-induced kynurenine aminotransferase II expression in the trigeminal system of the rat**

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Migraine belongs to the group of primary headaches, and it is one of the most prevalent neurological diseases, which affects 16% of the total population. Despite the intensive research, the exact pathomechanism is not known, but the role of glutamate seems pivotal.

One of the endogenous glutamate receptor antagonists is kynurenic acid (KYNA) formed from L-kynurenine by kynurenine aminotransferase II (KATII).

Systemic administration of nitroglycerin (NTG)-nitric oxide donor (NO)-is a human and animal model of migraine. On the other hand, NO leads to the downregulation of the respiratory chain complex activities and causes release of the mitochondrial protein cytochrome C, resulting in mitochondrial dysfunction. This phenomenon might be associated with decreased KAT-II activity.

Several studies have suggested that endocannabinoid system is involved in the processing of nociceptive signal. N-Arachidonylethanolamide or anandamide (AEA) is an endogenous agonist of the cannabinoid receptors and causes reduction of calcium-induced cytochrome C release protecting mitochondria against cytochrome mediated damage.

The aim of the present study was to examine the modulatory effects of AEA on the NTG-induced expression of KAT-II in the upper cervical spinal cord of the rat, where most of the trigeminal nociceptive afferents convey.

Adult male rats (n=24) were used. Half of the animals received intraperitoneal NTG (10mg/kg) four hours before the experiments, the remaining rats were treated with the placebo of NTG. Half of the NTG and placebo treated animals also received AEA (2x5mg/kg half hour before and one hour after the other treatment), whereas the remaining rats were injected only with vehicle of AEA. Then the animals were perfused transcardially and the cervical spinal cords were removed for KAT-II Western Blotting.

The results of the present experiment shows that NTG decreased KAT-II expression in the C1-C2 segments, and AEA attenuated this effect.

To summarize our data, we suggests that NTG may cause mitochondrial damage, which might be associated with the activation of the trigeminal system and AEA is able to inhibit this effect probably by the decrease of cytochrome C release.

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P1/16**Physiologically relevant activity patterns multiply the synaptic impact of single mossy fibers for several seconds**Máté Neubrandt¹, János Brunner¹, János Szabadics¹¹ *Institute of Experimental Medicine, Hungarian Academy of Sciences, Pharmacology, Budapest, Hungary*

Hippocampal mossy fibers (MF), the axons of DG granule cells (GCs) provide a major synaptic input to the CA3 network. GCs are usually quiescent but after several seconds long inactive periods they either fire single action potentials or short high-frequency bursts. Mossy fibers target primarily GABAergic cells in addition to pyramidal cells, and their synaptic output shows diverse postsynaptic cell-type specific short-term dynamics. We asked how single spikes and short, high-frequency burst firing affects the output of single MFs by paired recordings of anatomically identified postsynaptic CA3 neurons, and single presynaptic MFs either direct recordings from giant MF boutons or from CA3 GCs. Intriguingly, single bursts in single presynaptic MFs (15AP at 150Hz) increased the unitary amplitudes by $288 \pm 12\%$ ($n=52$ pairs) for a relatively long period (0.8-8 sec after the burst) in a subset of postsynaptic GABAergic cells including ivy, fast-spiking basket, axo-axonic and regular-spiking basket cells. This amplification developed only after several hundreds of milliseconds, the unitary amplitudes exceeded the maximal compound amplitudes during the high-frequency train (by $180 \pm 8\%$) and shorter burst were similarly effective ($319 \pm 34\%$ after 4-7 APs at 150Hz). Changes of the short-term dynamics of the connections suggest the involvement of presynaptic changes. However, the augmentation was not permanent as the amplitudes returned to their initial levels within 60 seconds and in postsynaptic pyramidal cells and other GABAergic neurons the effect of the bursts only resembled classical post-tetanic-potential. These results highlight that single, physiologically relevant short bursts of single MFs nearly maximize the efficacy of the synaptic transmission for almost ten seconds in a specific subset of postsynaptic GABAergic cells, which provide powerful feed-forward inhibition to the CA3; thus the feed-forward network between the DG and CA3 are efficiently rearranged after a single MF burst.

P2/58**The hippocampo-septal feedback controls the activity level of medial septal neurons**Litsa Nikitidou Ledri¹, Andor Domonkos¹, Edit Papp¹, Karl Deisseroth², Gabor Nyiri¹, Tamás Freund¹, Viktor Varga¹¹ *Institute of Experimental Medicine, Department of Cellular and Network Neurobiology, Budapest, Hungary;*² *Stanford University, Department of Bioengineering, Stanford, United States*

The hippocampus is an indispensable component of the brain's memory circuitry with activity patterns tightly coupled to stages of information processing. For normal hippocampal operation, input from the medial septum (MS) is vitally important. The septo-hippocampal cholinergic connection adjusts the activity state of the hippocampal network, the GABAergic component paces rhythmic fluctuation of inhibition, whereas the glutamatergic input modulates both inhibitory and excitatory neuronal activity. A subpopulation of somatostatin-containing GABAergic neurons of the hippocampus send a massive feedback to the MS, targeting GABA-releasing and to a lesser extent cholinergic neurons. Despite some theoretical and in vitro data the function of this prominent back-projection is still unknown. Here, we selectively manipulated the somatostatin-containing HS feedback in both urethane-anesthetized and freely moving mice, and recorded the response of MS neurons. Previously, we demonstrated a state-dependent, inhibition-based modulation of MS neuronal activity by stimulating the HS feedback in a temporally precise manner using optogenetic actuators. In the current phase of the study we observed a dramatic increase of activity in response to the blockade of HS fibers by the switchable chloride-selective channelrhodopsin SwiChR-CA during both theta and non-theta states. Theta burst of rhythmic MS neurons were also affected manifested as the lengthening of the bursts with the concurrent increase of intraburst spike number. Our results suggest that the function of the HS feedback is the state-dependent gain modulation of the MS network.

P2/108**Analysis of the role of dopamine in novelty processing**João Noutel¹, Sebastian Haesler²¹ *Nerf, , Leuven, Belgium;* ² *KU Leuven, Nerf, Leuven, Belgium*

Novel stimuli trigger orienting behaviors in animals. The capacity to detect changes and to learn invariances in the environment allows animals to adjust their behavior accordingly. It seems, then, to be of elementary importance for the understanding of brain functioning, to investigate the neural basis of novelty processing.

One of the brain regions implicated in novelty processing is the dopaminergic midbrain. We propose to dissect the role of dopamine in novelty processing by combining optogenetics and multi-electrode technology in behaving mice. Specifically, we aim at 1) determining the coding properties of optogenetically identified dopamine neurons in response to novel and familiar stimuli; 2) testing the causal contribution of dopamine for learning novel stimuli by optogenetically manipulating the activity of dopamine neurons during behavior; 3) using novel viral strategies, to search for possible routes of novelty information entry to dopamine neurons by recording and manipulating the activity of optogenetically-tagged inputs to dopamine neurons.

Here, we developed a behavioral paradigm in which novel and familiarized olfactory stimuli are delivered to mice while monitoring orienting respiratory behavior (exploratory sniffing). Experiments are performed in a context where dopamine novelty responses can be studied separately from other manipulations that are known to affect dopamine firing (i.e. rewards).

In these conditions, mice show exploratory sniffing to novel (first presentation, median respiratory frequency 0-5s poststimulation = 5.8 Hz) but not to familiar (2.9 Hz) stimuli. By decreasing the frequency of presentation, and therefore the expectedness, of one of the familiar stimuli, we find that respiratory behavior to the rare familiar stimulus is indistinguishable from that of more frequent familiar stimuli. This suggests that respiratory changes to novel odors are not due to surprise. Finally, respiratory response habituates after 2-3 presentations of a novel odor, indicating that novel odors are learned rapidly.

This study lays a framework for future electrophysiology studies of novelty processing mechanisms in mice.

P2/85

Investigation of complex effects of central insulin infusion on energy balance in male Wistar rats of different ages

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Background: Peripheral effects of insulin and peripheral insulin resistance have been subjects of numerous studies. Investigation of central metabolic effects of insulin and the role of central insulin resistance started only during the last decade. Similarly to leptin, central insulin administration decreases food intake and other studies described its hyperthermic effects, as well.

Objective: We aimed to study the complex effects of a central insulin infusion on energy balance in 2-, 4-, 6-, 12- and 24-month-old rats.

Methods: We analyzed the effects of a 7-day intracerebroventricular insulin infusion (Actrapid, 100mU/day) in male Wistar rats of different age-groups. Circadian values of core temperature, heart rate (indicator of metabolic rate) and horizontal locomotor activity have been recorded continuously in a biotelemetric system (MiniMitter) in freely moving rats. Food intake and body mass were measured manually every day.

Results: Food intake of 2- and 24-month-old (juvenile and old) animals was not affected significantly by central insulin infusion. Food intake of 4-month-old rats showed a decrease in the first 3 days, while that of 6- and 12-month-old ones was lowered significantly during the course of the infusion. Core temperature of 2-month-old animals did not show any change. Concerning 4-month-old rats, both their daytime temperature minima and nighttime maxima increased significantly during the infusion. In 6-month-old animals only the daytime core temperature values rose for 6 days. In 12-month-old rats the increase was significant only for 4 days. The temperature rise of old animals just reached the border of significance during the first night. In case of heart rate the daytime minimum values of 4- and 6-month-old rats increased significantly.

Conclusion: Central insulin infusion has a coordinated catabolic effect in young adult and middle-aged animals. Anorexigenic and hypermetabolic effects of central insulin change with different dynamics during aging. While its metabolic effects are stronger in young adult animals, the food intake suppressing effects are more pronounced in

the middle-aged ones. In old rats insulin resistance regarding both anorexigenic and hypermetabolic effects was observed. (34039/KA-OTKA/13-25)

P2/7**Comparison of 2D and 3D neuronal differentiation of patient specific induced pluripotent stem cells**

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Patient-derived induced pluripotent stem cells (iPSCs) are providing a useful tool to model the pathology of certain diseases, especially when the affected tissue is hard to study, like in the CNS. Pluripotent cells, carrying the genotype of a given patient, can be differentiated in vitro to neural precursor cells (NPCs) which are then used to obtain neuronal and glial cells in culture. These cells offer a valuable platform which allows the investigation of the patient-specific pathomechanism of a disease. Furthermore, NPCs and their derivatives can be used for in vitro drug testing assays. Finally, these cells serve as an unlimited source of immune compatible cells for cell replacement therapies to treat neurodegenerative disease such as Parkinson's or Alzheimer's disease.

Our aim is to develop a highly efficient and reproducible assay to generate neurons and glial cells from healthy and diseased iPSCs. Here we compared the usage of various small molecules and recombinant proteins in a step-wise manner, which selectively induces neural differentiation of iPSCs into NPCs and later into different neural lineages. The treatment induced neural rosette formation followed by the occurrence of neuroepithelial cells (NEPs) resulting in a homogeneous population of NPCs. At the NPC stage all small molecules were withdrawn allowing for the terminal differentiation of mature post-mitotic neurons. Neuronal lineage was confirmed by immunocytochemistry (ICC) and RT-qPCR for neural and neuronal specific genes. We have established a highly reproducible and efficient procedure to generate patient-specific neuronal cells from iPSCs. In future, 2D and 3D neuronal cultures will be investigated in electrophysiological measurements.

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P1/18**Functionally distinct populations within anatomically similar CCK-expressing hippocampal interneurons due to different availability of potassium conductances**

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Specific functions of various voltage-dependent ion channels might contribute to the functional divergence of GABAergic cell types. Distinct GABAergic cell types possess different firing properties, which are important for fulfilling their functions in neuronal networks. Here we show that within a morphologically homogeneous cell type, individual GABAergic cells have markedly different firing properties. Using whole-cell patch-clamp recordings and anatomical identification, we distinguished two populations of CCK-expressing GABAergic cells (CCK+ IN) in the CA3 region of the hippocampus based on the presence or absence of membrane potential-dependent rectifying firing properties. In some CCK+ INs, action potentials are inhibited at the onset when the firing is evoked from hyperpolarized membrane potentials (at -80mV). However, this transient outward rectification was absent in the same cells when firing is induced from depolarized membrane potentials (-60mV). The second subset of morphologically indistinguishable CCK+ INs did not show membrane potential-dependent inhibition of spiking (regular firing cells). Interestingly, the presence or absence of the rectifying firing characteristic did not correlate with any of the previously known anatomical subdivision of CCK+ INs; thus, the membrane potential-dependent rectification provides a novel level of complexity within this cell group. Voltage-clamp recordings revealed that rectifying CCK+ INs have substantial transient, A-type K⁺ currents at subthreshold potentials with leftward shifted inactivation kinetics. Voltage dependence, temporal dynamics, pharmacological profile and intracellular calcium

dependence of the low voltage activated potassium current in rectifying CCK+ IN explain the membrane potential-dependency of their firing and consistent with Kv4 channel-mediated function. Immunolocalization experiments revealed that both the Kv4.3 subunit and its associated protein, KCHIP1 are present in both functional groups, demonstrating that their presence alone cannot explain the rectification in CCK+ INs. Our results reveal that different availability of a single inactivating potassium conductance can establish different functionality among anatomically homogeneous CCK+ INs in the hippocampus.

P1/107**Development and validation of a simple procedural test battery for cognitive translational research**

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Cognitive decline is defined as a pathological deterioration of cognitive function. In 2013 there were an estimated 44.4 million people with pathological ageing and dementia posing a major public health threat worldwide. Up to date, there are no effective pharmacological interventions against the progress of dementia. In basic and applied translational research new animal models are needed to provide better predictability for clinical testing. In the present study, we set out to develop a procedural cognitive test battery for non-human primates (NHPs) as their performance is directly comparable to that of humans and data obtained with NHPs would greatly increase the translational power of non-verbal cognitive tests. Four male rhesus macaques were trained in two different procedural reaction time (RT) tasks. Both tasks involved responding to a visual target presented on a computer monitor with a key release for liquid reward. Animals played a maximum of 400 trials in a session. In the first task (simple reaction time task) a single target stimulus indicated key release with a fairly constant latency to enable testing sustained attention. In the second task (pavlovian visual discrimination task), different visual targets indicated different reward magnitudes and optimal task performance involved recall of information from memory. The tests were validated by means of prior systemic treatment with transient pharmacological amnesic agent scopolamine (15 ug/kg) and cholinesterase inhibitor donepezil (10 and 20 ug/kg b.w.). Scopolamine significantly decreased task performance (ratio of completed trials) and increased RT which measures were only partially reversed by donepezil. In the pavlovian discrimination task, scopolamine increased task errors mainly in trials with smaller rewards indicating a general detrimental effect caused by cholinergic antagonism on cognitive performance.

To sum up, the present test battery sensitively measures continuous task performance and the behavioral results may be directly comparable with similar simple vigilance tests which are widely used to test cognitive performance in humans. In addition, future research will determine whether neural (EEG) correlates of behavioral (RT) measures would similarly predict general cognitive abilities of humans and NHPs. Thus, the presently proposed test battery may greatly improve the predictability of pre-clinical testing of newly developed drug candidates in dementia research.

P1/102**Age-related peculiarities of the default mode and sensorimotor brain network interaction in human brain: fMRI study**

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Default mode network (DMN) has gained considerable attention due to its role of resting brain activity regulation, and the evidence of functional connectivity disruption in autism and Alzheimer's. Sensorimotor network (SMN) activation is accompanied by the DMN deactivation. SMN consists of primary (M1), supplementary (SMA), ventral and dorsal premotor (PMv, PMd) cortices, and cerebellum. DMN consists of posterior cingulate cortex (PCC), precuneus (pC), medial prefrontal cortex, bilateral inferior parietal lobuli, bilateral posterior medial temporal gyri. Considering age-related peculiarities of DMN pattern, we propose analysis of the SMN and DMN interaction during the movements in different age groups for brain maturing and ageing visualization.

Three groups of subjects were studied during the finger tapping motor task execution by fMRI with 1.5T SIGNA (GE, USA). Group1 - 8 healthy subjects (3M, 5F, 7-16 y.o.), Group2 - 12 healthy subjects (6M, 6F, 24-48 y.o.). Group3 - 9 healthy subjects (4M, 5F, 51-86 y.o.). EPI sequence was used for T2*W BOLD imaging (TR/TE=3000/71 ms, voxel size=4x4x5 mm). Anatomical T1W images were acquired with FSPGR sequence (TR/TE=11.6/5.2 ms, TI=450 ms, voxel size=1x1x1.5 mm). FMRI data processing was done with FSL software (Oxford, GB).

Data analysis revealed SMN (M1, SMA, PMd, PMv, cerebellum) activation in Group1, 2, 3 during the movement execution. Volumes of activation for three groups (V1, V2, V3, cm³) were (51.9, 102.5, 125.3). Additional activation of ipsilateral PMd, PMv regions were shown in the Group3. Deactivation pattern varied in three groups. Ipsilateral M1 deactivation was shown in Group1. Described DMN regions deactivation was shown in Group2. Only PCC and pC were shown to deactivate in Group3. Volumes of deactivation for three groups (V1, V2, V3, cm³) were (6.3, 125.6, 4.4). Ratios of brain activation/deactivation (V_{rat1}, V_{rat2}, V_{rat3}) were (8.2, 0.8, 28.5). The activation-deactivation balance is only shown in the adult age (Group2). The process of activation prevailed in the juvenile and elder groups. Increased volume of activation in older subjects supports the hypothesis of additional brain regions need for compensatory mechanism in movement execution. Absence of DMN deactivation in the juvenile is supported by the literature, where DMN is shown to mature with age in young children. Deactivation of PCC and pC in Group3 support DMN partial functioning in elderly and PCC 'hub' role in DMN network.

P2/31

Glucosylceramide synthase regulates the activation of TRPV1 and TRPA1 receptors in cultured dorsal root ganglion neurons

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Previous findings have revealed the involvement of gangliosides in the regulation of neuronal capsaicin sensitivity by showing a reduced activation of the transient receptor potential vanilloid type 1 (TRPV1) receptor following the inhibition of glucosylceramide synthase (GCS), the key enzyme of ganglioside synthesis, in cultured rat dorsal root ganglion (DRG) neurons. In the present study we examined the effects of inhibition of GCS on the activation of the TRPV1 and the transient receptor potential ankyrin type 1 channel (TRPA1) receptors by endogenous vanilloids and allylthiocyanate (AITC), respectively.

Cultures of rat DRG neurons were prepared and kept in culture media containing D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP, (30 nM), an inhibitor of GCS or its vehicle, for 4 days. The cobalt uptake technique was applied to study the activation of TRP ion channels by capsaicin (1 µM), N-oleoyl-dopamine (OLDA, 100 µM), N-arachidonoyl-dopamine (NADA, 100 µM), anandamide (ANA, 100 µM) and AITC (100 µM) in control and D-PDMP pretreated cultures.

The inhibition of GCS was demonstrated by a significantly reduced neuronal binding of cholera toxin B subunit in D-PDMP-exposed cultures. As compared to the controls, D-PDMP treatment resulted in significant reductions of the proportions of cobalt-labelled neurons regarding all agonists as follows: capsaicin 43.02±1.13 vs. 10.36±3.99; ANA 32.04±2.71 vs. 11.27±1.22; NADA 27.60±1.97 vs. 8.44±3.55; OLDA 24.42±2.34 vs. 8.37±1.86; and AITC 37.41±2.47 vs. 20.27±2.11 (P < 0.05 for all group comparisons).

The results confirm and extend previous findings implicating neural gangliosides in the regulation of the activity of the TRPV1 receptor. The present findings identify a novel molecular target of antinociception by demonstrating that activation of nociceptive TRP channels may be modulated by neuronal GCS possibly via changes in neuronal ganglioside level.

