

PL1. The Mirror Neuron Mechanism and Its Role in Understanding Others

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Mirror neurons have been described for the first time twenty years ago in the monkey ventral premotor cortex. In the first part of my talk, I will review the basic functional properties of monkey mirror neurons and discuss recent data on the mirror mechanism. I will describe first their *motor* properties. I will show that, as most neurons in the premotor cortex, mirror neurons code the *goal* of a motor act. I will review then their *visual* properties showing that mirror neurons represent a mechanism that allows a direct understanding of *what* the agent is doing. I will present then evidence that humans possess the mirror mechanism and that the anatomical location of mirror networks of the monkeys and of humans closely coincide. I will discuss subsequently the role of the mirror mechanism in emotions and in encoding the “vitality affects” of the observed actions. I will conclude discussing some implications of these findings for autistic spectrum disorder.

S1. Novel insight into fear-mediated structural plasticity at GABAergic synapses of the basolateral amygdala

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Learning and memory have been interpreted for years in the framework of Hebbian plasticity. However, recent accumulating evidence indicates that synaptic networks are also structurally plastic. Structurally plasticity has been reported for different learning paradigms and was principally observed at glutamatergic synapses. In associative fear learning both an increased rate of spine elimination in the medial prefrontal cortex as well as an increase in the volume of spines in the lateral nucleus of the amygdala have been described.

Only in the last year new compelling evidence has shown that altered sensory experience induces remodeling of spatially clustered inhibitory synapses and spines in the visual cortex. A number of studies have also shown that Pavlovian fear conditioning requires a reduction in GABA transmission, which was explained as a reduction in GABAA receptor expression.

We have undertaken a combined anatomical and physiological approach to examine at the synaptic level whether these alterations actually take place and distinctively involve specific GABAA receptors. We analyzed by means of the freeze-fracture replica immunolabelling technique (SDS-FRL) the density of the $\gamma 2$ and $\alpha 2$ GABAA subunits in the BA of mice that underwent fear conditioning as well as extinction. SDS-FRL also allowed to precisely measure the size of GABAergic synapses. We found a significant decrease in synaptic labelling density for the GABAA- $\gamma 2$ subunit, but not for the $\alpha 2$ subunit, in fear conditioned mice, whereas extinction reversed it back to control levels ($p < 0.01$; Kruskal-Wallis test). The average size of GABAergic synapses in control mice was $0.038 \pm 0.022 \mu\text{m}^2$ (mean $\pm$ sd; $n = 344$ full synapses from 4 animals). Notably, fear conditioned animals showed a significant shift in the frequency of synapses with larger area ($p < 0.001$, Kolmogorov Smirnov Z test; $n = 396$), whereas in fear extinction mice the frequency distribution of the area of GABAergic synapses was similar to controls ($p = 0.49$; $n = 432$). Analysis of $\alpha 2$ -labelled synapses confirmed the same pattern.

Alterations in synapse size upon fear conditioning and extinction were associated with functional changes. Miniature inhibitory postsynaptic currents (mIPSCs) recorded in slices obtained from fear conditioned animals were larger (increased charge transfer) compared to recordings from control animals and from animals subjected to extinction training.

Our results indicate that, in the BA, fear conditioning produces a structural and functional remodelling of GABAergic synapses that is specifically reversed by extinction training.

S2. Hippocampal and somatosensory inputs to the basolateral amygdala: role of identified GABAergic neurons

Marco Capogna

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I will focus on the contribution of specific GABAergic cell types of the amygdala to hippocampal network theta oscillation and somatosensory noxious stimulation. Theta rhythm (4-10 Hz) occurs during REM sleep, arousal, locomotion, mnemonic process, and can be partially disrupted during neurological and psychiatric diseases. I will show that different types of GABAergic cells of the basolateral amygdala are active at different phases of neuronal theta oscillations thereby controlling the activity of postsynaptic cells at different times (Bienvenu et al., Neuron, 2012). To define the cellular network underlying these hippocampal-amygdala interactions, I will show the responses of BLA neurons to optical stimulation at theta frequency of channelrhodopsin-expressing fibres originating from the ventral CA1 hippocampus. I will also show that various types of GABAergic neurons of the basolateral amygdala are activated by noxious somatosensory stimuli that represent salient events that reproduce stimuli used in fear conditioning experiments. The main message of the talk is that distinct types of GABAergic cells of the BLA contribute to the integration of hippocampal theta oscillations and salient stimuli in a cell type-specific manner.