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P1/67

The effect of light flashes on contrast thresholds of parvocellular and koniocellular pathway associated colors

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The role of the primary visual cortex (V1) in conscious vision has been widely discussed since the description of residual vision after damage to this cortical area by Riddoch in 1917. According to our current knowledge, conscious vision utilizes the signals of three subcortical pathways, called magnocellular (MC), parvocellular (PC) and koniocellular (KC). The PC and KC pathways can be stimulated selectively using isoluminant red-green and blue-cone isolating stimuli, respectively. The main goal of the present study was to investigate the contribution of V1 to the processing of stimuli specific to the PC and KC pathways using simultaneously presented light flashes that have been suggested to suppress V1 function (Schoenfeld et al. 2002). Eleven healthy volunteers with negative neurological and ophthalmological history were eligible for the study. Isoluminance of red-green and blue-cone isolating sine-wave gratings was set individually using the minimum motion procedure. The contrast thresholds for these stimuli as well as for achromatic gratings were determined by an adaptive staircase procedure where subjects had to indicate the orientation (horizontal, oblique or vertical) of the gratings. Thresholds were then determined again while strong white light flashes were presented 50 ms before each grating. We found that light flashes significantly ($p < 0.05$) increased the contrast thresholds of achromatic and blue-cone isolating gratings, however, it had no significant effect on the sensitivity to red-green gratings. Although these findings are surprising and difficult to explain, at least two hypotheses may be formulated. First, if we accept that light flashes suppress V1, pathways receiving PC input may be less sensitive to this manipulation. Second, the sensitivity difference may already be present at the subcortical level and in contrast to what has been described in the literature, the light flashes do not act on V1.

P1/92**Expression of Transforming Growth Factor beta receptors following MCAO in the rat brain**

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Transforming growth factor- β s (TGF- β s) regulate cellular proliferation, differentiation, and survival. TGF- β s bind to type I (TGF- β RI) and type II receptors (TGF- β RII), which are transmembrane kinase receptors, and an accessory type III receptor (TGF- β RIII). TGF- β may utilize a second type I receptor, activin-like kinase receptor (Alk1). TGF- β is neuroprotective in the middle cerebral artery occlusion (MCAO) model of stroke. Recently, we reported the expression pattern of TGF- β 1-3 after MCAO. To establish the involvement of TGF- β receptors in the neuroprotective effect of TGF- β s following MCAO, the present study describes the induction of TGF- β RI, RII, RIII and Alk1 at 24h, 72h and 1 month after transient 1h MCAO as well as following 24h permanent MCAO using in situ hybridization histochemistry. In the intact brain, only TGF- β RI had significant expression: neurons in layer IV of the cerebral cortex contained TGF- β RI. At 24h after the occlusion, none of the TGF- β receptors showed any induction. At 72h following MCAO, all four types of TGF- β receptors were induced in the infarct area, while TGF- β RI and RII also appeared in the penumbra. Most cells with elevated TGF- β RI mRNA levels were microglia as the signal co-localized with microglial marker. TGF- β RII co-localized with both microglial and endothelial markers while TGF- β RIII and Alk1 were present predominantly in endothelial cells. All four TGF- β receptors were induced within the lesion 1 month after the occlusion. In particular, TGF- β RIII was further induced as compared to 72h after MCAO. At this time point, the TGF- β RIII signal was predominantly not associated with blood vessels suggesting its microglial location. These data suggest that TGF- β receptors are induced after MCAO in a timely and spatially regulated fashion. TGF- β receptor expression is preceded by increased TGF- β expression. TGF- β RI and RII are likely to be co-expressed in microglial cells while Alk1, TGF- β RII, and RIII in endothels within the infarct where TGF- β 1 may be their ligand. At later time points, TGF- β RIII may also appear in glial cells to potentially affect signal transduction via TGF- β RI and RII.

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P1/28**Molecular mechanisms coupling the long lasting, label-free intrinsic optical signal to short time neuronal activity**

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Voltage sensitive dye (VSD) imaging and intrinsic optical signals (IOS) are widely used for monitoring spatiotemporal neural activity in extensive networks. Identification of their major cellular and molecular components, however, has not been concluded yet.

We addressed these issues by imaging spatiotemporal spreading of IOS and VSD transients initiated by Schaffer collateral stimulation in rat hippocampal slices with temporal resolution similar to standard field potential (FP) recordings using a 464-element photodiode-array. By exploring the potential neuronal and astroglial molecular players in VSD and IOS generation, we identified multiple astrocytic processes that significantly contribute to both VSD and IOS signals, in addition to the expected neuronal targets.

Glutamate clearance through the astroglial glutamate transporter EAAT2 has been shown to be a major player in VSD generation even in a very short (<2 ms) time-scale, indicating that astrocytes do contribute to the development of spatiotemporal VSD transient. Moreover EAAT2 has found to be the major component of IOS as well. Under control conditions, role for neuronal K⁺/Cl⁻ cotransporter KCC2, but not for glial Na⁺/K⁺/Cl⁻ cotransporter NKCC1 was observed, therefore IOS previously supposed to reflect predominantly glial activation has considerable neuronal origin. In addition, non-specific anion channels, astroglial K⁺ clearance through Kir4.1 channel and astroglial Na⁺/K⁺ ATPase also contribute to the spatiotemporal IOS and VSD transients. Therefore, VSD imaging cannot be considered as spatially extended field potential measurement with predominantly neuronal origin, but it also reflects a fast communication between neurons and astrocytes.

Major players disclosed this way imply that IOS thought to reflect glial activation has neuronal contribution and the VSD signal with suggested predominantly neuronal origin has astroglial contribution. Our model may help to better interpret in vivo IOS and VSD signals and may support diagnosis in the future.

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P2/55

Comparisons of intrinsic and associative connections of the primate somatosensory cortex

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Somatosensory cortical areas 3b and 1 have a strong connectional relationship and their topographically organized body representations provide the opportunity to study structure-function relationships within and between these cortical areas. However, areas 3b and 1 exhibit functional differences as shown e.g. by the different magnification factors and receptive field sizes in the two regions. We hypothesize that a different size of horizontal and perhaps laminar spatial distribution of connections within and between areas 3b and 1 can be in part responsible for these observed differences. To investigate this possibility we used bidirectional tract tracing via injections of BDA into supragranular and upper deep layers of distal finger pad representations of area 3b and area 1 of the squirrel monkey. The sizes of injections were approximately 300 µm in diameter, matching the size of submodality specific tactile modules in areas 3b and 1. Irrespective of the origin, both retrograde and anterograde labeling exhibited supragranular dominance, making these layers the main site of interactions between the two areas. Interestingly, thick axonal processes, which could be a structural correlate of fast information exchange, connected predominantly the homotopic distal finger pad representations of areas 3b and 1. Kernel density analysis of retrograde labeling suggests that neuronal populations of a similar size provide input to column-size cortical regions within and between areas 3b and 1. In area 3b intrinsic connections formed significantly higher densities of bouton-like structures than afferents originating from area 1. However, in area 1 densities of bouton-like elements originating from intrinsic and inter areal afferents showed similar densities. Therefore, in contrast to area 3b where local (intrinsic) processing may dominate, area 1 shows higher integration of intrinsic and interareal information at least when considering the interactions of these two somatosensory areas. While these findings do not support the

idea that the spatial distribution of connectivity reflects differences of cortical magnification factors of areas 3b and 1, differences in the density of bouton-like structures of these two areas could signify differences in the amount or strength of integration within 3b vs 1 and therefore be an important anatomical substrate of their functional differences.

P1/31**GABA potency is determined by loop-F of the GABAA receptor alpha subunit**

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The alpha subunits of the GABAA receptor (GABR) are relevant in determining the function and pharmacology of these receptors. Following computer modeling studies, we have investigated the effect of exchanging amino acid residues in the F-loop of the human GABR alpha5 (GABRA5) subunit with those of the human GABRA2 (GABRA5_FL2) and vice versa (GABRA2_FL5). The properties of these subunits were analyzed in HEK 293 cells in the context of co-expressed GABAA beta2 and gamma2 subunits by voltage-sensitive dye assay and patch-clamp electrophysiology. GABA had significantly higher EC50 on receptors containing GABRA2_FL5 than GABRA2. Exchange of a single residue, D176K, largely reproduced these effects. GABRA5_FL2 was significantly more responsive to low of GABA than GABRA5, while having similar maximal effects. Male mice homozygous for knock-in of GABRA5_FL2 showed a deficit in the novel object recognition (NOR) test but had intact spatial working memory. The deficit in NOR was reversed by a negative allosteric modulator of GABRA5. These data show that changes in Loop-F can bring about up to 5-fold changes in the potency of GABA on GABRA2 receptors (EC50 1.2 vs 5.9 μ M). However, at synaptic concentrations of GABA (100 μ M) this is unlikely to be physiologically relevant. More significantly, Loop-F also shapes the response of GABRA5 receptors to low concentrations of GABA that are relevant for the predominantly extra-synaptic localization of these receptors. Indeed, selective changes in the cognitive performance of mice expressing "sensitized" GABRA5_FL2 subunits suggests, that these molecular mechanisms of ligand recognition are operative in vivo.

P1/70**GABAergic input from the basal forebrain controls spiking of GABAergic interneurons in the basolateral amygdala**

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The amygdalar nuclei play a key role in coding of fear and extinction memory in the mammalian brain. During information processing in the basolateral amygdala (BLA), local inhibitory cells can shape the output of amygdalar networks by controlling principal cell function. While experiencing an aversive cue, the activity of interneurons in the BLA has been shown to be suppressed, but the source of this inhibition remains unclear. One candidate for this inhibitory supply might originate from the basal forebrain, projecting GABAergic afferents into the BLA. However, it is still unknown what the targets of this extra-amygdalar GABAergic fibers are and how this subcortical input affects local interneuron function in the BLA.

In this study we first investigated the targets of GABAergic projection from the basal forebrain into the BLA by combining tracing experiments with electron microscopy. We found that GABAergic fibers from the basal forebrain selectively innervated local GABAergic cells in the BLA forming multiple synaptic contacts on their somata and dendrites. Next, by selective expressing channelrhodopsin 2 (ChR2) in parvalbumin-containing cells in the basal forebrain, we examined the effects of these subcortical afferents on the function of target cells in the BLA. We observed that stimulation of fibers by blue light in amygdalar slices evoked GABA-A receptor-mediated large amplitude postsynaptic currents preferentially in the interneurons. These light evoked events could powerfully inhibit or pace the spiking of local amygdalar GABAergic cells.

These results suggest that the GABAergic afferents from the basal forebrain can specifically control the spiking of local GABAergic interneurons in the BLA, providing an effective gating mechanism of amygdala function.

P1/44

Epileptic reorganization of GABAergic inhibitory interneurons in the cortex and hippocampi of Genetic Absence Epilepsy Rats from Strasbourg (GAERS)Péter Papp¹, Péter Szocsics², Zsolt Kovács³, Gábor Juhász⁴, Zsófia Maglóczy²

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Temporal lobe epilepsy (TLE) and generalized absence epilepsy are syndromes with unique etiology, clinical features and seizure activity with independent neuroanatomical sources. However, recurrent pathological excitation in epileptic seizures may induce reorganization processes in the affected networks, possibly bridging the neuropathological characteristics of the two conditions. We aimed at exploring potential epileptic reorganization in two functionally distinct classes of GABAergic inhibitory interneurons in Genetic Absence Epilepsy Rats from Strasbourg (GAERS) compared to non-epileptic (NEC) and normal Wistar (WISK) controls. Interneuron-specific and perisomatic inhibitory interneurons expressing the calcium-binding proteins calretinin (CR) and parvalbumin (PV), respectively, were examined in two regions of interest: the S1 somatosensory cortex and the dentate hilar subregion of the hippocampus. GAERS and, interestingly, WISK displayed a significantly lower density of CR-immunolabeled interneurons in the dentate hilus when compared with NECs. In addition, GAERS showed the same pattern in the infragranular layers of the S1 cortex. PV-immunolabeled interneurons were markedly reduced in density in the hilar regions of GAERS, but not in WISK. In the S1 region, no significant difference was found when comparing the PV-density of either group to NECs. Our results suggest significant levels of epileptic reorganization to follow from generalized absence seizures of GAERS and spontaneously occurring high-voltage rhythmic activities in normal Wistar rats. The severe loss of CR-interneurons involved in synchronizing dendritic inhibitory processes may lead to network hyperexcitability and an increased chance of developing individual epileptic foci. These results resemble our earlier findings in TLE patients, hinting that the reduced efficiency of interneuron-specific inhibition may be a common neuropathological feature in different forms of epilepsy.

P1/73**Brainstem projections of neurons located in various subdivisions of the dorsolateral hypothalamic area**Rege Sugárka Papp^{1,2}, Miklós Palkovits^{1,2}

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The projections from the dorsolateral hypothalamic area (DLH) to the lower brainstem have been investigated by using biotinylated dextran amine (BDA), an anterograde tracer in rats. The DLH can be divided into 3 areas (dorsomedial hypothalamus, perifornical area, lateral hypothalamic area), and further subdivided into 8 subdivisions. After unilateral stereotaxic injections of BDA into individual DLH subdivisions, the correct sites of injections were controlled histologically, and the distribution patterns of BDA-positive fibers were mapped on serial sections between the hypothalamus and spinal cord in 22 rats. BDA-labeled fibers were verified more than 100 brainstem areas, nuclei, or subdivisions. Injections into the 8 DLH subdivisions individually established distinct topographical patterns of BDA-labeled fibers. In general, the density of labeled fibers was low in the lower brainstem: highly dens accumulation of fibers was seen only 4 of the 116 areas: in the lateral and the ventrolateral parts of the periaqueductal gray, the Barrington's, and the pedunculo pontine tegmental nuclei. All of the biogenic amine cell groups in the lower brainstem (9 noradrenaline, 3 adrenaline, and 9 serotonin cell groups) received labeled fibers, some of them from all, or at least from 7 DLH subdivisions, mainly from perifornical and ventral lateral hypothalamic neurons. Some of the tegmental nuclei and cell groups within the reticular formation were also innervated, although the density of the BDA-labeled fibers there was generally low. No definitive descending BDA-positive pathway, but long-run, rostro-caudal solitaire BDA-labeled fibers were seen in the lower brainstem. These descending fibers joined some of the fibers of large tracts or fasciculi in the brainstem. In conclusions, the distribution pattern of BDA-positive fibers from the DLH was comparable to the distribution of previously published orexin- or melanin-concentrating hormone-immunoreactive fibers throughout the lower brainstem with somewhat differences in the topography, as well as the density of the labeled fibers.

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P2/52**Effect of an AMPA receptor antagonist, Talampanel, in vulnerable and resistant regions of the central and peripheral nervous system of amyotrophic lateral sclerosis SOD1 G93A mice model**

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Increased intracellular calcium (Ca) which might be the consequence of an excess influx through Ca-permeable AMPA-receptors has been implicated in the pathomechanism of amyotrophic lateral sclerosis (ALS). Previously we demonstrated that non-competitive AMPA-receptor antagonist (Talampanel; Ta) applied to transgenic mice with mutant Cu/Zn superoxide dismutase (SOD1) modeling familial ALS has significantly reduced Ca of spinal motoneurons (MNs) in Ta-treated presymptomatic transgenic group, which suggest that Ca-mediated toxicity in MNs can be reduced. The present study examined whether this protective effect could be observed in the executive regions of these MNs. Since Ca-mediated toxicity is thought to be reduced by elevated Ca-binding protein (CaBP) content in resistant type of MNs, additionally, possible synergetic effect of Ta-treatment on the Ca-level was tested in the oculomotor neurons and in their axon terminals. SOD1 mice were treated with Ta or Vehicle (Ve) at presymptomatic and symptomatic stages of the disease. Non-transgenic (NTG) mice treated either with Ta (n=6) or with Ve (n=5) served as controls. Ca-levels were estimated electron microscopically using the oxalate-pyranthionate technique and were expressed as volume percent (mean±s.e.m.) of the precipitates. Counts from the nerve endings in the interosseus muscle of presymptomatic mice show that, similarly to the cell bodies, Ta significantly reduced the Ca-levels in SOD1 mice compared to Ve-treated groups (SOD1+Ta: 10.01±1.15; SOD1+Ve: 12.36±1.10; NTG+Ta: 11.06±1.06; NTG+Ve: 9.91±0.77). Analysis of the late-stage Ca-level changes of motor nerve terminals show again similar results as in perikarya, i.e. significant differences between SOD1 groups (SOD1+Ta: 17.96±0.78; SOD1+Ve: 17.87±0.82) compared to the controls (NTG+Ta: 10.13±0.93; NTG+Ve: 10.24±0.82). Results from resistant cell types show that Ca-content either in nerve endings (SOD1+Ta: 10.13±0.085; NTG+Ve: 10.32±0.22) or in oculomotor neurons (SOD1+Ta: 6.21±0.38; NTG+Ve: 5.60±0.23) cannot be influenced with Ta-treatment. These findings demonstrate that Ta-treatment is able to reduce Ca-levels in cell bodies and also in axon terminals, which suggests that functional integrity of vulnerable MNs could be preserved. Our results also suggest that the protective effect of CaBPs against Ca-mediated toxicity is sufficient to avoid Ca increases in the presymptomatic phases of the disease, which cannot be further facilitated by Ta-treatment.

P1/62**Responses of rat medial prefrontal cortical neurons to Pavlovian conditioned stimuli and to delivery of appetitive reward**

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Environmental stimuli, by means of the incentive motivation, direct the organism to reward and lead the behavior to reach its goal, the successful consummation. In the present experiments, medial prefrontal cortical (mPFC) neurons were extracellularly recorded in freely moving rats by means of tetrode electrodes to examine their possible role in the prediction of appetitive reward.

In a Pavlovian trace conditioning paradigm (CS: 2 sec; trace interval: 2 sec; US: 12 sec; intertrial interval: 6 sec) two different tone CSs were associated with two different rewards (sugar solution or water), respectively. Neuronal activity changes in the mPFC were analyzed: (i) during CSs; (ii) just before the first licks of licking clusters; (iii) during consummation of the different rewards. Approaching movements of the rats to the drinking bottles were measured with an accelerometer mounted to the headstage amplifier. Early occurring approaches (during the first 2 sec of

reward delivery) were demonstrated to appear as CS related approach behavior: (i) significantly higher accelerations were observed during approaches to the drinking bottle containing sugar solution than to the water containing one; (ii) for both rewards significantly higher accelerations were observed during early approaches, then during late occurring approaches (2 to 12 sec of reward delivery). Population activity of neurons discriminated between trials with different US types (sugar solution or water) during CS tones, CS evoked approach behaviors and consummation. Analysis of single neuron activity during early and late approaches suggests that some responses are related to conditioned approach behavior (higher response during the early phase) while other responses code reward prediction independently from approach behavior.

These results proved that the activity of medial prefrontal cortical neurons represent consummation and prediction of different rewards. Our data also suggest that multiple representations of the appetitive reward are present in the rat mPFC during Pavlovian trace conditioning.

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P2/46

Analgesic effect of polysulfide compound dimethyl trisulfide is mediated via TRPA1 and somatostatin sst4 receptors

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Polysulfides initiate S-sulfhydration of proteins. They were reported to activate TRPA1 receptors on astrocytes. TRPA1 receptors are mostly expressed in nociceptive primary sensory neurons and contribute to mechanical hyperalgesia. Activation of these neurons might lead to neuropeptide release, including somatostatin (SOM) and calcitonin gene-related peptide (CGRP). Systemic antinociceptive and anti-inflammatory effects of SOM are mediated by sst4 receptors. The working hypothesis of this study was that polysulfide dimethyl trisulfide (DMTS) might activate primary sensory neurons and induces SOM release. SOM might elicit analgesic effect.