S3. Structural and functional organization of inhibitory microcircuits in the basolateral amygdala

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The cortical part of the amygdalar nuclei, the basolateral amygdala complex (BLA) is one of the key structures in the brain, which region has an essential role in fear and extinction memory formation. Microcircuits of the BLA are composed of principal glutamatergic neurons, which give rise to the output of this structure, and of GABAergic interneurons. During information processing, local inhibitory cells provide an effective control of principal cell function, thereby shaping the signal transfer to downstream areas. The main aim of this presentation is to show how the distinct types of GABAergic interneurons are embedded into the local neuronal networks, and how the individual inhibitory cells affect the principal cell operation at single cell levels. To this end, we combine neuroanatomical techniques with in vitro electrophysiological recordings. Our results show that distinct type of interneurons, synapsing on the different somato-dendritic membrane surface of principal cells, very efficiently control the spiking of postsynaptic excitatory neurons. Our investigations are the first attempts to clarify the local connectome in the BLA microcircuits, which should gain insights into the understanding how this cortical circuit operates locally.

S4. Dendritic inhibition protects hippocampal output during fear learning

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Fear memories guide adaptive behavior in contexts associated with aversive events. The hippocampus forms a neural representation of the context that predicts such aversive events. Representations of context incorporate multisensory features of the environment, but must somehow exclude sensory features of the aversive event itself. Here we investigate the mechanisms of this selectivity, using cell type-specific imaging and inactivation in hippocampal area CA1 of behaving mice. We find that aversive stimuli activate CA1 dendrite-targeting interneurons via cholinergic input, leading to inhibition of pyramidal cell distal dendrites that receive aversive sensory excitation. Inactivating dendrite-targeting interneurons during aversive stimuli increases CA1 pyramidal cell population responses and prevents fear learning. We propose subcortical activation of dendritic inhibition as a novel mechanism for selective exclusion of aversive stimuli from hippocampal contextual representations during fear learning.

PL2. Cellular and molecular basis of spinal pain control

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The spinal dorsal horn constitutes the first site of synaptic integration in the pain pathway. At this site, a local network of inhibitory and excitatory interneurons serves as a “gate control” unit for incoming somatosensory and nociceptive signals. In particular inhibitory interneurons serve a pivotal function by controlling the output of dorsal horn neurons to higher CNS areas, by limiting spontaneous activity in the dorsal horn and by keeping different sensory modalities apart. Although a huge amount of data have firmly established these critical roles, it has not been possible to exploit this concept for the treatment of pain. In a series of experiments involving GABA-A receptor point mutated mice, we could demonstrate that targeting a specific subtype of GABA-A receptors (those containing the $\alpha 2$ subunit) allows to reverse pathological pain in mouse models of inflammatory and neuropathic pain while avoiding typical side-effects of classical GABAergic drugs such as sedation and tolerance development (Knabl et al. *Nature* 2008). The antihyperalgesic effects of $\alpha 2$ -GABA-A receptors occur exclusively through a spinal site (Witschi et al., *J Neurosci* 2011; Paul et al., *Neuropsychopharmacology* 2014) and fit nicely with the strategic localization of these receptors in the spinal dorsal horn (Paul et al., *J Comp Neurol*, 2012). In more recent studies, we have addressed the identity of the inhibitory interneuron subtypes of the dorsal horn neuronal network. Using genome-wide expression profiling of dorsal horn tissue obtained from mice lacking dorsal horn interneuron populations we identified previously unknown marker genes for distinct sets of dorsal horn interneurons (Wildner et al., *J Neurosci*, 2013). In order to study their function, we established viral techniques allowing diphtheria toxin-mediated ablation, tetanus toxin-mediated silencing, and DREADD-dependent activation of defined dorsal horn interneuron populations. This armamentarium will greatly facilitate the analysis of the function of the dorsal horn circuitry and of its malfunctioning in chronic pain states.

S5. Successful spinal cord regeneration in adult zebra fish

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In contrast to mammals, adult zebrafish regenerate different neuronal cell types from local progenitor cells in the lesioned spinal cord. We have been studying the cellular and molecular key events following a lesion. Ependymo-radial glial progenitor cells, with a soma contacting the central canal and endfeet at the pial surface, are quiescent in the unlesioned spinal cord and start to proliferate after a lesion. Different domains of ependymo-radial glial cells then generate motor neurons and a range of interneuron cell types, re-deploying developmental signals, such as sonic hedgehog. Using drug screening strategies and expression profiling of highly purified progenitor cells in embryos, we have identified novel signals that promote motor neuron generation during development and adult regeneration. Some of these signals are derived from descending axons, such as dopamine (Reimer et al., *Dev. Cell*, 2013, 25, 478-91), which acts by augmenting hedgehog signalling. We find an unexpectedly large array of long-range and local signals interacting to promote development and regeneration of different neuronal cell types in the spinal cord. Hence, the zebrafish model is ideally suited to discover signalling pathways that are active during spinal cord differentiation and neuronal regeneration in a vertebrate, which may ultimately be translatable to mammals.