Ca²⁺ influx in TRG neurons of TRPA1 receptor wild-type (WT) and knockout (KO) mice was detected by ratiometric Ca²⁺ imaging with fura-2-AM. SOM release from isolated tracheae of Wistar rats was measured by radioimmunoassay. Heat injury was induced in one hind paw of TRPA1 or sst4 WT and KO mice by submerging the paw into 51°C water for 15 s under diethyl ether anaesthesia. Mechanical pain threshold of the hind limbs was then determined by dynamic plantar aesthesiometry. DMTS or vehicle was administered i.p. 30 min before heat challenge.

DMTS induced reproducible elevations of [Ca²⁺]_i in TRG neurons of TRPA1 WT animals. The response was inhibited by TRPA1 receptor antagonist HC-030031 and was absent in neurons from TRPA1 KO mice. DMTS induced concentration-dependent SOM release from isolated rat tracheae. SOM release was not affected by HC-030031. However, CGRP release measured from the same samples was inhibited by HC-030031. DMTS in 100, 250 and 500 µmol/kg doses had analgesic activity in mild heat injury-evoked mechanical hyperalgesia in TRPA1 WT mice. DMTS lacked analgesic effect in TRPA1 KO animals. DMTS had similar effect in sst4 receptor WT mice. The influence of 100 and 250 µmol/kg DMTS on mechanical pain threshold was abolished in sst4 KO mice, whereas that of 500 µmol/kg DMTS was unaffected.

The present study provides the first data on the effect of polysulfides on neuropeptide release, as well as on their in vivo analgesic effect and in vivo activation of TRPA1 by polysulfides. Analgesic effect of DMTS is mediated via TRPA1 receptors. SOM released from sensory neurons might mediate this effect at 100 and 250 µmol/kg doses, but other mechanisms are involved at 500 µmol/kg.

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P1/54

An ascending glycinergic pathway regulates the organization of movements via the intralaminar thalamus

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Coordination of movements and organization of behavior requires a communication channel which allows the activation signals from the brainstem and the incoming information from the environment to reach the forebrain and alter its state of alertness.

The intralaminar thalamic nuclei (IL) can represent a key hub in this task due to their rich connectivity with the frontal cortex, basal ganglia, brainstem motor centers and the reticular activating system (RAS) of the brainstem. However, direct evidence for the involvement of IL in motor control is still lacking due to the inability to transiently modify the activity of the irregularly shaped IL in a physiologically relevant manner.

Here we describe a GABAergic/glycinergic pathway ascending from the pontine reticular formation (PRF) of the brainstem and selectively innervating the IL via large multisynaptic terminals. The anatomical and physiological characteristics of the pathway allows fast and precise control of IL activity. An afferent system with comparable morphological features was also found in the human IL.

Optogenetic activation of the inhibitory PRF axons (30 Hz, 10-30 sec) in the IL of virus injected, freely moving, glycine transporter type 2::Cre (GlyT2::Cre) transgenic mice led to immediate behavioral arrest and the emergence of slow cortical oscillation for the duration of the stimulus. Weaker laser intensities resulted in bradykinetic rotation contralateral to the stimulation site. Under urethane anesthesia the stimulation either diminished IL firing or synchronized it with the cortical slow rhythm.

These data are consistent with a central role of IL thalamus in motor control and indicate that the ascending inhibitory influence may convey a behavioral arrest signal and promote the resting (idle) state of the thalamocortical network. Moreover the emergence of slow rhythms in the cortex during the activation of the pathway raises the possibility of the existence of a reticular inhibitory system (RIS) ascending parallel to the RAS and influencing the balance between conscious and inattentive state.

P2/77

Regulation of energy homeostasis in high fat diet fed Cx3CR1 deficient and control mice

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Energy homeostasis is maintained by a balance between food intake and energy expenditure.

Brown adipose tissue (BAT) plays an essential role in metabolic- and thermoregulation using fatty acids for heat production via uncoupling of mitochondrial oxidative phosphorylation.

Because BAT uses fuels stored in white adipose tissue (WAT), there is an increasing interest on the relationship between BAT and obesity. Both BAT and WAT contains significant population of immune cells, tissue specific macrophages, which have an impact on body metabolic and inflammatory functions.

Adipose tissues are under sympathetic nervous system control. Norepinephrine (NE) and NPY are co-released from sympathetic varicosities, but these signal molecules can be also expressed locally by macrophages.

Fractalkine (Cx3CL1, FKN) is a chemokine that attracts macrophages into several tissues, including adipose tissues. Fractalkine receptor (Cx3CR1) is expressed mainly by macrophages.

The aim of this study was to reveal the role of macrophages in regulation of energy homeostasis in obesity. We used male transgenic mice wherein Cx3CR1 was replaced by gfp reporter gene. Mice were fed with fat enriched diet (FatED, 2:1 mixture of standard chow and lard) or normal diet (ND) for 10 weeks starting at PND 35.

+gfp FatED - but not gfp/gfp FatED group – gained more weight and had higher relative BAT and WAT weight than ND fed mice.

Based on rt-PCR analysis in response to FatED adipose tissues expressed FKN and MCP-1, macrophages accumulated into BAT and WAT and produced inflammatory cytokines. In BAT both UCP-1 and its regulator PPAR γ expression were elevated. TH (the key enzyme in NE synthesis) did not, but NE receptor Adrb3r and NPY expression were increased in +gfp mice.

Lack of FKN signaling prevented mice from excessive weight gain, macrophage accumulation and inflammatory cytokine expression. Similar to control mice, increased mRNA levels of UCP-1, PPAR γ and Adrb3r were detected in BAT but NPY expression has not been elevated in response to FatED in Cx3CR1 gfp/gfp mice.

These results suggest that FKN signaling plays a significant role in energy homeostasis via NPY production by macrophages. In response to FatED, macrophages in BAT express NPY, which shifts lipolysis/lipogenesis balance

towards lipogenesis. In FKN receptor deficient mice macrophages do not accumulate into BAT, and do not express NPY, hence elevated number of UCP-1 can use excess fatty acids for heat production.

P1/6**Fos expression in the rat olfactory system neurogenic area following single exposure to odor deprivation during early postnatal stages**

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The subventricular zone (SVZ) of the lateral brain ventricles represents the largest site of neurogenesis in the adult mammalian brain. From the SVZ, neuroblasts migrate tangentially via the rostral migratory stream (RMS) to the olfactory bulb (OB), where they differentiate into local interneurons within the granule and periglomerular layers and integrate into synaptic circuits. It has been confirmed that individual processes of neurogenesis in the SVZ/RMS/OB system may be influenced by various factors of an environment. However, the mechanisms involved in regulation of neurogenesis by exogenous factors remain unclear. The aim of our study was to contribute to the understanding of these mechanisms by immunohistochemical assessment of Fos protein induction in the olfactory system neurogenic region of juvenile rats. Nuclear protein Fos is encoded by the immediate early gene c-fos, which is rapidly and transiently expressed in neurons in response to stimulation. To evaluate possible activation of Fos protein induction in cells of the SVZ/RMS/OB system, single exposure to early olfactory deprivation has been used. Olfactory deprivation in juvenile rats was induced using of well-described model of maternal separation. Rat pups of different ages (P7, P14 or P21) were separated from the dam for 120 minutes. Immediately after separation, the rats were deeply anesthetized and perfused. Serial, sagittal sections from the brain were incubated with the primary antibody against Fos protein. Our results demonstrate that single exposure of juvenile rats to odor deprivation induces a powerful activation of some olfactory system areas associated with postnatal neurogenesis. Quantitative analysis showed that individual areas of the olfactory system neurogenic region respond to maternal odor absence differently on dependence of the animal age. Significant increase of the number of Fos positive cells has been observed in all OB layers of P7, P14 as well as P21 animals. There were no Fos positive cells present in the RMS of juvenile rats neither under physiological conditions nor after exposure to maternal separation. However, we have observed some Fos producing cells in the SVZ of P21 control rats and the significant increase of them following maternal separation. This suggests that some SVZ cells of juvenile rats have complete prerequisites necessary for the Fos signal transduction cascade.

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P2/22**Effect of fructose feeding on the synaptic organization of the hippocampal CA1 neuropil**

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Energy balance is crucial for survival, but excessive energy intake may compromise health; it can also lead to cognitive deficits. Recent evidence suggests that different carbohydrate sources have different biological and metabolic properties, and they may influence cognitive function, implying synaptic plasticity in brain areas not directly linked to metabolic regulation. Fructose has been shown to cause impaired hippocampal-dependent spatial learning, but it remains unclear what structural changes in the hippocampal neuropil may underlie the observed cognitive impairment. Therefore, using a fructose-feeding protocol, we studied the synaptic neuropil of CA1 hippocampus using quantitative electron microscopy. We analysed the density, size, morphology and internal structure of asymmetric spine synapses of excitatory synaptic connections in the CA1 hippocampus. Our findings revealed previously unrecognized structural changes within dendritic spines as a function of fructose feeding, and suggest that the hippocampal synaptic neuropil is sensitive to changes in carbohydrate balance, supporting a link between metabolic balance and cognitive functions.

P1/2**Development of electrophysiological properties within hippocampal cell cultures: analysing burst and network activity by a multielectrode array system**Erika Anikó Rátkai¹, Krisztián Tárnok², Sándor Borbély¹, Katalin Schlett¹¹Eötvös Loránd University (ELTE), Department of Physiology and Neurobiology, Budapest, Hungary; ²Eötvös Loránd University (ELTE), Dept. Physiology and Neurobiology, Budapest, Hungary

Multielectrode (or microelectrode) array (MEA) is suitable for the non-invasive and long term electrophysiological analyses of neuronal cell cultures. Neuronal networks develop on the surface of 59 recording electrodes, therefore local field potentials (LFPs) can be recorded repeatedly either during in vitro development or upon pharmacological treatments. Based on LFP data, multi-unit activity (MUA) is calculated by cluster analysis. Additionally, network burst activity as well as the time-dependence and cross-correlation of spikes can be investigated.

We have analysed the development of network activity within mouse embryonal hippocampal dissociated cell cultures between the 6th and 13th day after plating (DIV6-13). In parallel with dendritic maturation and formation of dendritic spines during this period, neuronal networks are intensively redefined. Network burst activity was already present on DIV6. Bursts became longer, while interburst intervals (IBIs) were significantly reduced during development. Blocking of voltage gated Na⁺ or Ca²⁺ channels almost completely abolished burst or neuronal activity, respectively. GABAA receptors were involved predominantly in the regulation of IBIs and did not affect significantly the length and the spike frequency of bursts. AMPA receptors were involved in spontaneous network activity but NMDA receptors became active only after depolarisation of the cultures.

Long-term depolarisation of cultivated neurons is known to promote their maturation. After 3 days of KCl treatment, spontaneous burst activity was completely blocked and individual spikes appeared. Selective inhibition of protein kinase D, a serine-threonine kinase known to regulate synaptic and cellular plasticity, led to redistribution of network burst activity as bursts appeared in fast trains, divided by long (>30s) intervals. These results show that the MEA method is an effective tool to analyse the electrophysiological outcome of treatments regulating cellular plasticity.

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P2/74**Volume density of pituitary ACTH cells is modulated in gerbils submitted to global cerebral ischemia and exposed to extremely low frequency magnetic field (50 Hz, 0.5 mT)**Snežana Rauš Balind¹, Vesna Selaković², Milica Manojlović-Stojanoski³, Ljiljana Nikolić⁴, Dajana Todorović⁵, Verica Milošević³, Branka Petković⁴¹Institute for Biological Research, Department of Neurophysiology, Belgrade, Serbia; ²Institute for Medical Research, Military Medical Academy, Belgrade, Serbia; ³Institute for Biological Research, University of Belgrade, Department of Cytology, Belgrade, Serbia; ⁴Institute for Biological Research, University of Belgrade, Department of Neurophysiology, Belgrade, Serbia; ⁵Institute for Biological Research, University of Belgrade, Department of Insect Physiology and Biochemistry, Belgrade, Serbia

Cerebral ischemia, as a one of the categories of stroke, occurs after severe reductions in cerebral blood flow. As a consequence of insufficient oxygen and glucose supply, brain suffers dramatic changes (e.g. massive release of glutamate leads to excitotoxicity and damage of neurons especially in the hippocampus and striatum). Cerebral ischemia is detected by the hypothalamo-pituitary system through neuronal inputs in the paraventricular nucleus (PVN), upon whose stimulation adrenocorticotrophic cells release hormones (ACTH). ACTH acts on adrenal glands to secrete glucocorticoids (GC). GC among other functions affect glutamate concentration and apoptosis that is pronounced in GC receptors rich brain regions such as the hippocampus. Thus, the interaction between damaged neurons, PVN, ACTH cells and GC feedback has an important role in neurological function and outcome. Based upon mentioned, the aim of our study was to determine if the extremely low frequency magnetic field (ELF-MF), as an unavoidable environmental factor, effects pituitary ACTH cells in 3-month-old Mongolian gerbils submitted to global cerebral ischemia. After a 10-min occlusion of both common carotid arteries, the gerbils were continuously exposed to ELF-MF (50 Hz, 0.5 mT) for 7 days. Volume of adenohypophysis, as well as a volume density of immunolabeled pituitary adrenocorticotrophs, was analysed stereologically in gerbils sacrificed immediately (the

7th day after reperfusion) and 7 days after cessation of exposure (14th day after reperfusion). The results showed that volume of adenohypophysis was increased in ELF-MF group sacrificed immediately and 7 days after cessation of exposure for 39.8% and 30.9%, respectively, as well as in ischemic gerbils on the 7th (31%) and 14th reperfusion day (30.9%). In ischemic gerbils exposed to ELF-MF on the 7th reperfusion day, the decrease of adenohypophyseal volume was 24.4%. Volume density of ACTH cells was reduced by 19.2%, 6.2%, 10.9% and 33.2% in ELF-MF exposed groups, as well as group of gerbils after 7 days of reperfusion and ischemic gerbils exposed to ELF-MF for 7 days. Increase of measured parameter was observed in ischemic gerbils on the 14th reperfusion day (24.4%) and ischemic gerbils exposed to ELF-MF on the 14th day after reperfusion (12.6%). Based on the results, we may conclude that the pituitary ACTH cells are disturbed by global cerebral ischemia, ELF-MF and that ELF-MF modulates the ACTH response in the ischemic gerbils.

P1/69**Knocking out GAD67 from parvalbumin containing interneurons deplete gamma component of visual steady state response in the visual cortex**

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The coherence of gamma oscillation between local and distant brain areas is correlated with cognitive functions in the visual system. Parvalbumin-containing, fast spiking GABAergic interneurons are important component of gamma oscillation generator cortical local neuronal network. The high GABA level in fast spiking cells is maintained by GAD67 to some extent. However, it is unclear whether GAD67 is fundamentally needed for the generation of gamma oscillations or sufficient concentration of GABA can be produced by synaptic GAD65. To answer this question, we tested the gamma component of visual steady state responses (SSRs) evoked by 25 Hz flickering LED light in PV+ GAD67 knockout (KO) mice in vivo. Control (Balb/c) mice performed stable gamma component of SSRs (70-80 Hz) representing that the cortical activity accurately follows the 25 Hz flash and a gamma component steadily sits on the responses. In contrast, in GAD67 KO mice, SSRs become clearly unstable and fragmented in time and frequency as well. In addition, their gamma component becomes inconsistent in time and varies in frequency. Our results suggest that GAD67 is an essential enzyme in maintaining stability of gamma oscillations of the GABAergic interneurons in the visual cortex. Thus, GAD67 could be a target in revealing the pathomechanisms of mental disorders with gamma decrease, like schizophrenia and bipolar disorder.

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P1/12**Targeted differentiation of human pluripotent stem cells into dentate gyrus Prox1-positive neurons and medial ganglionic eminence GABA interneurons**

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Human embryonic stem (ES) cells and induced pluripotent stem (IPS) cells represent unique model systems for investigating the molecular machinery of neurodevelopment. Pluripotent stem cell-based methods have become an important research tools for developmental biology and molecular medicine. These self-renewable cell lines can be used to generate different neuronal types in vitro, and to study the differentiation process into various neuronal

types. Along this line, individual cell lines generated from different healthy or diseased individuals can also be compared.

We used established and published protocols to generate hippocampal dentate gyrus granule cells and forebrain GABA interneurons. The dentate gyrus protocol is based on dual SMAD inhibition, Wnt- and SHH inhibition after generating embryoid bodies from pluripotent stem cells, which procedure results in forebrain specific Sox2 and Nestin positive neuronal progenitor cells (NPCs). This intermediate cell type can be further differentiated into Prox1-expressing mature neurons. Using this protocol we compared the Wnt-antagonist effects of recombinant Dkk1 and XAV939, a small molecule used in other protocols. The forebrain GABA protocol exploits SMAD inhibition combined with SHH signaling to generate Nkx2.1-expressing medial ganglionic eminence precursors, which give rise to GABAergic interneurons. We characterized these cells by immunocytochemistry, Ca-imaging, single cell electrophysiology and qPCR.

The prospect of investigating neuropsychiatric disorders by reprogramming somatic cells of patients into IPS cells and using these differentiation protocols is tantalizing. Several such studies on disease models have been able to recapitulate certain aspects of these disorders in vitro. The introduced protocols will be discussed in the context of IPS cell based in vitro disease modeling.

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P2/102

Maturation of magnocellular and parvocellular visual pathways across the school-age years

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Much is known about the development of visual functions in infancy and early childhood. However, the age-related changes of subcortical visual pathways during school years and adolescence are not comprehensively characterized. The aim of this study was to examine the development of magnocellular and parvocellular visual pathways from the age of 7 to adulthood by investigating the contribution of these two subcortical channels to the recognition and categorization of natural images. We asked children aged 7-15 years and adults to perform an animal vs. vehicle categorization task with intact grayscale stimuli and luminance-matched images with high spatial frequencies (HSF) or low spatial frequencies (LSF) only. Our results indicate significant changes in magnocellular and parvocellular pathways across childhood, with a greater advantage of processing parvocellular channel-associated HSF stimuli and the primacy of detecting animals over vehicles, especially for LSF stimuli. These findings are discussed within the framework of proactive top-down effects on visual processing.

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P2/103

The effect of spatial frequencies on the electrophysiological correlates of visual categorization

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The goal of the present study is to investigate how low and high spatial frequencies that are primarily conveyed by the magnocellular and parvocellular pathways respectively, modify event-related potentials during the first 200 milliseconds of visual analysis. Thirteen healthy subjects participated in an animal vs. vehicle categorization task with intact grayscale stimuli and luminance-matched images with high spatial frequencies (HSF) or low spatial frequencies (LSF) only. A 32-channel EEG was recorded and changes of the P1 and N1 visual event-related

components were analyzed at posterior electrode sites. Moreover, scalp maps of category-specific neural activities were compared between intact vs. HSF images and intact vs. LSF stimuli in the 120-160 ms and 170-210 ms intervals post-stimulus. The results indicate that neural signatures of processing are modified differently by low and high spatial frequencies during the first 200 milliseconds. Specifically, P1 is modulated by low spatial frequencies, while the N1 is modified by high spatial frequencies.

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P1/23

GABAergic signalling from individual interneurons to astrocytes in the cerebral cortex

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Direct communication between individual neurons and astrocytes is limited by the absence of synaptic junctions between these cell types. Astrocytes express GABA receptors and GABAergic neurogliaform cells in the neocortex were suggested to reach target neurons through a unitary form of volume transmission. Testing several cortical interneuron types, here we show selective unitary coupling from neurogliaform cells to astrocytes with a fast, GABAA receptor and GABA transporter mediated component and a slow component requiring the indirect activation of GABAB receptors on neurons. Unitary GABAergic responses failed to produce Ca²⁺ influx in astrocytes. Our experiments identify a presynaptic cell type specific, GABA mediated communication pathway from individual neurons to glial cells suggesting a role for unitary volume transmission in the control of ionic and neurotransmitter homeostasis.

Symp I. - Thursday

Hippocampus-derived estradiol and its role in synaptic plasticity

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All enzymes active in steroidogenesis are also expressed in the brain and thus the brain is capable of synthesizing sexual steroids that exert local effects on the circuitry and the animal's performance. Sexual steroids, which are synthesized in the brain and function in a paracrine manner, are defined as sexual neurosteroids (SN). In final SN synthesis testosterone is either irreversibly converted to 17 β -estradiol (E2) by activity of aromatase or testosterone is irreversibly metabolized to dehydrotestosterone (DHT) by activity of 5 α -reductase. Spine synapse density in females, specifically in the hippocampus, widely depends on aromatase activity in hippocampal neurons, in other words depends on hippocampus-derived E2. Inhibition of aromatase activity in hippocampal slice cultures originating from females and systemic treatment of female mice with aromatase inhibitors reduced significantly the number of spines and spine synapses, the expression of synaptic proteins, and LTP could not be induced in the presence of aromatase inhibitors. Impairment of LTP and as a consequence spine and spine synapse loss was specifically seen in females but not in males. In contrast to females, in males only minor impairment of LTP was found in response to reduced E2 levels in the tissue and synapse density was not affected. Vice versa, DHT induced synapse density in hippocampal slice cultures of male animals but not of female animals. In addition, finasteride, an inhibitor of 5 α -reductase decreased synapse density in "male" slice cultures but not in "female" cultures, suggesting that the maintenance of hippocampal synapses in "male" hippocampal cultures depend on hippocampus-derived DHT.

Synthesis of SN, such as 17 β -estradiol and dehydrotestosterone, is regulated by GnRH. In hippocampal neurons of females GnRH upregulates E2 synthesis and increases synapse density via activation of aromatase. This effect was not found in neurons originating from males, very likely due to the higher degree of phosphorylation of aromatase in "male" than in "female" neurons. Together SN regulate synapse density in the hippocampus in a sex-specific paracrine/autocrine manner.

P1/76**Estradiol and isotype-selective estrogen receptor agonists modulate the mesocortical dopaminergic system in gonadectomized female rats**

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The mesocortical dopaminergic pathway projecting from the ventral tegmental area (VTA) to the prefrontal cortex (PFC) contributes to the processing of reward signals. This pathway is regulated by gonadal steroids including estradiol. To address the putative role of estradiol and isotype-selective estrogen receptor (ER) agonists in the regulation of the rodent mesocortical system, we combined fMRI, HPLC-MS and qRT-PCR techniques. In fMRI experiments adult, chronically ovariectomized rats, treated with either vehicle, estradiol, ER α agonist 16 α -lactone-estradiol (LE2) or ER β agonist diarylpropionitrile (DPN), received a single dose of D-amphetamine-sulphate (10mg/kg, i.p.) and BOLD responses were monitored in the VTA and the PFC. Ovariectomized rats showed no significant response to amphetamine. In contrast, the VTA of ER agonist-substituted ovariectomized rats showed robust amphetamine-evoked BOLD increases. The PFC of estradiol-replaced animals was also responsive to amphetamine. Mass spectroscopic analysis of dopamine and its metabolites revealed a two-fold increase in both dopamine and 3,4-dihydroxyphenylacetic acid content of the PFC in estradiol-replaced animals compared to ovariectomized controls. Quantitative real-time PCR studies revealed upregulation of dopamine transporter and dopamine receptor in the VTA and PFC, respectively, of ER agonist-treated ovariectomized animals. Collectively, the results indicate that E2 and isotype-selective ER agonists can powerfully modulate the responsiveness of the mesocortical dopaminergic system, increase the expression of key genes related to dopaminergic neurotransmission and augment the dopamine content of the PFC. In a broader sense, the findings support the concept that the manifestation of reward signals in the PFC is dependent on the actual estrogen milieu of the brain. This work was supported by grants from the Hungarian Scientific Research Fund (OTKA K100722, K83710, K101326), National Development Agency of Hungary (NFU-BONUS-HU08/2-2011-0006) and the European Community's Seventh Framework Programme (FP7/2007-2013, No.245009).

P2/11**In vivo calcium imaging in freely moving mice**

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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 2 dimension network measurements none of them provided the high spatial and temporal resolution that can be realized with 2D-Resonant Two Photon Laser Scanning imaging.

We used a high-resolution, resonant two-photon microscope with resonant scanning mirrors that are capable of gathering images up to 1000 FpS. Resonant scanning allows imaging with high acquisition speed at rates from 30,5 frames per second (512 x 512 pixels) to 490 frames per second (512 x 32 pixels). With these tools we measured the neuronal activity of hundred of cells in the visual cortex in 2D with millisecond temporal resolution.

We show calcium imaging of visual stimulation-evoked activity in neurons of the mouse visual cortex in in vivo head restrained freely moving mice.

P1/88**The somatostatin 4 receptor is involved in chronic variable mild stress-induced behavioural changes in mice**

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The somatostatin 4 receptor (sst4) is widely expressed in stress-related brain areas (e.g.: hippocampus, amygdala, prefrontal cortex) and has been shown to play a role in acute stress-related behaviour. Since its importance in chronic stress susceptibility has not been investigated, we examined the involvement of sst4 in the behavioural alterations evoked by chronic variable mild stress (CVMS).

Male Sstr4 gene-deficient (Sstr4^{-/-}) mice and their wildtype counterparts (Sstr4^{+/+}) were used in the experiments. Anhedonia was evaluated in the sucrose preference test (SPT), anxiety in the light-dark box (LDB) test while depression-like behaviour in the tail suspension (TST) and forced swim (FST) tests before and also after the CVMS and the difference of behavioural parameters were also compared in addition to absolute values in each group. Six different stressors (restraint stress, wet bedding, cold exposure, etc.) were used in our CVMS paradigm, two per day for 3 weeks. The adrenal and thymus weights were measured to confirm the effect of the stress.

In wildtype mice, the only behavioural outcome of CVMS was a slight increase in the time spent in the light and in the number of entries into the lit compartment in the LDB, but only the difference, not the absolute values were significantly different when evaluating the stressed and non-stressed groups. In contrast, the behaviour of the Sstr4^{-/-} mice did not change in the LDB upon CVMS, but their immobility time in the TST significantly increased (both absolute values and the difference). In the FST, Sstr4^{-/-} animals showed increased immobility compared to wildtypes before CVMS and their immobility time decreased after the chronic stress. The sucrose preference of Sstr4^{-/-} mice did not differ from the wildtypes and did not change after CVMS. The adrenal weights of stressed animals were significantly increased, while the thymus weights were decreased in comparison with non-stressed groups, but there was no difference between Sstr4^{-/-} and wildtype mice.

It can be concluded that the sst4 is involved in the behavioural changes induced by chronic stress, which was remarkably different in the used tests. The slightly increased light-preference of stressed wildtypes was not seen in knockouts. While immobility in TST was increased by CVMS in Sstr4^{-/-} mice, the elevated baseline immobility in FST decreased.

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P2/24

Protein kinase D promotes cellular plasticity and memory formation by regulating actin dynamics in dendritic spines

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Protein kinase D (PKD) is a serine/threonine kinase which is activated by members of the novel PKC family and is recruited transiently to the plasma membrane or intracellular membranes via binding diacyl-glycerol. So far, neuronal PKD activity has been shown to affect dendrite development and maintenance, intracellular transport and Golgi functions as well as modulation of transmembrane receptors. In this work, we investigated PKD-mediated effects on dendritic spines and the consequences of altered PKD activity regarding synaptic plasticity and memory formation.

In non-neuronal cells, PKD has important role in stabilizing F-actin via multiple molecular pathways. Using in vitro models of cellular plasticity, we show that actin stabilization needed for the enlargement of dendritic spines is dependent on PKD activity. Additionally, we provide in vivo evidence that impaired PKD functions block activity-dependent changes in hippocampal dendritic spines, including morphological alterations as well as LTP formation, and has deleterious consequences on spatial memory formation. Thus, PKD controls synaptic plasticity and learning by regulating actin turnover and stability in dendritic spines.

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P2/101

Electrophysiological examination of the double-flash illusion with the help of two time-windowsJúlia Simon¹, Gábor Csifcsák²¹*Institute of Psychology University of Szeged, Cognitive and neuropsychology, Szeged, Hungary;* ²*University of Szeged, Department of Cognitive and Neuropsychology, Szeged, Hungary*

A double-flash illusion occurs when a single flash of light is accompanied by two brief auditory stimuli - properly close in time. In this case, the subjects frequently report perceiving two flashes. The measured susceptibility of this illusion can differentiate between people, but for the examination of the sources of these differences, a reliable experimental protocol is needed. Early electrical responses measured with electroencephalography (EEG) are less influenced by decisional strategies and therefore, they can be assessed for investigating the underlying neural mechanisms of this illusion. In our study we measured the EEG correlates of the illusion with two types of illusory conditions that differ in the inter-sounds interval: a 67 ms (short condition, SC) and a 133 ms (long condition, LC). Based on the results of 18 participants we found correlations between the behavioral data and the difference waves' amplitudes in the early 110-140 ms interval, more consistently in the case of the LC. This condition's other advantage over SC is, that there are more variability in the behavioral responses. Furthermore, we used two different sound intensities to demonstrate that in healthy individuals a slight difference in perceived loudness does not robustly influence the behavioral or EEG measures, so it can not be accounted for the individual variability of responses. This fact strengthens the reliability of our paradigm as a promising new tool for examining multisensory interactions.

P1/82**Neuropeptide co-expression in kisspeptin neurons of the human hypothalamus**Katalin Skrapits¹, Beáta Á. Borsay², László Herczeg², Philippe Ciofi³, Zsolt Liposits^{1,4}, Erik Hrabovszky¹¹*Institute of Experimental Medicine, Hungarian Academy of Sciences, Laboratory of Endocrine Neurobiology, Budapest, Hungary;* ²*Faculty of Medicine of the University of Debrecen, Department of Forensic Medicine, Debrecen, Hungary;* ³*Neurocentre Magendie, INSERM U862, Bordeaux, France;* ⁴*Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Department of Neuroscience, Budapest, Hungary*

Hypothalamic kisspeptin (KP) neurons use peptidergic signaling to regulate reproduction and puberty. Here we have carried out a series of immunofluorescent experiments on formalin-fixed post mortem histological samples to identify the neuropeptide co-transmitters of human KP cells. In these colocalization studies using confocal microscopy, we found no evidence for neurotensin, cholecystokinin, proopiomelanocortin-derivatives, agouti-related protein, neuropeptide Y, somatostatin or tyrosine hydroxylase (dopamine) expression in KP neurons. In contrast, neurokinin B (NKB) was present in a large subset of these cells, as reported earlier in laboratory animals. Dynorphin, which has also been described in KP neurons of rodents and sheep, was observed rarely in human KP cells and their axons, and similarly, human KP neurons did not contain signal for galanin, a neuropeptide colocalized earlier with KP in mice. To the opposite, 30-50% of KP and NKB neurons in humans expressed immunoreactivity for substance P (SP) and cocaine- and amphetamine-regulated transcript (CART), unlike in laboratory species. In addition, we have provided evidence for a sexually dimorphic co-expression of proenkephalin (pENK) with KP and NKB. The pENK signal was detectable in 12.5±5.1% of NKB-IR and 1.9±1.0% of KP-IR neurons and in 5.7±2.5% of NKB-IR and 4.9±1.8% of KP-IR axon varicosities in human males. This colocalization was absent in postmenopausal women. Altogether, these data identified the neuropeptide co-transmitters of human KP cells. The presence of substance P, CART and pENK in human KP neurons, together with the absence of dynorphin and galanin in most of these cells, indicate that humans use considerably different neurotransmitter mechanisms than rodents, to regulate fertility.

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P2/50**Altered dendritic impulse propagation in principal cells of Tg2576 mice, a model animal for Alzheimer's disease**Attila Somogyi¹, Zoltán Katonai¹, Ervin Wolf¹

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Morphological changes, including a drop in spine densities, of principal cells have been reported in human amyloid beta precursor protein overexpressing mutant Tg2576 mice, a widespread animal model of human Alzheimer disease. In our earlier computer simulations we found significant differences in dendritic impulse propagation properties of principal neurons of wild-type (WT) and mutant (TG) mice. However, the exact surface area of dendritic spines and the extent of inhomogeneity in properties of the soma-dendritic membrane are uncertain but can affect dendritic impulse propagation. Here we aimed to investigate the effects of these factors on the dendritic impulse propagation of TG and WT principal cells.

We used morphologically detailed passive segmental cable models of these cells (n=58) within NEURON simulation environment. Current was injected to dendritic points of modelled TG and WT neurons to simulate local activity of synapses and generate PSPs. Dendritic current and voltage transfers to the soma were computed, in both homogeneous and inhomogeneous soma-dendritic membrane models, where somatic and dendritic specific membrane resistances were equal or different. We used a wide range of dendritic spine surface areas (0,3-1,5 μm^2) in our models. We quantified the extent of differences between healthy and pathological neurons by counting the ranges with significantly different transfer properties of WT and TG neurons in the following functions: the rates of voltage and current transfers as a function of path distance from the soma, percentage of dendritic surface area as a function of voltage and current transfers in the apical and basal dendrites.

We found that the dendritic impulse propagation of WT and TG cells differed more in the inhomogeneous than in the homogeneous membrane models. However, the size of spines hardly affected the extent of differences in signal transfers of WT and TG neurons except in the case of the smallest spines.

Our simulations strengthen our earlier results suggesting an increase in somatopetal current transfer in apical dendrites of TG neurons independently on the size of spines, especially in neurons with electrically inhomogeneous soma-dendritic membrane. The predicted increase in TG neurons is in agreement with the hyperexcitability of pyramidal neurons found both in TG mice and in humans with Alzheimer disease.

The authors are grateful to Dr. Alán Alpár for the files containing the reconstructed morphology of neurons.

P1/34

Inference of causal relationships at the seizure initiation, based on measurements by subdural grid electrodes

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The traditional method for seizure onset zone determination is based on the temporal order in the appearance of pathological waveforms. However, this method assumes, that the well observable pathological waveforms are really the first signs of those processes, which play important role in the seizure generation. New mathematical analysis methods, such as Approximate Entropy and Convergent Cross Mapping raise the possibility of objective determination of the causal relationships at the initiation of the seizure, without specific assumptions about the waveforms. Application of these new methods to data measured by subdural electrode grid on 48 channels with 1024 Hz sampling frequency marked well the seizure onset zone and revealed new interdependence between the hippocampus and the neocortex at the initiation and in specific stages of the seizure.

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Plenary Lecture II. - Thursday

The Neurobiology of Need

Scott Sternson

Janelia Farm Research Campus, HHMI

Neural circuits that are essential for survival, such as those that mediate hunger, are shaped by selective pressure, but they control flexible goal-directed actions. This is exemplified by starvation-sensitive AGRP neurons that are sufficient to rapidly coordinate voracious food seeking and consumption behaviors. However, the mechanisms by

which localized brain stimulation can give rise to goal-directed but flexible motivated behavioral responses remain poorly understood. We have used cell type-specific techniques for neural circuit manipulation and imaging to examine the motivational properties and neuronal dynamics of AGRP neuron hunger circuits. We find that AGRP neurons transmit a signal with negative valence that can serve as a negative reinforcement teaching signal for food-seeking behavior. Similar characteristics are also found for a separate neuron population that controls thirst. Using in vivo calcium imaging in freely moving mice, we also find the dynamics of AGRP neuron activity is consistent with a negative reinforcement function. Taken together, the properties of these homeostatic neurons provide a framework based on negative reinforcement learning that could explain how a single molecularly defined neuron population can elicit a flexible motivated behavior.

P2/42**Ameliorative effects of Pituitary Adenylate Cyclase Activating Polypeptide in diabetic retinopathy of rats: analysis of synaptic proteins and ultrastructure**

Krisztina Szabadfi¹, Alíz Szabó², Bálint Szalontai³, György Ifj. Sétáló⁴, Péter Kiss⁵, Dóra Reglődi⁵, Krisztina Kovács², Andrea Tamás⁵, Róbert Gábrriel⁶

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a neurotrophic and neuroprotective peptide that has been shown to exert protective effects in different neuronal injuries, such as retinal degenerations. Diabetic retinopathy (DR) is a leading cause of adult blindness and is the most common complication of diabetes. As we have previously described severe degeneration of dopaminergic amacrine cells was counteracted by intraocular PACAP treatment in streptozotocin-induced DR with regarding both number and morphology. Some of these cells started to express PAC1-Rs as a response to the PACAP treatment. Pro-apoptotic pathways were suppressed while anti-apoptotic factors induced or activated by intravitreal PACAP treatment. The aims of our study were to analyze the synaptic structure of PACAP-treated diabetic retina.

Streptozotocin-treated Wistar rats received intravitreal PACAP injection 3 times into the right eye 2 weeks after the diabetes induction. Morphological and molecular biological (qRT-PCR; Western blot) methods were used to analyze retinal synapses (ribbons, conventional) and related structures.

Electron microscopic analysis revealed that retinal ribbon synapses and other synaptic profiles suffered alterations in diabetes. However, in PACAP-treated diabetic retinas more bipolar ribbon synapses were found in the IPL than in DR animals. The ribbon synapse marked with CtBP2/Bassoon/Syntax3 and formed horseshoe-shaped ribbons which were more retained in PACAP-treated diabetic retinas than in DR rats. These results are supported by molecular biological data (qRT-PCR and Western blot for CtBP2, Syntax3). The related structures such as amacrine (glycinergic) and ganglion cells elective degeneration could be ameliorated by PACAP treatment.

In conclusion, intravitreal administration of PACAP may have therapeutic potential in streptozotocin-induced DR.

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P2/1**Long-term organotypic culture of the human retina**

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Purpose: Corneal transplantation has become a routine procedure in ophthalmology nowadays. The anterior segment with the cornea of the donor eyeball is removed shortly before transplantation, and the otherwise intact posterior segment containing the retina that could be used for scientific investigations is usually disposed of. Here

we present a potent in vitro application of these retinas that allows the long term survival of retinal cells with maintained retinal integrity.

Methods: Approximately 5x5 mm pieces of central retina were harvested from donor adult eyes 2-4 hours after enucleation. The pieces of the neural retina without the pigment epithelium were placed onto a nitrocellulose membrane and kept in culture using serum-free medium for up to seven weeks. The cultures were fixed at different time points, processed and analyzed as histological sections or whole mounts by immunohistochemistry using cell type-specific antibodies.

Results: Although slight degenerative signs were present, the overall retinal architecture was well preserved even after 49 days in culture and the retinal thickness was comparable to that of in vivo controls. As expected, we detected signs of moderate glial activation after 7 days in culture which gradually increased thereafter. The cones showed a near normal morphology with occasional swelling of the outer segments. Although in reduced number, both M/L- and S-cones survived for seven weeks. The rod outer segments gave a strong anti-rhodopsin immunoreaction, but they were shorter than in in vivo controls and signs of opsin mislocalisation were present. Some pyknotic nuclei in the outer and inner nuclear layers were detectable after prolonged culturing, but no severe decrease in the number of bipolar, amacrine and horizontal cells was found. Even some ganglion cells survived for 49 days in culture.

Conclusions: Our results indicate that the adult human retina can be maintained in an appropriate culture system for at least seven weeks. Despite the minor changes detected, the retina shows normal lamination, the retinal cells preserve their normal morphology and staining characteristics. The culture method presented here can be reliably reproduced and can be adapted to pharmacological and toxicological applications.

P1/93

Behavioral changes in mother and maternally sensitized female mice

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Behavioral changes in virgin female, maternally behaving sensitized virgin female, and primiparous mother mice were investigated using three behavioral tests measuring active maternal behavior, maternal motivation and depression-like behavior. Although peripartum changes in estrogen and progesterone contribute to the initiation of maternal responsiveness, inputs from pups are required for its maintenance. Maternal responsiveness can actually develop in response to pup exposure even in the absence of hormonal stimulation, in a process termed sensitization. In the pup retrieval test measuring active maternal behavior, latency to retrieve pups was not significantly different between the 3 female groups. In the second experiment, which tested maternal motivation, the conditioned place preference (CPP) test was used. Mother and maternally sensitized female mice spent significantly more time in the pup-associated cage than in the control cage, while the virgin female mice significantly preferred the control cage during the 1 h test period when they could freely choose between the cages. In the third experiment, the forced swim test (FST) was used to examine depression-like behavior. The time spent with active (swimming and struggling) and passive (floating) behavior was measured for 6 min. The active behavior in FST showed significant differences among the maternal status: mother mice spent more time with active behavior than virgin and sensitized female mice. The results indicate that control mice have more pronounced spontaneous maternal behavior than the much better described rats. Still, behavioral alterations can be detected in mice, too, when they become mothers in particular regarding maternal motivation and depression-like behavior, similar to rats. In addition, a maternal sensitization procedure can have measurable effects in mice even with a substantially reduced sensitization procedure as compared to rats. Interestingly, however, the anti-depression-like behavior characteristic of mother mice is not present in the sensitized animals.

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P1/61

Medial Orbitofrontal Cortex: Complex Functional Attributes of Glucose-Monitoring Neurons and Their Metabolic Significance

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The orbitofrontal cortex (OBF) – important cortical region in the central regulation of feeding and metabolism – is a major constituent of the forebrain glucose-monitoring (GM) system. The present study was designed to characterize the endogenous and exogenous chemical sensitivities of these GM cells, and to elucidate their role in the maintenance of glucose homeostasis after selective destruction of GM neurons by streptozotocin (STZ).

In this study, extracellular single neuron activity was recorded in the medial OBF (mOBF) of male Sprague-Dawley rats by means of tungsten wire multibarreled glass microelectrodes, during microelectrophoretic administration of various chemicals, as well as during gustatory stimulations with the five primary taste qualities and orange juice as complex taste solution.

In addition, i.p. glucose tolerance test (GTT) was performed in male Wistar rats 20 minutes (acute) and 4 weeks (subacute) after bilateral microinjection of the GM neuron selective neurotoxin streptozotocin (STZ) or vehicle solution into the mOBF.

One fifth of the tested neurons changed their activity in response to microelectrophoretic administration of D-glucose, that is, these cells proved to be elements of the forebrain GM neural network. Acetylcholine altered neuronal activity in 80% of all cells, whereas DA did so in 40% of the neurons. Approx. 20% of the examined cells changed in firing rate to microelectrophoretic application of noradrenaline. More than half of the examined cells were inhibited by GABA. One fourth of the tested neurons changed their activity during gustatory stimulation with sweet taste solution, whereas this ratio was one third in case of the sour taste. Approximately 60% was the alteration in neuronal activity in case of the other primary taste qualities and orange juice.

In the acute GTT, the maximum of blood glucose levels of STZ treated animals was higher and it was reached later, than in the control group. There was no significant difference between the two groups 4 weeks after the microinjection.

Our present findings demonstrate complex functional attributes of GM neurons in the mOBF. These chemosensory cells play an important role in the adaptive mechanisms of the organization of feeding and metabolism by the integration of relevant signals arising from the endogenous and exogenous environments.

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P2/13

CAG-GCaMP2 transgenic rat model for in vitro and in vivo brain activity imaging

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Due to their superior cognitive functions, better performance in learning and memory tasks and the availability of several animal disease models, a transgenic rat expressing a calcium-sensitive fluorescent protein in the CNS might represent a better alternative to the widely used mouse models. Here we show that the Sleeping beauty transposon-mediated transgenesis is a versatile tool to establish stable expression of the fluorescent calcium indicator protein GCaMP2 driven by the CAG promoter in virtually all tissues of rat. We characterized the CAG-GCaMP2 line for brain expression in acute and organotypic hippocampal slices at different developmental stages.

Organotypic slices displayed massive GCaMP2 expression in 3-4 days after tissue preparation, which reached its plateau level within the first week. Application of the Ca²⁺ ionophore ionomycin resulted in increased GCaMP2 fluorescence, demonstrating the ability of the model to reliably measure intracellular Ca²⁺ concentration. Importantly, Ca²⁺ influx into neural cells could also be triggered by pharmacological activation of neural activity by

glutamate or ATP. 100 μ M glutamate initiated short-term Ca^{2+} fluctuations in both the soma and processes of neurons, while 1 mM ATP increased the intracellular Ca^{2+} concentration simultaneously in all cells.

Additionally, we performed functional in vivo two-photon imaging in the visual cortex (V1) of the CAG-GCaMP2 transgenic rats. Displaying moving grating patterns to head-fixed rats anesthetized by fentanyl resulted in 20-60 % changes in the GCaMP2 fluorescence of labeled layer2 pyramidal neurons. The responses showed clear orientation selectivity. Besides, we detected high-amplitude ($dF/F_0 > 2$) Ca^{2+} responses corresponding to the up and down states of spontaneous synchronized network oscillations in ketamine-anesthetized rats.

Overall, the transgenic CAG-GCaMP2 rat model demonstrates widespread distribution and appropriate sensitivity of the Ca^{2+} sensor, which can facilitate analyses of activity-driven cellular network states in healthy and diseased conditions in the future.

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P2/6

Fast three-dimensional imaging of neuronal assemblies in the mouse visual cortex using genetically-encoded neuronal indicators and two-photon microscopy

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The understanding of brain computation requires novel methods that read out neural activity on different spatial and temporal scales. Two-photon calcium imaging is a powerful means for monitoring the activity of distinct neurons in brain tissue in vivo. Genetically-encoded fluorescent calcium sensors are widely used to image neural activity; therefore we imaged calcium indicator-expressing neurons in the mouse visual cortex (V1) through a cranial window in vivo.

We present multiple 3D, acousto-optical, two-photon laser-scanning technologies to monitor neuronal activity at different scales with genetically-encoded calcium indicators in a near-cubic-millimeter scan range (up to 700 μ m $\times$ 700 μ m $\times$ 1,400 μ m), with a high scanning speed (up to 500 points/kHz in 3D trajectory scanning mode), with high (<500 nm) resolution in the center core, and less than 1.9 x 1.9 x 7.9 μ m resolution throughout the whole scanning volume.

We used volumetric random-access calcium imaging of spontaneous and visual stimulation-evoked activity from hundreds of neurons. We applied 3D trajectory scanning technique to measure multiple dendritic segments in vivo with motion compensation possibility. Finally we introduced an expansion of trajectory scanning technique to monitor the activity from more than 100 cells in vivo while obtaining information for motion correction.

P1/66

Paired acoustic and MGB electrical stimulation evoked potential oscillations in the cat

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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 and wideband noise 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 microstimulation of the MGB. Recording electrodes were chronically implanted to the surface of the auditory cortex and 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 and multiunit activity (MUA) were recorded and

analyzed. Position of the MGB electrodes was verified by recording tonotopic responses and post mortem histology. 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. Paired presentation of acoustic and electric stimuli with different delays revealed mutual modulation of both early and late cortical evoked components. We have analyzed the amplitudes of responses evoked by the paired stimulations and found that the superposition of evoked potential components is not linear. This non-linearity depends on the distance between the two stimuli in the paired stimulation.

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P1/32

Setting up mouse models of different forms of sensorineural hearing loss

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Sensorineural hearing losses (SNHLs) are among the most frequent sensory deficits, but they lack effective drug therapy. Lack of a readily available set of in vivo models of different SNHLs hinders drug development. Although disturbed redox homeostasis seem to play a crucial role in the pathomechanism of all forms of SNHLs, potency of protective drug candidates needs to be tested individually in the different forms of the disease.

We have set up mouse models of the most frequent forms of SNHLs, i.e., ototoxic drug-, and noise-induced hearing loss (NIHL) and age-related hearing loss (AHL) to test the in vivo protective effect of compounds, that showed otoprotective potential in vitro.

Aminoglycoside antibiotic-induced hearing loss (HL) model was set up by testing two different mouse strains, three different aminoglycosides in one-three different concentration. Finally 800 mg/kg kanamycin and the BALB/c strain was chosen. Rasagiline, a MAO-B inhibitor type antiparkinsonian drug, alleviated dose-dependently the kanamycin-induced shifts in the hearing thresholds determined by auditory brainstem response (ABR). The neuroprotective, antioxidant and protective dopamine release enhancer effects of rasagiline may explain the otoprotection.

To test the effect of drugs against NIHL, we developed a ventilated noise box and exposed CD1 and BALB/c mice to 90, 98 and 110 dB, 8 – 16 kHz white noise for 15 and 30 min. Temporary (TTS) and permanent (PTS) threshold shifts were determined by ABR. Based on the measured threshold shifts, the 30 min, 98 dB noise exposure was chosen for further experiments. D-Met and N-Acetylcysteine, compounds shown to be protective against NIHL, were used to validate the model. Our candidate compound exerted a slight, but significant protection against both TTS and PTS.

Protective effect of the candidate compound against AHL was started to test in two inbred strains of mice (DBA/2J and BALB/c), which carry the *ahl8/ahl2* genes and show early/late onset AHL, respectively. Hearing threshold changes are planned to follow for a year by measuring ABR in every month. Early results demonstrated the progressive impairment of hearing and a moderate protection by the test compound.

The complex, multifactorial pathomechanism of SNHLs most likely requires drugs acting on multiple targets for effective therapy. On the other side, drugs with multi-target action might be useful in different SNHL forms, what needs to be tested in vivo.

P1/80

The localization of connexin43 gap junctions in tanycytes.

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Tanycytes are special, glial cells lining the lateral walls and the floor of the third ventricle behind the optic chiasm. The cell body of tanycytes is located in the ependymal layer, while their long basal process projects into the median eminence (ME) and into the arcuate, ventromedial or dorsomedial nuclei. Earlier, tanycytes were considered as supporting and blood-brain barrier forming cells, but it was demonstrated during the last decades that these cells play important role in the regulation of energy homeostasis and in the control of neuroendocrine axes.

To determine whether tanycytes communicate with each other via connexin43 (Cx43) gap junctions, we have loaded tanycytes with lucifer yellow (LY) and biocytin through patch pipette. In all cases, biocytin labeled only a single tanycyte, while LY filled a larger group of tanycytes. Furthermore, neuronal axon and the wall of blood vessels were also labeled through their connection with tanycyte end processes. Injection of Cx43-blocker carbenoxolone inhibited the spreading of LY between tanycytes.

Using immunocytochemistry, the highest density of Cx43-immunoreactive spots were observed in the cell bodies of α -tanycytes. In much lower density, but Cx43-immunoreactivity was also observed in β -tanycyte cell bodies. The processes of both types of tanycytes were surrounded by Cx43-immunoreactivity.

At ultrastructural level Cx43 immunoreactivity was present among tanycyte cell bodies in all tanycyte regions, however, gap junctions were more frequent among the α -tanycytes. In addition, Cx43-immunoreactivity was frequently found on the ventricular surface of tanycytes and on their protrusions that project into the ventricle. Cx43 immunoreactivity was also observed among tanycyte end feet processes and between tanycyte end feet process and axon varicosity in the external zone of the median eminence. Cx43 hemichannels were also present between tanycyte end feet processes and small capillaries in the arcuate nucleus, as well as between tanycyte end feet and fenestrated capillaries in the lower part of the ME.

Our results suggest that gap junctions are present not only among tanycytes, but also between tanycytes and the axons of hypophysiotropic neurons. In addition, Cx43 hemichannels may also facilitate the material transport between tanycytes and extracellular fluids, like the cerebrospinal fluid, extracellular space of the median eminence and the circulating blood.

P1/52

Studies on the possible role of long non-coding RNA molecules in neurodegenerative disorders

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It has been previously established that only a small part of the human genome (appr. 1-2%) encodes proteins, but half of our genome is transcribed to RNA molecules. The function of these non-coding RNAs is subject of extensive investigations. One of these newly described RNA classes are the so called long non-coding RNAs (lncRNAs). These molecules are defined as transcripts longer than 200 nucleotides without an extended open reading frame. Their maturation processes are similar to that of messenger RNAs, however, compared to mRNAs, they possess a higher cell- and tissue specificity.

lncRNAs are particularly abundant in the central nervous system and due to their strict spatial- and temporal expression, they have important roles in brain development. The role of lncRNAs is also prominent in the adult brain: an emerging number of publications support that their dysfunction might promote neurodegenerative disorders such as Parkinson-, Alzheimer- and Huntington-disorder. Our aim is to investigate whether single nucleotide polymorphisms (SNPs) of genes coding for lncRNAs contribute to the pathogenesis of Parkinson disorder.

For that we performed an in silico analysis of a publically available RNA-Seq database (http://www.broadinstitute.org/genome_bio/human_lincnas/) and genes that are highly expressed in CNS were selected. In addition, six lncRNAs were chosen, which have been previously proven to be implicated in neurodegenerative disorders: HAR1F, Uchl1-AS, PINK1-AS, Sox2-OT, BC200 and ANRIL. With the help of the ENSEMBLE Database, we are searching for the non-coding exonic variants of these transcripts, which possess a

Global Minor Allele Frequency (MAF) higher than 0.1. As a pilot study, we aim to compare the genotype distribution and allele frequency of 15 selected polymorphisms in healthy volunteers and patients with Parkinson disorder, to show whether these SNPs are associated with the development of Parkinson disease.

Symp IV. - Friday

Passive properties and electrical coupling of cerebellar Golgi cells

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The strength of chemical and electrical interactions shows large heterogeneity. We have previously demonstrated a large diversity in the strength of electrical coupling among cerebellar Golgi cells (GoC), and showed that this diversity underlies GoC network desynchronization. Despite its functional importance, the underlying mechanisms are largely unknown. By combining paired in vitro whole-cell recordings with correlated electron microscopy, here we investigate how the number and dendritic location of gap junctions (GJs) affect the strength of electrical coupling among GoCs. Dual somato-dendritic recordings and multi-compartmental modelling were performed to determine the passive electrical properties of GoCs, such as R_m , C_m and R_a . Paired recordings between electrically coupled GoCs followed by electron microscopic identification of the number and location of the GJs formed the basis of our multi-compartmental modelling to estimate the average conductance of a GJ plaque. Immunofluorescent localization of connexin36 (Cx36) positive GJs revealed a large variability in the size of individual GJ plaques. Freeze-fracture replica immunogold labelling demonstrated that a GJ plaque contains on average 340 Cx36 channels. Given our GJ plaque conductance estimate, the single channel conductance and the total number of Cx36 molecules per a GJ plaque, our results predict that not all available channels are open. Our results also demonstrate that variability in the number, dendritic location and size of GJs underlies heterogeneity in the strength of electrical coupling between cerebellar GoCs.

P1/45

Impairment of giant pyramidal cells in the primary motor cortex of schizophrenia patients

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Schizophrenia is one of the major psychiatric disorders without fully understood pathogenesis – despite of the more than 100 years of intensive research. Several post mortem and MRI studies reported morphological and cytoarchitectural changes in the cortex of schizophrenia patients. Wide range of neurotransmitter systems is also affected by this disorder. The early descriptions of schizophrenia have already mentioned several disturbances of motor skills (e.g. fine motor function, eye movements, speech); in addition, the antipsychotic medication may cause impairments in the motor function.

In order to clarify whether the functional loss in motor skills is associated to neuromorphological alterations, we have investigated the primary motor cortex (Brodmann's area 4) of 5 schizophrenic and 5 control post mortem human brains using immunohistochemistry against parvalbumin (PV), labeling perisomatic inhibitory interneurons and giant pyramidal cells (Betz cells), and SMI32 (labels the pyramidal cells in layers III and V). To analyze the possible synaptic changes the areas of the primary motor cortex were examined in the electron microscope. Furthermore, we have established a rat model to examine the effect of the antipsychotic medication in the same area, using daily peritoneal haloperidol and olanzapine treatment in rats for 4 weeks.

We found a decrease in size and density of the giant motoneurons (Betz cells) in the schizophrenia patients. Other pyramidal cells of this area have been also investigated and show no alterations in these parameters. In the animal model no decrease was found in the size or density of giant pyramidal cells. It may suggest that the antipsychotic medication could not explain our findings in human samples. Therefore, the impairment of the giant motoneurons might be related to the disorder itself. Electron microscopic analysis show degenerative changes of the Betz cells.

The conclusions are limited by the small sample size; however, our result may show a unique sensitivity of Betz cells in schizophrenia, as a possible neuroanatomical background of the motor impairment, which is most likely independent from the medication.

P2/51

Evaluation of dendritic spine density and detection of amyloid-beta plaques in 6 and 9 months old APP/PS1 transgenic mice

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Alzheimer's disease (AD) is a very complex, heterogeneous and multifactorial neurodegenerative decline. Beside the two major histopathological features (amyloid-beta plaques and neurofibrillary tangles), mitochondrial dysfunction, endoplasmic reticulum-stress (ER-stress) and malfunction, synaptic vulnerability and failure, lysosomal deterioration and toxic proteins are the cellular pathophysiological properties of AD. Moreover, synapse loss is strongly correlated with cognitive impairment thus synapse number is the best indicator of cognitive decline in AD. The senile plaques are associated with local synapse and dendritic spine loss.

Several rodent models have been used for studying the pathomechanism of AD and the progress of the disease. The APP/PS1 (Tg(APPswe, PSEN1dE9)85Dbo) transgenic mice strain is one of the most commonly used animal models in AD research.

The aim of the study was to evaluate the dendritic spine density and to monitor the progress of the amyloid plaques in the hippocampus CA1 region in male APP/PS1 transgenic mice and their wild type littermates of different ages (6 and 9 months).

To measure the dendritic spine Golgi impregnation technique (FD Rapid GolgiStain™) and ImageJ software were used. Amyloid plaques were studied by immunohistochemical analysis (4g8 antibody, brightfield microscopy) and immunogold-labelling method (AB5078P antibody, G7277-5 nm gold particles, transmission electron microscopy).

Significant difference was not found in dendritic spine density of 6 months old APP/PS1 mice but the dendritic spine density of 9 months old APP/PS1 mice was decreased significantly compared to the controls. Interestingly, senile plaques were already detected in the 6 months old transgenic animals by immunohistochemical and immunogold techniques.

Different plaque morphology was detected by TEM: dystrophic neurites were showed around the senile plaques in 9 months old transgenic mice but these morphological structures were not seen in 6 months old APP/PS1 mice.

Our results showed that AD pathology (senile plaques) appeared already in the 6 months old APP/PS1 mice, however significant differences were not found in the density of dendritic spines. It can be assumed, that the early presences of the amyloid plaques do not yet influence the dendritic spine density.

P2/60

Activation of TRPA1 ion channel by hydrogen sulfide and polysulfides on trigeminal sensory neurons and sensory nerve endings

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Hydrogen sulfide (H₂S) is an endogenous gasotransmitter, modulates various biological functions, including nociception. It is known that H₂S causes neurogenic inflammation and elicits hyperalgesia. The Transient Receptor Potential Ankyrin repeat domain 1 (TRPA1) channel is expressed on sensory neurons and sensory nerve terminals and is involved in inflammatory pain. It responds to a wide variety of stimuli, including cold temperature, pungent natural compounds and environmental irritants. Recently showed that H₂S induces Ca²⁺-influx in astrocytes. Therefore the aim of these study to determine the molecular targets of H₂S by investigating the effects of H₂S donors (NaHS and Na₂S) and polysulfides as possible H₂S -derived signaling molecules (DMTS, K₂SX) on trigeminal sensory neurons from rats and mice (wild-type and TRPA1(-/-) and sensory nerve endings of rat tracheae.

Ratiometric technique of intracellular calcium concentration ($[Ca^{2+}]_i$) measurement with the fluorescent indicator fura-2-AM on cultured trigeminal cells (rat and mice) was performed. Calcitonin gene related peptide (CGRP) release from the stimulated peripheral sensory nerve terminals of the isolated rat trachea was measured by radioimmunoassay.

Polysulfides (DMTS 100 μ M, K₂SX 1mM), and NaHS (1 mM, 10 mM) and Na₂S (500 μ M, 1mM) increased $[Ca^{2+}]_i$ in TRG cells from wild-type mice sensory neurons. These evoked robust $[Ca^{2+}]_i$ increase in a concentration dependent manner. Most of the NaHS-responsive cells were also sensitive to AITC (200 μ M). Neither compound had any effect on $[Ca^{2+}]_i$ on TRPA1(-/-) mice trigeminal neurons. The TRPA1 selective antagonist HC-030031 (10 μ M) could inhibit the DMTS-evoked Ca^{2+} -influx. CGRP release after DMTS (100 μ M) administration was measured. Significant decrease in the DMTS-evoked CGRP release was observed after HC-030031 administration.

Our results indicate that H₂S and polysulfides activate TRPA1 ion channel on the cell bodies of sensory neurons and on sensory nerve terminals in concentration dependent manner.

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P1/26

Differential effects of static and dynamic inputs on neuronal excitability

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The intrinsic excitability of neurons is known to be dynamically regulated by activity-dependent plasticity and homeostatic mechanisms. Such processes are commonly analyzed in the context of input-output functions that describe how neurons fire in response to constant levels of current. However, it is not well understood how changes of excitability as observed under static inputs translate to the function of the same neurons in their natural synaptic environment. Here we performed a computational study and hybrid experiments on rat bed nucleus of stria terminalis neurons to compare the two scenarios. Our goal was to analyze the effects of various voltage-dependent membrane currents on the firing responses of the model and biological neurons that receive either conventional current stimuli or simulated synaptic bombardment. The inward rectifying K-current (I_{Kir}) and the hyperpolarization-activated cation current (I_h) were found to be considerably more effective in regulating the firing under synaptic inputs than under static stimuli. This prediction was experimentally confirmed by dynamic clamp insertion of a synthetic inwardly rectifying K_{ir}-current into the biological neurons. At the same time, ionic currents that activate with depolarization, such as the slowly inactivating K-current (I_D) and the persistent Na-current were more effective regulating the firing under static inputs. When two intrinsic currents are concurrently altered such as the ones under homeostatic regulation, the effects in firing responses under static versus dynamic inputs can be even more different. Our results show that plastic or homeostatic changes of intrinsic membrane currents can shape the current step responses of neurons and their firing under synaptic inputs in a contrasting manner. In this respect, analyzing the I-O relationship of neurons under conventional current protocols alone might be of limited predictive value.

P2/63

Complex pre- and postsynaptic effects of DAMGO on excitability of spinal lamina I projection and local-circuit neurons

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Spinal application of mu-opioid receptor (MOR) agonists results in analgesia. Immunohistochemical and electrophysiological studies suggest that functional post-synaptic MORs are expressed mainly on lamina II (substantia gelatinosa – SG) neurons while pre-synaptic MORs are located in primary afferents and axon terminals of unknown origin in the spinal cord.

MOR positive SG neurons are not frequent (about 10%), have a tonic firing pattern and are mainly excitatory. Administration of a potent MOR agonist [D-Ala2, N-Me-Phe4, Gly5-ol]-enkephalin (DAMGO) hyperpolarizes these neurons via activation of G-protein-coupled inward-rectifier K⁺ conductance, and can also convert their firing pattern from tonic to adaptive.

At the same time very little is known about the effects of opioids on lamina I neurons that include projection neurons transmitting processed nociceptive information to supraspinal centers. Thus, we combined whole-cell patch-clamp recordings, primary afferent stimulation and immunocytochemistry in the in vitro intact spinal cord preparation to shed light on opioid actions in lamina I.

We found that a subpopulation of lamina I neurons, that included both projection and local-circuit neurons showed reduced primary afferent excitation with or without hyperpolarization indicating a complex effect of opioids. We hypothesize that unbalanced input from lamina II excitatory and inhibitory neurons may also contribute to the opioid action. Our results provide new insights into how opioid analgesia affects superficial dorsal horn network function and nociceptive information flow.

The work was supported by the KTIA_NAP_13-2-2014-0005 grant of the Hungarian Government (PSz), the OTKA PD 101248 (ZP), the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (PSz) and by the grant from the Portuguese Foundation for Science and Technology funded by COMPETE and FEDER (BS).

P2/32

The paired-domain transcription factor Pax2, as a possible somatic marker for adult GABAergic spinal dorsal horn neurons

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Confirmation of the inhibitory nature of biocytin labelled interneurons in the spinal dorsal horn is technically challenging. The most accepted method, demonstration of vesicular inhibitory amino acid transporters (e.g. VGAT) in axon terminals of the neuron, has several pitfalls. The method requires sufficient labelling of the axon in recorded neurons and, furthermore, the demonstration of the fact that the VGAT positive axon terminals are indeed connected to the neuron to be identified. An ideal and more convenient way to identify inhibitory neurons, even in case of partially labelled neurons, would be to use somatic or nuclear markers.

Several lines of evidence support the model where differentiation of GABAergic interneurons in the dorsal cord depends on the paired-domain transcription factor Pax2, with several other factors (including Lhx1 and Lhx5) helping to activate and maintain Pax2 expression in these cells. Pax2 as a marker has already been used to identify future GABAergic neurons in development studies and recently it was also utilized to identify neuronal groups labelled with intrauterine electroporation in the postnatal period.

In this study we systematically tested the expression of Pax2 in adult rodent spinal dorsal horn neurons to quantify the overlap with the inhibitory population defined by the presence of GAD2 or VGAT in the soma and terminals, respectively. We found that Pax2 labels inhibitory neuronal somata in the adult rodent spinal cord with good reliability making it a putative marker for individually labelled inhibitory neurons.

The work was supported by the KTIA_NAP_13-2-2014-0005 grant of the Hungarian Government (PSz, ZM) and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (PSz)

P1/53

Aged-related decline and axonopathy in the cholinergic brain in rats

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Objective. The decline of cholinergic brain functions during aging is a risk factor for Alzheimer disease (AD). In the present study the aged-dependent changes in the function of cholinergic neurons was followed in rats and the degree of axonal aberrations (axonopathy) were estimated in both aging rats and in AD brains. In addition, physical exercise as preventive treatment was also introduced in aging rats.

Methods. Wistar rats were sacrificed 12-, 24-, and 32-months of age after either sedentary condition or long-term forced exercise treatment. Ir-ChAT positive cholinergic fibre density was measured in the hippocampus in rats and the axonopathy in several brain areas of rats and also in AD brains. Morphological assay of ChAT fibre density was performed either by Quantimet (Leica; measuring fiber density above threshold) and by manually estimating all stained fibres irrespective of their staining density.

Results. First, the ir-ChAT fiber density was only declined with age measured above threshold with Quantimet which suggested that the synthesis and/or the transport of ChAT to fill up axons declined. Second, the six-months long previous exercise did not influence the cholinergic decline in either ages, but a life-long exercise could attenuated it in the hippocampus in old age. Third, cholinergic axon aberrations were present in both senescent (32-month old rats) and AD brain and the development of this pathology could be reduced by exercise in rats.

Conclusion. During aging the cholinergic neurons keep their preformed axons structurally while the synthesis/transport of ChAT is gradually slowing down. This may provide possibility for preventive and drug treatment options for the cholinergic brain. Chronic exercise may attenuate cholinergic decline with age if the duration of exercise is sufficiently elongated. Axonal pathology developing during the advance aging was more effectively repressed by exercise.

Symposium II. -Thursday

"Neuropharmacology of attention in the visual cortex"

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Attention influences almost all aspects of cognitive behaviour. It enables enhanced processing of behaviourally relevant stimuli at the expense of irrelevant stimuli. At the cellular level this is mediated by alterations in cell activity, alterations of neuronal firing rate correlations, and a reduction in the variance of firing rates. Using iontophoretic methods of drug application during extracellular recordings in V1 of the macaque monkey, we find that attention induced firing rate changes depend on the action of acetylcholine (mediated through muscarinic receptors), but not on NMDA receptor mediated glutamatergic signaling. Conversely, attention mediated reduction in firing rate variance and firing rate correlations depend on NMDA receptor mechanisms, but not on cholinergic mechanisms. We thus show that different transmitter systems contribute to different aspects of attentional modulation in primary sensory cortex. We propose that acetylcholine adjusts the V1 network such that it is adequately sensitive to feedback mediated attentional modulation, which likely targets NMDA rich synapses. Conversely, the neuropharmacology of attention in the frontal eye field (FEF) distinctly differs from that in V1. In FEF acetylcholine contributes to attentional modulation through nicotinic receptors, which enable attention induced variance reductions. While glutamatergic ionotropic receptors enable cellular excitation in FEF, they do not affect any of the attentional signatures investigated. Our findings demonstrate that a range of different mechanisms affect attentional control, which differ between cortical areas.

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P2/33

Schizophrenic symptoms accompanied by epigenetic changes in the male vasopressin-deficient Brattleboro rat

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Despite the high prevalence schizophrenia remain enigma because there is no single factor that account for a large number of patients. Therefore it has been widely speculated that epigenetic changes may play a role in its etiology. Our previous studies confirmed that the vasopressin-deficient (AVP-) Brattleboro rats show schizophrenia-like (SZ) behaviors. We aimed to clarify if epigenetic changes contribute to their behavior.

We compared the SZ behavioral profile (hyperlocomotion by openfield, memory disturbances by object and social discrimination tests, attention deficit by prepulse inhibition (PPI)) and epigenetic (DNA-methylation enzyme by PCR, global histone (H3 and H4) acetylation (ac) by Western-blot and H3 lysine9 (K9) ac by immunocytochemistry) differences in prefrontal and hippocampal brain areas between wild type and AVP- animals.

As a positive symptom AVP- animals were more active. Social withdrawal was confirmed as a negative sign and memory and attention disturbances were also present representing the cognitive deficit. There was no significant difference between the DNA methyltransferase mRNA and H4 acetylation level between the groups. The pan-H3ac immunoreactivity was lower in the homogenized brains' prefrontal region and higher in the hippocampus of AVP- animals. Thus, we examined the region specific distribution of a specific acetylation site, the H3K9ac, as one of the most studied epigenetic mark. We observed lower immunopositive cell number in the ventral part of the lateral septum and dorsal peduncular prefrontal cortex in AVP- rats and significant differences in all hippocampus regions (CA1, CA2 and CA3) between AVP- animals and wild type.

All in all, AVP- Brattleboro rat showed a disturbances in all symptom domain of SZ, thus this animal seem to be a reliable preclinical model of SZ. Epigenetic changes in the prefrontal cortex and hippocampus may explain the behavioral alterations. Indeed, the reduced acetylation may contribute to disturbances of many neurotransmitter systems (for example GABA, acetylcholine). The multifactorial origin of SZ may become unified under the epigenetic umbrella.

P2/96

Transcranial direct current stimulation over the right vs. left prefrontal cortex differentially affects consolidation of implicit statistical learning

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Picking up statistical regularities of patterns from the environment is essential for predictive and adaptive behavior. One of the most important challenges is to understand how statistical learning occurs and how the acquired information consolidates and stabilizes in the brain. Evidence suggests that the prefrontal cortex (PFC) has a critical role in these processes; the division of labor between hemispheres, however, is less characterized. The aim of the present study was to directly investigate the causal role of the right and left PFC in implicit/incidental statistical learning and its consolidation. Transcranial direct current stimulation (tDCS) over the right or left dorsolateral PFC (DLPFC) was applied during implicit statistical learning in order to modify learning-related cortical plasticity in the targeted brain regions by increasing neural excitability. Performance was tested during stimulation and 12-hour later. In order to investigate how brain stimulation during learning affects sleep-dependent memory consolidation, we compared experimental groups who did or did not have sleep in the 12-hour consolidation period (AM-PM vs. PM-AM design). Here we show no sleep effect on consolidation of implicit sequence memories in sham condition, however, the interaction between sleep/no sleep condition and tDCS on right vs. left DLPFC was significant. Our results can lead to a deeper understanding of the hemispheric specialization in sleep-dependent memory consolidation.

Keywords: implicit learning, direct current stimulation (tDCS), sleep, memory consolidation, statistical learning, prefrontal cortex

P2/67

Determination of the essential number of motoneurons required to produce functionally useful limb locomotion

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An avulsion injury inflicted upon the spinal cord induces a critical loss of motoneurons followed by irreversible locomotor function impairment ranging from inadequate limb movement to complete paralysis. Recent surgical approaches may provide improvement in limb function, however, the question remains how many motoneurons are needed to survive and grow new axons to achieve sufficient muscle reinnervation. The aim of this study was to determine the minimum motoneuron numbers, required to reinnervate the denervated skeletal muscles of the limb and produce a functionally satisfactory locomotor pattern. In order to determine the exact threshold of satisfactory functional reinnervation we have developed and employed a very sensitive movement recording and analysing system as none of the commercially available methods/equipments was able to provide a quantifiable and in-depth analysis system about the motor pattern of the whole hind limb. Therefore we combined the use of video-based footprint and hind limb motion analyses to achieve a new and more reliable assessment. Sprague-Dawley rats that underwent a lumbar 4-5 (L4-5) ventral root avulsion had their L4 ventral root reimplanted and received different doses of riluzole (0.4, 0.8 and 1,6 mg/kg every second day for two weeks postoperatively) treatment in order to rescue incremental numbers of the damaged motoneuron pool. Control animals received no treatment. The locomotor pattern of the rats was recorded with high resolution (1080x720 pixels) and high speed (100 frame/s) digital cameras from both lateral and caudal aspects simultaneously. All together nine parameters of the hind limb movement pattern were evaluated by measuring specific joint angles, footprints and gait parameters in single video frames. Four months after the operation we carried out Fast Blue retrograde tracing in order to label and count the reinnervating motoneurons. A correlation between the numbers of the reinnervating motoneurons and the functional improvement was made. Our results confirmed a strong relationship between functional restoration of the original movement pattern and morphological reinnervation.

P1/63

Single unit analysis during auditory sensory gating in medial prefrontal cortex in freely moving rats

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The medial prefrontal cortex is described as one of the main regions involved in the regulation of sensory gating process. Prepulse inhibition (PPI) has been found to be reduced in schizophrenia. The purpose of our experiment was to examine how sensory gating can be altered by PPI associated with an acoustic startle response. In the present research behavioral and electrophysiological data have been integrated during acoustic startle and PPI in medial prefrontal cortex (mPFC) in freely moving rats. For startle response measuring a new headstage with 3D-accelerometer was invented. Single unit activity was recorded by means of chronically implanted tetrodes. A neuronal database was created for single units, that recorded in the mPFC. 72 of 273 units (26.37%) met the criteria for being significant responses during the first three recording sessions. These single units were grouped according to 2 classes of tone responses. Single units that were found to have a tone related increase in firing rate were classified as excitatory units and those single units which responded to tones with a decrease were classified as inhibitory units. Within the excitatory category short duration and long duration unit subgroups have been identified. In this study we provided the first characterization of the multiunit activity of mPFC during PPI paradigm. Our data demonstrate that mPFC neuronal responses correlate with PPI induced alteration of startle responses. Supporte by the Pécs University Medical School and the Talentum Foundation of Richter Gedeon Pharmaceutical Co.

P1/40

Laminar organisation of high frequency oscillation in the human brain

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High frequency oscillations (HFOs) have been implicated in the generation of epileptic seizures and HFOs may serve as biomarker for epileptogenic cortex in being able to epilepsy, also being capable to localize the epileptogenic zone.

Methods

We used laminar microelectrodes (MIELME) to record potential gradient, current source density (CSD) and multi-unit activity (MUA) in wakefulness in 15 cases. Time frequency and wavelet analysis was used to detect ongoing or event related spectral perturbations. ANOVA analysis was used for statistical analysis.

Results

The majority of HFO events (over 90%) were coincident with interictal epileptiform discharges (IED). Such HFO bursts were maximal after the onset of the IED. Conversely, HFO was present only in 30% of all IEDs. When present, HFOs with a central frequency of 80-130 Hz had greatest power in upper cortical layers. CSD analysis showed the presence of an alternating sink-source pair in these upper cortical layers. Faster oscillations and activities (>150Hz) were more prevalent in middle cortical layers. In general, this faster, deeper activity preceded the slower activity seen in superficial layers (10-30 ms).

Conclusion

These results suggest that different cortical domains may participate in HFO generation. The further characterization of these domains may clarify the role of HFOs as biomarkers for epilepsy.

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P1/72

Impact of satiety state and testosterone on the metabolic lateralization in the male rat hypothalamus

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The hypothalamus is a crossroad and center of numerous homeostatic regulatory pathways. It is unclear, however, how this relatively small brain area can regulate such an abundance of functions. Although the hypothalamus, despite of, or for that matter, due to its identical bilateral neuronal populations, has usually been investigated, presented and interpreted as a functional unit of midline brain structures, we have generated evidence to argue for the functional sidedness of this forebrain structure in female rats with respect to the functional lateralization of reproductive regulatory functions. Using male animals, in the present study we provide evidence that when cyclicity in reproductive functions does not exist, satiety-dependent hypothalamic regulatory functions are just as lateralized as demonstrated with regard to reproduction in females. Besides the hypothalamic asymmetry in the regulation of feeding, the present data suggest that it is the satiety state, and probably its humoral background, rather than the presence or absence of gonadal steroids that accounts for the detected differences between the intensity of mitochondrial metabolism measured in the left and right sides of the male rat hypothalami.

P2/23

Investigating the effects of protein kinase D on the endocytosis of dendritic transferrin receptor

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Protein kinase D (PKD) is a serine/threonine kinase, which mediates the somatodendritic distribution of transferrin receptor and the receptor-mediated transferrin uptake in neuronal cells.

This study aims to investigate the effects of PKD on the endocytosis of transferrin in embryonal hippocampal neurons. Fluorescently labelled transferrin was used to follow the kinetics of endocytosis. PKD activity was influenced genetically or pharmacologically, either via overexpressing a constitutively active PKD mutant or by the application of a PKD specific inhibitor. The early, late and recycling endosomes were labelled by EGFP-tagged members of the small GTPase Rab protein family, namely Rab5, Rab7 and Rab11, respectively. The colocalisation between internalized transferrin molecules and Rab positive vesicles was analysed on confocal images.

Our experiments show that enhanced PKD activity increased transferrin uptake and the colocalisation between internalized transferrin and Rab5 vesicles from the first minute. In case of recycling endosome associated Rab11 labelling, this effect was observed only during the first minutes. Inhibition of PKD activity decreased the transferrin uptake of cells as well as the level of colocalisation between transferrin and Rab5 or Rab11 vesicles. Enhanced PKD activity did not influence the lysosomal pathway, as PKD had no effect on the colocalisation between transferrin and Rab7 vesicles. Our results show that PKD plays a role during the early steps of transferrin-transferrin receptor complex endocytosis at the plasma membrane, and stimulates the recycling endosomal system.

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P1/49

Immunohistochemistry of cerebellar seizures: mossy fiber afferents play important role in seizure spread and initiation in the rat

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Clinical reports suggest the participation of the cerebellum in epilepsy. Mossy fibers are the main excitatory afferents of the cerebellar cortex; most of them use glutamate and strongly excite granule cells through NMDA- and AMPA receptors. The role of the pontocerebellar mossy fibers in cerebellar neuronal hyperactivity was investigated in the present study. We detected neuronal hyperactivity through the expression of the glutamate induced c-fos protein, by means of immunohistochemistry and immunoblotting in the vermis and in the hemispheres. Generalized seizures were induced by means of intraperitoneal 4-aminopyridine injections. Following the 4-aminopyridine seizures, the c-fos expression of cerebellar granule cells was significantly elevated at 1.5 h in every lobule. Maximum c-fos expression was seen at 3 h. The role of the pontocerebellar mossy fiber afferents in the induction of c-fos expression was examined after the transection of the middle cerebellar peduncle on the left side. Immunohistochemical analysis 14 days after the surgery revealed that the synapsin I immunoreactivity was significantly reduced in the cerebellar cortex on the operated side, compared to the sham operated controls and to the non-operated cerebellar hemisphere of the operated animals; indicating the degeneration of mossy fiber terminals. Transection of the middle cerebellar peduncle suppressed cerebellar c-fos expression in the vermis and in the hemispheres significantly. These findings suggest the strong involvement of the middle cerebellar peduncle and the pontocerebellar mossy fibers in the pathophysiology of cerebellar epilepsy.

Symp. III. - Friday

The plasticity of the self-other relation: from body-ownership to social cognition

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Abstract: Awareness of one's body is intimately linked to self-identity, the sense of being "me". Converging evidence suggests that the integration of exteroceptive signals related to the body, such as vision and touch, produces or even alters the representation of self-identity. Are these multisensory-induced changes limited to the representation of the self? I will present recent evidence that extends this line of research into social cognition

processes. Recent studies have asked whether it is possible to *change* fundamental social cognition processes such as stereotyping, perceived similarity and body resonance by experimentally changing the relation between self and other. I will present recent results from behavioral, neuroimaging and neurostimulation experiments that show how multisensory input changes not only the representation of one's self but also the relation between self and others. This approach can in turn be used to disambiguate the functional role of areas that have been shown to underpin both self-awareness and social cognition.

P1/97**The new Behavioral Studies Unit in the Institute of Experimental Medicine invites cooperation**

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The Behavioral Studies Unit (BSU; Hungarian: Viselkedésvizsgáló Egység, VVE) is a new core facility of the Institute of Experimental Medicine created and equipped between 2012 and 2014. The project was funded by an Infrastructure Development Grant of the Hungarian Academy of Sciences.

Main objectives of the BSU are (i) general behavioral phenotyping of animals, (ii) to investigate the role of different neural processes on behaviors shown in models of psychiatric disorders, (iii) to test drug effects in order to develop novel treatment approaches for psychiatric disorders.

In the BSU, numerous types of experiments can be carried out in laboratory rodents. These include classical and state-of-the-art behavioral tests for studying animal models of anxiety, depression, learning and memory, prosocial and antisocial behavior, symptoms of schizophrenia, drug addiction, nociceptive and motor functions, as well as continuous monitoring of home-cage behavior. The instrumentation and software products available in the BSU are the latest developments of leading companies of the international market. Thanks to the high level of automation and information technology, biases can be eliminated, and experiments can be conducted time-efficiently. The overall capacity of the BSU allows as many as 10 different experiments to be performed simultaneously, which also increases the overall speed of obtaining data. Surgery, perfusion, local administrations and other interventions can be done in the surgery room of the BSU. The BSU also has a high capacity for hosting animals during the period of behavioral testing. Expertise in animal maintenance in general and specifically in transgenic animals is guaranteed by the Medical Gene Technology Unit of the Institute.

The Behavioral Studies Unit is unique in the Hungarian academic research sector and allows the translation of basic neurobiological research into animal disease models and new treatments.

BSU offers its facilities for collaboration with research groups of the Institute as well as with other researchers from Hungary, Europe and the world. Such cooperative work is already being performed with in-house and external collaborators.

P1/90**Synaptic protein changes in the maternal hypothalamus**

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The postpartum physiological and behavioral changes in females are critically important parts of mammalian reproduction. Our hypothesis is that alterations associated with the postpartum behavioral and hormonal state present at the proteome level. The hypothalamus has a pivotal role in the coordination of these maternal adaptational mechanisms. A huge part of protein level changes in the brain takes place in the synaptic compartment. Therefore we isolated synaptosome fractions from the maternal hypothalamus to investigate

proteomic changes in dam rats. We used dams which were deprived from their pups immediately after the parturition as control animals.

The synaptosome preparation was performed using Ficoll gradient centrifugation, and the validation of the method was conducted with electronmicroscopy. 2D-DIGE full stain technique combined with LC MS/MS was used to separate proteins and determine protein level differences. We have identified 21 significant protein changes among which we found 7 increases and 14 decreases in dams with their pups compared to the control animals. We identified acetyl-CoA acetyltransferase which is involved in the lipid metabolism and showed one of the greatest protein level increases. Two other proteins with outstanding protein level increase, alpha-internexin and cytochrome b-c1 complex subunit 2 are implicated in cell differentiation and cellular respiration, respectively. Nucleosid diphosphate kinase A, which functions in energy metabolism, and the the myelinization regulating myelin basic protein showed the largest protein level decreases. Furthermore, the decrease of complement componens subcomponent binding protein 1 and the phosphatidylethanolamin binding protein 1 was validated with Western blot technique. The extensive functional classification of the altered proteins was manually conducted with protein databases. Lots of these proteins participate in citric acid cycle, oxidative phosphorylation, protein folding, lipid metabolism and cell differentiation according to the classification. The interactions of these proteins were determined and visualized with Elsevier Pathway Studio Platform. The bioinformatics analysis showed that several proteins are connected with hypothalamic hormones.

According to our results pup nursing is associated with synaptic proteins level changes which may play a role in the hormonal alterations of dams.

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P1/20

Structural and intrinsic plasticity tunes synaptic integration in dendritic branches to the statistics of their inputs

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The dendritic tree of a cortical neuron is the locus of a dazzling array of powerful and puzzling forms of plasticity. Beside more traditional forms of synaptic plasticity, dendrites also exhibit experience-dependent forms of structural as well as intrinsic plasticity, changing the set of presynaptic neurons targeting a dendritic branch and its intrinsic nonlinearities, respectively. While synaptic plasticity has been studied extensively, little is known about the functional principles underlying the other two forms of plasticity. Here we derive optimal rules for structural and intrinsic plasticity, and their biological approximations, based on the idea that they tune sub-cellular connectivity and dendritic nonlinearities to the statistics of population activity in the presynaptic ensemble to allow for robust computations in spiking circuits.

Our theory predicts that presynaptic neurons from the same ensemble innervate the same dendritic branch, and hence the stabilization or elimination of a newly formed synapse depends on the evidence that it belongs to the same ensemble as the neighboring synapses. We show that computing this evidence requires two signals readily available at the synapse: one that is local to the synapse (glutamate bound to the NMDA receptors) and another that is local to the dendritic branch (its membrane potential). Both the local nature and the timing requirements of the derived plasticity rule match those observed in dendrites of cortical neurons. Our theory also predicts that the nonlinearity of a dendritic branch depends on the correlations among the neurons of the presynaptic ensemble. Thus, we derive a plasticity rule to learn these correlations, and the resulting nonlinearity, from spiking inputs and show a close correspondence with experimentally observed forms of branch strength potentiation. Our approach provides a novel framework for studying plasticity within the dendritic tree from the perspective of circuit-level computations.

P2/109

A novel behavior-analyzing system demonstrates alterations of social behavior of mice under group-housed conditions by growing conditions of mice

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Mental disorders have been reported to increase in the modern societies. As their risk factors, not only a genetic factor but also an environmental factor, such as excessive dieting during pregnancy, neglect, bullying, adolescent stress are considered to be involved. However, it remains unclear how growing environments influence mental disorders. Although the signs and symptoms of social behavior disorders of mental disorders manifest in the social context, behavioral tests that have been used to analyze social behaviors in basic research using rodents are to assess a relationship between only two animals. To establish methodology which can accurately assess the social abnormality of animals under more naturally-formed social context, we developed a novel assay system that can automatically analyze behaviors of mice on the individual basis under group-housed conditions. This novel system can identify an individual mouse and record the location of each mice as an XY coordinates. Then, we applied it to examine influences of various growing environments on behaviors. C57BL/6J mice were divided into two groups, a group-reared (GR) group and an individually-reared (IR) group at weaning, followed by further rearing until adulthood. For the behavioral test, four male adult mice, which were strangers to each other, were introduced into a test cage and observed at least 3 days. In the experiments, three combinations of mice, i.e., 4 mice from GR only (GR-4), 4 mice from IR only (IR-4), and 2 mice each from GR and IR (GR-2+IR-2), were used. Mice have a tendency to huddle together with time in a home cage. IR mice in IR-4 needed more time until huddle than GR mice in GR-4 in a test cage. In GR-2+IR-2, the huddling behavior occurred first in GR mice, and then IR mice joined the group. Furthermore, a number of access of IR mice to GR mice was greater than that of the opposite direction. These results suggest that environments of growing mice may affect a way of social communication within a group. In addition, the process of building a new relationship with strangers are affected by not only own growing environment but also characters of others. In conclusion, our novel system can be used to identify each mouse and characterize not only behaviors of an individual mouse but also social interactions under group-housed conditions.

P1/35**Interictal spikes preferentially occur during descending and down-states of delta waves**Péter Ujma¹, Péter Simor², Raffaele Ferri³, Péter Halász⁴, Loránd Erőss⁵, Dániel Fabó⁴

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Low frequency components of sleep EEG, such as delta waves (0.5-4 Hz) synchronize higher frequency oscillations, which preferentially arise in the up-states of such low-frequency waves. Sleep and delta activity has also been associated with increased epileptiform activity in epileptic patients. However, no previous study has explicitly investigated the co-occurrence of epileptiform activity with specific phases of delta waves. We investigated the occurrence of interictal spikes in corticographic recordings of 3 epileptic patients during delta waves on frontal scalp EEG channels. Delta waves were detected according to the methods described by Piantoni et al. (2013) and interictal spikes were detected using an individual amplitude threshold on 8 Hz high-pass filtered corticographic EEG. Delta waves were separated into four quarters separated by zero-crossings and peaks: descending down-phase (1), ascending down-phase (2), ascending up-state (3) and descending up-state (4). Spikes were not symmetrically distributed across the quarters of delta waves. Spikes occurred mostly during the descending phases of delta waves (quarters 1 and 4), much more rarely during ascending down-states (quarter 2) and almost never during ascending up-states (quarter 3). Our results demonstrate that the synchronizing activity of delta waves is also present in terms of epileptic interictal spikes. Surprisingly, however, interictal spikes did not occur during up-states like high-frequency oscillations, but during descending and down-states. This suggests that the relative cortical silence in down-states facilitates epileptiform activity.

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P1/96**Calcium-dependent AMPA receptor antagonism diminish conditioned fear response and facilitate extinction 28 day after footshock**

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Fear conditioning is a widely used animal model of human anxiety. Rats learn to associate aversive electrical footshock with neutral environment resulting in expression of fear responses (freezing) to the originally neutral stimulus. Recent in vitro data suggested that blockade of the glutamatergic calcium-permeable AMPA receptors (CP-AMPA) by IEM-1460 might be involved in fear extinction. We aimed to test this theory in an in vivo system.

For studying the effect of IEM-1460 (1 and 3 mg/kg i.p. 60 min before fear conditioning), trials were conducted 1 or 28 days after footshock trauma. Glutamate receptor subtype (GluA1 and GluA2) mRNA levels were measured by RT-PCR at these timepoints in the prefrontal cortex (PFC) and in amygdala (basolateral-BLA, central-CeA) regions. Extinction was studied by placing the animals back to the neutral environment after a single IEM-1460 treatment, 5 times a day on the 1st day, once next day and 28 days later, started 1 or 28 days after trauma.

1 mg/kg IEM-1460 reduced the expression of freezing at the 1st day and accelerated extinction 28 days after footshock, which effect lasted at least 28 days, with some tendencies with the dose of 3 mg/kg. Shock induced an elevation in GluA2 levels of the PFC and in GluA1/GluA2 ratio of the BLA the later symbolizing the CP-AMPA. There were no difference in mRNA levels between 1 or 28 days after footshock.

Our in vivo data confirmed the involvement of CP-AMPA in appearance and extinction of conditioned fear with a bell-shaped dose-response curve. These results suggest that antagonism of the CP-AMPA might be a potential target of a combined pharmacological and exposure based therapy in posttraumatic stress disorder.

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Pupil size predicts the modulation of visual working memory capacity by reward

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Recently, it has been shown that reward increases the capacity of visual working memory (VWM), however, the neural mechanisms underlying this effect are less known. Here we investigated whether the reward-related increase in working memory capacity is related to the well-known electrophysiological marker of VWM retention, the contralateral delay activity (CDA) component of the event-related potential. We also examined the putative relationship between pupil dilation, which has previously been shown to track reward processing and mental effort, and the reward-based enhancement of VWM. Participants performed a delayed matching-to-sample task with colored squares while pupillometric and electroencephalographic (EEG) recordings were administered. In the beginning of each trial, a visual cue indicated whether correct response is rewarded or not. Our results showed an increase in VWM capacity in the rewarded compared to the non-rewarded condition. An increase in pupil diameter during the delay period was also observed. The increase in pupil size showed a positive correlation with VWM capacity enhancement – the larger the dilation of the pupil, the greater the behavioral improvement in rewarded trials. Interestingly, the CDA, which is assumed to reflect the maintenance of visual representations in working memory, did not show any modulation by reward. Our results are suggestive of the role of the noradrenergic system in the reward-related improvement of visual working memory capacity, presumably reflected in pupillary dilation.

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Effect of a high-dose L-Kynurenine sulphate treatment on behavior and basal c-Fos expression in the adult C57Bl/6 mice

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L-kynurenine (L-KYN) is a central metabolite of the tryptophan degradation, called Kynurenine pathway (KP). Systemic administration of L-KYN sulphate (L-KYNs) leads to rapid elevation of the concentration of a neuroactive KP metabolite kynurenic acid (KYNA); while concomitant changes in the concentration of extracellular glutamate, acetylcholine and dopamine can also be observed in the murine brain. This increased brain KYNA level exert multiple action on synaptic transmissions resulting in complex behavioral changes, like hypoactivity and impaired memory functions. However, these studies focus on rats, using medium-dose L-KYNs. Our aim was to investigate the effect of a systemic high-dose L-KYNs treatment on moving patterns and neural activity on corresponding brain areas in the C57Bl/6j mouse. First, we examined the effect of systemic administration of L-KYNs (300mg/kg) on behavior of animals in open field arena, evaluating the locomotor activity and anxiety-like behavior, compared to the control group (0,1M PBS). Then, to evaluate the changes in neuronal activity after L-KYNs treatment, in a separated group of animals we estimated c-Fos expression levels in the dorsal striatum. L-KYNs treatment significantly elevated the moving speed, as well as altered the moving pattern of the mice. As an anxiety-like behavior peripheral zone preference of the open field arena was emerged and rearing activity was attenuated. Significant difference in c-Fos expression was found in the dorsal striatum compared to basal value. Taken together, we can posit that L-KYNs treatment doesn't affect general ambulatory activity but alters the moving pattern of the mice, elevating the portion of high-speed movement, and increased anxiety-like behavior. These changes can be corresponded partly to the imbalance of striatal neuronal activity.

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P1/5

Changes in D and L-aspartate levels in relation to learning-induced neurogenesis in young domestic chicks

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The D enantiomer of aspartate occurs in substantial amounts in the developing embryonic nervous system. In the postembryonic brain D-aspartate (D-Asp) has been found in immature neurons. In numerous learning models, training triggers proliferation of progenitor cells and results in an increased generation of new neurones. Passive avoidance learning of young precocial birds is such a model. According to our hypothesis, learning-induced neurogenesis in young domestic chicks is accompanied with marked elevation of D-Asp concentration, especially in brain regions with high proliferative activity.

In the present experiment, brains of passive taste aversion-trained and naive chicks were compared 48 hours after training. We measured the density of BrdU and doublecortin immunolabelled immature neurones in regions of high (subventricular zone, SVZ) or low (lateral nidopallium) proliferative activity. The free amino acid concentrations in homogenates of tissue samples from the same regions were also measured by capillary electrophoresis coupled with laser induced fluorescence detection.

More new neurones were detected in the SVZ of the learned than in that of naive chicks or in the nidopallium of both groups. The concentration of D- but not L-Asp showed a similar pattern: highest in SVZ of trained individuals. Our data suggest that D-Asp facilitates the maturation and survival of new neurones, even triggering the proliferation of their progenitors. Several studies have suggested that D-Asp is released by newly generated neuroblasts themselves, contributing to their own survival and maturation, together with their neighbours. Furthermore, it seems that D-Asp might also participate in the recruitment of novel neurones demanded by learning.

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P2/88

Crosstalk between retinoid metabolism and inflammation within the CNS

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It is well known that all-trans retinoic acid (RA) is a crucial regulator of embryonic development of the nervous system. It had also been validated that the postnatal central nervous system – after the maturation of the blood-brain barrier - has got its own enzymatic apparatus to serve retinoid metabolism. In the adult mouse brain RA synthesis is mainly localized in the hippocampus, plexi choroidei, the leptomeninx and the dura mater. These latter structures not only produce and bathe in the cerebrospinal fluid – as we, and others have determined by HPLC-MS-MS and immunohistochemical studies - but they form important border structures between the CNS and the periphery. RA influences both normal and pathophysiological neural functions. For example it is a major differentiation signal for neurons and has anti-inflammatory and antioxidant effects on neural cells. Besides the direct neuronal action the anti-inflammatory impact targets microglia – the most important resident macrophages of the brain. RA also affects the closure of the blood-brain barrier, therefore it might be an important conductor of the influx of immune-related cells from the periphery and might play a role in the restriction of brain oedema during inflammatory stimuli. It is also known from in vitro studies that RA-catabolic enzymes are upregulated in activated microglia and in contrast, the direct RA exposure attenuates the release of pro-inflammatory cytokines from these cells. In order to understand the role of RA during brain inflammation we introduced gram-negative bacterial endotoxin (LPS) to the right lateral ventricle of adult C57Bl mice. We collected samples after 24 hours from various intracranial tissues (such as plexus choroideus, pia mater, dura mater, hippocampus) to examine the RA-metabolism related expression changes with Q-PCR analysis. We also collected and analyzed cerebrospinal fluid with FACS to determine the cytokine dynamics. Then we performed immune-histochemistry on paraformaldehyde fixed brain sections: we stained the most abundant anabolic and catabolic enzymes of the retinoid pathway. As described, RA mediated functions are very important in maintaining physiological as well as pathological conditions so any changes in the RA metabolism might affect a high variety of CNS cells. Nevertheless, very few in vivo models had been created to study the retinoid metabolism during inflammatory events. Our goal is to perform an in vivo study to answer these questions.

P1/86

Examination of bioactive factors in human milk samples with different methods

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Breast milk contains several bioactive compounds that play important roles in the development of the nervous system and in gaining immunocompetence. PACAP is a neuropeptide with important functions in reproductive and developmental processes. Recently, we have shown that PACAP38 is present in high levels in breast milk and we have described changes of PACAP levels during lactation in the first 17 months. Earlier we have also examined the presence of MIF, a proinflammatory cytokine, in human milk samples, but previous prospective studies have only focused on the water phase of breast milk in the first 3 months of nursing. In the present experiment we aimed to examine the changes of MIF and other bioactive factors both in the water and lipid phase of milk samples during the first 6 months of lactation.

It is well-known that the constitution of breast milk is influenced by numerous external agents, e.g. the gender of the newborn. Therefore, in the second part of our research we analyzed the difference between the milk samples of male and female newborns. We collected 5 ml milk every month during the first 6 months of nursing. First we separated the milk samples to lipid phase and water phase by centrifugation. We used ultrasonication to factor the lipid phase, with this method we obtained an additional lipid fraction and water fraction. We measured the MIF concentration with ELISA technique from each sample. Afterward, with Luminex technique we examined the concentration of Fractalkine, MIP-1-Beta, Eotaxin, MDC, RANTES, EGF, MCP-1, GRO, Flt-3L, CD40 in these samples. The PACAP level of the milk samples was measured by RIA examination.

In our experiment we detected the presence of MIF in the lipid phase of human milk for the first time. We measured higher MIF concentrations in the water fraction than in the lipid fraction. We were also the first to show an increasing tendency of the MIF concentration in the lipid layer of human milk during a long-term 6-month follow-up period. With Luminex technique we also detected significant changes in the level of other bioactive factors in 3 different milk fraction during the first 6 month of lactation. Our preliminary examinations did not find significant differences between milk samples of male and female newborns.

Our future aim is to establish the exact influence of the above-mentioned factors, in the process of lactation with additional clinical and molecular biological experiments. Support: PTE-MTA "Lendület" Prog., OTKA K10498, Arimura Foundation

P1/79**Dimorphic cell signaling in GnRH neurons of male and female mice**

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Gonadotropin-releasing hormone (GnRH) neurons play pivotal role in regulation of the reproductive system. The gonadal hormonal milieu and the physiology of reproduction differs between sexes. We hypothesized that a sex-specific operation of GnRH neurons exists behind their different functional properties. To test this, the gene expression profiles of GnRH neurons obtained from metestrus female and male GFP-GnRH transgenic mice were compared. The unbiased systematic sampling of GnRH neurons by laser capture microdissection technique and the linear amplification of the isolated mRNAs by the whole transcriptome amplification offered accurate gene expression profiling of hypothalamic GnRH cells. In each sex, about 1500 GnRH neurons were micro-dissected from 6 individual mice. We identified 546 differentially expressed genes by Affymetrix Mouse Genome 430 PM microarray analysis under stringent selection criteria (fold change > 1.6, adjusted p-value: 0.01). Majority of them were upregulated in the male. The sexual dimorphism in gene expression was most apparent in gene clusters affiliated with synaptic communication, signal transduction, cell adhesion, transport mechanisms and cell metabolism, as revealed by functional annotation. For validation of the microarray data, 63 genes of interest were selected and studied by real-time PCR. The confirmation rate was 84.1%. Using independent GnRH neuron samples obtained by patch pipette harvesting of 30 GnRH neurons from each sex validated 82.5% of the results. High-throughput comparative analysis of gene expression profiles of GnRH neurons of male and metestrous female mice revealed a striking sexual dimorphism that may affect neurotransmitter and intracellular signaling, transport and release mechanisms of these neurosecretory cells.

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P2/27**Morphological characteristics of three distinct GABAergic interneuron types targeting the perisomatic region of principal cells in the mouse basolateral amygdala**

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Similar to most cortical networks, the basolateral complex of the amygdala (BLA) is composed of excitatory, glutamatergic principal cells (PCs) and inhibitory, GABAergic interneurons (INs).

In the present study we have studied the morphological characteristics of BLA interneurons targeting the perisomatic region of PCs. Using whole-cell patch-clamp technique in slice preparations, we filled recorded neurons intracellularly with biocytin, followed by the visualization of neuronal processes with immunocytochemistry. We could distinguish axo-axonic cells (AACs) and two kinds of basket cells (BCs): i) parvalbumin (PV)-containing fast-spiking BCs and ii) regular-spiking BCs expressing cholecystokinin (CCK) and type 1 cannabinoid receptors (CB1). Based on light and electron microscopic analysis, PV-containing AACs innervated exclusively axon initial segments (AISs) of PCs. In contrast, both types of basket cells targeted the somata and the dendrites of PCs to variable extent, showing similar postsynaptic target distribution. Using Neurolucida software, we reconstructed and compared the dendritic and axonal arborizations of labeled interneurons and found that PV-BCs had the longest axonal arbor bearing the most varicosities, suggesting that this interneuron type might control the activity of a higher number of cells in the local network. In addition, BCs had almost twice as long dendritic trees than AACs, which might endow them to receive and integrate more incoming inputs.

Our findings revealed previously unrecognized synaptic properties of interneurons innervating the perisomatic region of PCs within the local BLA circuitry, which likely underlie their different function in amygdalar operation.

P1/50

Central nervous system-specific alterations in tryptophan metabolism in 3-nitropropionic acid model of Huntington's disease

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Huntington's disease (HD) is a rare neurodegenerative disorder caused by trinucleotide repeat expansion within the gene encoding huntingtin protein. Experiments on human brain samples and on genetic rodent models of HD suggest that several neuroactive metabolites of the kynurenine pathway (KP) of tryptophan (TRP) catabolism may play a role in disease development. In this study we examined the effects of 3-nitropropionic acid (3-NP), a widely used toxin to model HD, on the concentration of TRP, kynurenine, kynurenic acid and 3-hydroxykynurenine (3-OHK) in the serum and brain of 5-month-old C57Bl/6 mice. In addition, we investigated the behavioural changes in open-field, rotarod and Y-maze tests, as well. We found increased TRP catabolism via the KP in the mirror of the elevated KYN/TRP ratios in the striatum, cerebellum and brainstem. With regard to the other examined metabolites, we found significant decrease only in 3-OHK levels in the cerebellum of 3-NP-treated mice. The open-field and rotarod tests showed that treatment with 3-NP resulted in reduced motor capabilities, which however almost totally disappeared a week after the last injection, similarly to that observed previously in most of murine 3-NP studies. However, we could demonstrate a still significant reduction in the ambulation distance of toxin-treated animals in the last 5 minutes of the open-field test with 15 minutes duration. We propose that the identified alterations could serve as applicable therapeutic endpoints for experimental drug development on delayed-type neurodegeneration in a relatively fast and cost-effective phenotypic model of HD.

P2/26

Characterization of synaptic connections between cholecystokinin-expressing interneurons and between cholecystokinin- and parvalbumin-containing interneurons in the mouse basolateral amygdala.

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The cortical parts of the amygdalar nuclei, the basolateral complex (BLA) play a key role in the formation of fear and extinction memory. During information processing in the BLA, several different types of local inhibitory interneurons may contribute to computation within the amygdalar network. GABAergic cells expressing cholecystokinin (CCK) have been shown to innervate excitatory principal cells in the BLA, however, it is currently unknown whether these GABAergic cells also target other local inhibitory cells.

To address this question, we performed electrophysiological recordings from monosynaptically coupled CCK-CCK and CCK- parvalbumin (PV) pairs. The properties of the monosynaptic connection between these interneuron types have been examined in acute slices prepared from BAC-CCK-DsRed transgenic mice and BAC-CCK-DsRed x BAC-PV-eGFP mice, respectively. Using whole-cell patch-clamp technique, action potentials were evoked in a presynaptic interneuron, while synaptic events were recorded in its postsynaptic partner, in another interneuron. We found that i) there was a large probability to find connections between CCK interneurons and between CCK and PV interneurons and ii) CCK interneurons were often reciprocally connected, while no functional connection from PV interneurons onto CCK cells has been identified. Immunocytochemical analysis showed that all CCK interneuron expressed the type-1 cannabinoid receptor (CB1). These receptors were functional at the connections between CCK cells, because, in some cases, depolarization of the postsynaptic CCK interneuron induced suppression of synaptic inputs from the presynaptic interneuron, a phenomenon that could be blocked by a CB1 receptor antagonist, AM251.

The results of these experiments suggest that, in addition to principal cells, CCK interneurons innervate interneurons expressing CCK or PV in the BLA.

P2/93

Implicit sequence learning in temporal lobe epilepsy patients with bilaterally implanted foramen ovale electrodes

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Background

Although implicit sequence learning (ISL) has been associated with front-striatal processes, recent data indicate the shared participation of the medial temporal lobe (MTL) also, despite its most prominent role in encoding and consolidation of explicit memories. Possible changes in ISL have not yet been measured in patients with mesial temporal lobe epilepsy (mTLE). The aim of the present experiment is the better understanding of the role of the MTL in ISL with the help of the Alternating Sequence Learning Task (ASRT) that consists of active sequence learning periods interrupted by passive resting unattended epochs. With this task we were able to measure two separate components of implicit learning: the sequence-specific and the general-skill learning components.

Methods and aims

All included patients (n=6) had implanted foramen ovale electrodes amended by scalp electrodes. Patients conducted the ASRT task in two steps: first in the night (session 1), followed by a 12 hour retention period, and a morning session (2). We used the difference between the high and the low frequency triplets to measure ISL. EEG was first divided into active and passive segments, followed by Fourier transformation. Differences between active and passive sections were evaluated using the frequency spectrums, and dominant frequency bands or characteristic changes were looked for. Paired T-test and ANOVA was used for statistical analysis.

Results

Our results show significant sequence specific learning and average skill learning in the first session. Sequence specific learning remained intact after the 12 hour retention period in both observed groups, however general skill learning was slightly deteriorated in the TLE group compared to healthy controls. Frequency analysis showed a varying elevation of theta and/or gamma band power during active learning compared to passive rest periods.

Conclusion

Our results show that temporal lobe epilepsy has a differing effect on certain aspects of ISL. The present experiment contributes to a more accurate mapping of the brain networks engaged in the encoding and consolidation of memories, furthermore to the assembling of a more effective cognitive rehabilitation program.

P2/94

Competition between frontal lobe functions and implicit sequence learning - evidence from the long-term effects of alcohol-dependency

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Background

According to the framework of competitive neurocognitive networks, disrupting executive functions and the related frontal areas increases performance on an implicit learning tasks. The aim of our study was to explore this relationship by investigating the effect of long term alcohol intake on implicit sequence learning. Since alcohol dependency impairs executive functions, we expected intact or even better implicit learning in the patient group compared to healthy controls based on the competitive nature of the above mentioned networks. To our knowledge, this is the first study to examine the long-term effects of alcohol dependency both on implicit learning and executive functions requiring different but partly overlapping neurocognitive networks.

Methods and aims

Fourteen alcoholic patients (11 male, 3 female) and 16 controls (11 male, 5 female) participated in the experiment. Implicit sequence learning was measured by the ASRT task (Howard and Howard 1997). Executive functions were measured by classical neuropsychological tests.

Results

Here we show weaker executive functions but intact implicit learning in the alcohol dependent group compared to the controls. Moreover we found an inverse relationship between sequence specific learning and executive functions - such that participants with lower executive functions showed higher learning performance in both alcohol dependent and control groups.

Conclusion

Our results confirm the competitive relationship between the fronto-striatal networks underlying implicit sequence learning and executive functions and suggests that the functional integrity of this relationship is unaltered in the alcohol dependent group despite of the weaker frontal lobe functions. Our results shed light on the different or partly overlapping fronto-striatal networks which have different roles in implicit processes and executive functions moreover showing a competitive relationship among them.

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Synaptic mitochondria: a brain mitochondria cluster with a specific proteome

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The synapse is a specifically important compartment of neurons in metabolic sense so we posed the question how far the protein composition of synaptic mitochondria reflects its functions. To reveal the potential molecular differences we isolated synaptic (sm) and non-synaptic mitochondria (nsm) from mouse brain, then validated their purity with electron microscopy and functional assay based fluorescence activated cell analysis and sorting (FACS). Two-dimensional differential gel electrophoresis and mass spectrometry based proteomics experiment revealed 22 proteins of significantly higher and 34 proteins of significantly lower level in sm compared to nsm. Expression differences in some tricarboxylic acid cycle proteins, such as isocitrate dehydrogenase subunit alpha (Idh3a) and ATP-forming β subunit of succinyl-CoA ligase (SucIA2) were verified by Western blot and immunohistochemistry. The data suggest an altered tricarboxylic acid metabolism in the energy supply of synapse. Further functional clustering demonstrated that proteins with higher synaptic levels are involved in pathways including synaptic transmission, lactate and glutathione metabolism. In contrast, mitochondrial proteins associated with glucose, lipid, ketone metabolism, signal transduction, morphogenesis, protein synthesis and transcription were enriched in the nsm. The protein expression difference pattern was in accordance with already known functional specialties of sm. The marked differences in complement component 1 Q subcomponent binding protein (C1qbp) and alpha-synuclein (Snca) support the high sensitivity of synapses to oxidative stress. The changes in the composition of

mitochondria in the synapse can contribute to its altered functions including increased efficiency of energy production but also a concomitantly elevated vulnerability to oxidative stress.

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Conductance measurements and simulations of electrical fields generated by focused transcranial electrical stimulation (TES)

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Transcranial electrical stimulation can modulate the ongoing brain activity. The efficacy and safety of TES is determined by the strength of the generated electrical fields at tissue. However, these electrical gradients in the brain are also influenced by numerous factors (e.g. homogeneity and impedance of the tissues, etc), thus the in silico predictability of intracranial gradients generated by TES is weak. In principle, TES can be also spatially selective (as deep brain stimulation is) by limiting the extent of the electrical field gradient. In our experiments, we set out to measure the TES generated electrical fields and to test the possibility of a spatially focused TES protocol. Our assumption is that the effect of repetitive delivery of high frequency (>1 kHz) Gaussian pulses on consecutive diagonal electrode pairs may be temporally integrated by the neurons, leading to a stronger neuronal entrainment at the cross point of the diagonal gradients than at the periphery.

We recorded TES-generated field potentials in human cadavers and anesthetized rats. Stimulation was applied by placing Ag/AgCl EEG electrodes over the external surface of the skull. We used independently isolated stimulation pairs to deliver sinusoidally modulated TES with various parameters and repetitive high-frequency Gaussian stimulus trains in two different arrangements (linear and crossed alignment). Custom made multiple-site electrodes (>200 contact points) were used to thoroughly sample the field potentials in the brain. We also measured the shunting effect of the skin and the connective tissue during transcutaneous stimulation in contrast to the stimulation directly through the skull.

In addition to our earlier results, we found that the skin dramatically reduces the generated intracranial electrical gradients, and alters its geometry. The high-frequency pulsed stimulation generates a relatively small diameter axial voltage gradient in the geometrical axis of the stimulator electrodes (<50% gradient strength off-axis vs in-axis). We also found that our multiple stimulation pairs protocol can result a spatially focal effect after temporal integration (>30% larger gradient strength at the crosspoint than at the periphery).

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Unilateral vestibular lesion modifies the tenascin-R expression in the perineuronal nets of the vestibular nuclei in the rat

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The vestibular system or system of balance provides information about the motion, equilibrium, and spatial orientation of the body. Lesion of vestibular system results in various static and dynamic symptoms which are restored spontaneously during the vestibular compensation. Vestibular compensation involves multiple, parallel mechanisms in the vestibular nuclei and in various parts of vestibular networks. We have previously observed modification of hyaluronan and chondroitin sulfate proteoglycan expression in the perineuronal net (PNN) of the lateral vestibular nucleus (LVN) during the vestibular compensation suggesting the role of extracellular matrix in

the compensatory mechanisms. In the present work we studied the consequences of unilateral labyrinthectomy (UL), model of vestibular lesion, on the expression pattern of tenascin-R (TN-R) in the vestibular nuclei of the rat. Under anesthesia, the left vestibular receptors were mechanically destroyed and immunohistochemical reaction for TN-R was performed on the vestibular nuclei after 1, 3, 7 and 14 days of survival.

In the control animals the TN-R immunoreactivity was intense in the PNN of each vestibular nucleus, except the caudal part of descending vestibular nucleus (DVN) which showed weaker staining. During the postoperative days 1 and 3 the TN-R staining decreased in the superior (SVN) and LVN vestibular nuclei and in the rostral part of DVN on the operated side, whereas the immunohistochemical reaction in the unoperated one remained normal. The staining intensity returned to the control level by the 7th postoperative day in the SVN, and by the 14th days in the LVN and the rostral part of DVN. The staining intensity has not been modified in the medial vestibular nucleus during the postoperative period studied.

Our results showed that the UL is accompanied by the modification of TN-R staining pattern in the vestibular nuclei. The time course of re-establishment of PNN is being attained parallel to the improvement of vestibular symptoms.

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Microglial fractalkine signaling is involved in hypothalamic control of glucose homeostasis

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Energy homeostasis is a sensitive process regulated by the central nervous system. There is an emerging role of glial cells in sensing and mediating of various homeostatic functions. Our aim was to reveal the role of hypothalamic microglia in the regulation of energy homeostasis focusing on state of negative energy balance (fasting, insulin-induced hypoglycemia).

Homozygote CX3CR1gfp/gfp transgenic mice, in which a certain part of the CX3CR1 (fractalkine receptor) gene was replaced by a green fluorescent protein (GFP) and their wild type controls (C57Bl6/J) were used. Animals were fasted overnight and sacrificed 1 hour after intraperitoneal insulin injection (1 IU/kg) in the next morning. Non-fasted and fasted, control animals received vehicle injection.

Mice which are deficient in fractalkine signaling between neuron and microglia did not develop hypoglycemia in response to insulin load after overnight fast. c-fos is an immediate-early gene that is expressed rapidly and transiently within the challenged neurons. Using c-Fos based functional anatomical mapping strategy we identified distinct cellular activation patterns in the hypothalamus of C57Bl6/J vs. CX3CR1gfp/gfp mice following insulin-injection. c-Fos positive neurons were counted in the arcuate nucleus (ARC) of the hypothalamus. ARC plays a crucial role in the regulation of food intake and energy homeostasis. In ARC we observed significantly less c-Fos positive neurons in CX3CR1gfp/gfp mice, than in wild type mice. Furthermore, using double immunocytochemistry we have identified activated neurons (by c-Fos) and activated microglia by ionized calcium-binding adaptor protein-1 (IBA-1). We have revealed recruitment of microglia to hypoglycemia- activated arcuate neurons.

Our results highlight the role of microglia in general-, and fractalkine signaling in particular, mediating hypoglycemic challenges to the hypothalamic neuronal circuit that regulates energy homeostasis.

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Secretagogin in the rat brain stem

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Calcium-binding proteins protect against excess intracellular calcium ion levels or act as sensor proteins thereby regulating downstream cascades. Secretagogin, a recently described calcium sensor protein, has been identified in several organs including brain. Whilst the distribution pattern of secretagogin-containing neurons in the forebrain is mostly known, the expression pattern of this calcium-binding protein in the brainstem has been largely neglected. Here, we show that secretagogin is present in the rat brainstem with a distinct distribution pattern

largely concentrated in noradrenergic centres. Additionally, secretagogin-containing neurons were identified in rather heterogeneous fields including, but not restricted to, superior colliculus, dorsal nucleus of vagus, medial vestibular nucleus or interpeduncular nucleus. Tyrosine hydroxylase-containing neurons typically co-express secretagogin both in their somata and axonal fibres. In stress, we identified a local increase of secretagogin expression in the locus coeruleus as shown by micropunch-technique based protein analysis. We suggest that secretagogin is a useful neurochemical marker to identify neuronal subsets in the mammalian brain stem and could play a role in stress reactions.

P2/95**The relationship between sleep and memory in childhood**

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There are several studies proving the relationship between sleep and memory. However a relatively small number of studies investigated this relationship in children in spite the fact that sleep promotes brain plasticity and is critical for children's cognitive development. The aim of our study was to investigate declarative and non-declarative memory in children with sleep-disordered breathing (SDB). We used a story recall task to measure declarative memory and Alternating Serial Reaction Time (ASRT) task to assess non-declarative memory. This latter enables us to measure two aspects of non-declarative memory, namely general skill learning and sequence-specific learning. There were two sessions: a learning phase and a testing phase, separated by a 12-hour offline period with sleep. Polysomnography was used in order to record sleep parameters. Our results showed that children with SDB exhibited a generally lower declarative memory performance both in the learning and testing phase, but the consolidation during the offline period was similar among the groups. In addition, we observed association between snoring events and declarative memory performance. The memory performance could further associate with other sleeping characteristics, so we examined the EEG data in detail. In contrast we showed intact non-declarative learning and consolidation in SDB group in both sequence-specific and general skill. In order to investigate the underlying memory processes we analyzed the within block position effect which enable to separate hippocampal and striatal dependent memory processes. Here we show that children with SDB demonstrated weaker performance in the former index compared to the control indicating disturbed hippocampal functions. Our findings suggest that disruption of sleep in childhood has a differential effect on different memory types (declarative vs. non-declarative) and stages (online vs. offline).

Keywords: declarative memory, non-declarative memory, sleep, development, sleep disordered breathing