S6. The role of serotonin in locomotor recovery after spinal cord injury in rats

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Serotonergic neurons located in the medulla and projecting to the spinal cord are able to initiate locomotor activity by action actions on motoneurons and on coordinating interneurons of the locomotor CPG. Thus the medullary serotonergic nuclei play multiple roles in the control of locomotion, and they must be differentially controlled to be effective. Replacement of serotonergic neurons by grafting must allow for such differential control, and knowledge of the specific defined populations for each functions served by the serotonergic descending system is required to achieve this. Serotonergic neurons destined to form specific defined populations with distinct roles in the control of locomotion must be included in the grafts, and they must project to the appropriate target neurons in the sublesion spinal cord. Their actions are normally mediated by a limited number of 5-HT receptors, including 5-HT₂ and 5-HT₇ receptors. We grafted various combinations of fetal serotonergic neurons destined to become specific defined populations of the descending serotonergic system after spinal cord injury, and found that the capacity to reinnervate crucial parts of the spinal cord locomotor system differed depending on the source of the grafted cells. Reinnervation of both motoneurons and CPG neurons was essential for recovery, and reinnervation of other targets was not effective, even though such grafts presumably provided considerable release of serotonin.

S7. The role of serotonin in locomotor recovery after spinal cord injury in rats

Larry Jordan

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S8. New perspectives for the treatment of spasticity and chronic pain after spinal cord injury

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Most spinal cord injury (SCI) patients suffer from spasticity and chronic pain. This is a major therapeutic challenge since medical therapies often are ineffective. It is now widely accepted that spasticity results from profound modifications of both the intraspinal inhibitory synaptic transmission and the electrical properties of neurons. We identified the mechanism responsible for a reduced inhibition within the networks below the lesion. Briefly, a low $[Cl^-]$ is a pre-requisite for inhibition mediated by GABA and glycine to occur. This concentration is maintained at low levels in healthy adult neurons by K-Cl co-transporters (KCC2), which extrude Cl^- ions. The expression of KCC2 is down-regulated at the level of both motoneurons and laminae I-II neurons after SCI, thereby causing spasticity and pain, respectively. Concomitantly, the excitability of neurons is increased due to the up-regulation of voltage-dependent persistent inward currents (PICs). Two important mechanisms characterize these PICs: they require long depolarizations to fully activate and they are inactivated by hyperpolarization of motoneurons. We showed that the down-regulation of KCC2 and the up-regulation of PICs play a synergistic role in the physiopathology of spasticity. Targeting KCC2 and sodium persistent currents open new avenues for the treatment of both spasticity and chronic pain after SCI.

S9. Recovery and regeneration in the injured rodent cord induced by grafted stem cells: multiple mechanisms

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Spinal cord contusion injury leads to severe loss of grey and white matter and subsequent deficit of motor and sensory functions below the lesion. The murine clonal embryonic neuroectodermal stem cell (NE-GFP-4C) line has been shown to prevent the loss of spinal motoneurons after avulsion injury. Here we studied the effect of these cells on the injured spinal cord following contusion injury. We grafted NE-GFP-4C cells intraspinally or applied them intravenously immediately or one week after thoracic (T11) spinal cord contusion injury. Control animals received physiological saline intraspinally or fibrin one week after injury. Stem cells applied either locally or intravenously induced significantly improved functional recovery accompanied by considerable morphological reorganization and regeneration compared with control animals. The stem cells integrated into the host tissue and differentiated to neurons, astrocytes and oligodendrocytes. In intraspinally grafted animals corticospinal tract axons regenerated along the ventral border of the cavity and have grown several millimeters below the lesion. The extent of regeneration and functional improvement was inversely related to the amounts of chondroitin sulphate and ephrin-B2 molecules found around the cavity and to the microglial and astrocytic reactions in the injured segment early after injury. Our data suggest that the grafted stem cells prevent the secondary damage and are able to replace some of the lost cells.

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PL3. Dendritic integration in cortical neurons in vivo

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Neurons in sensory regions of the cortex receive sensory modality-specific information through synapses that are located on their dendrites. The use of two-photon microscopy combined with whole-cell recordings has helped identify visually-evoked dendritic calcium signals in mouse visual cortical neurons *in vivo*. The calcium signals were restricted to small dendritic domains ('hotspots') and represented visual synaptic inputs that were highly-tuned for orientation and direction (Jia et al., Nature, 2010). An improved variant of two-photon imaging with a better sensitivity for fluorescence signaling and a reduced risk of phototoxicity, termed LOTOS (= low power temporal oversampling), allowed the detection of sensory-evoked calcium transients in dendritic spines of mouse neurons in the auditory cortex (Chen et al., Nature, 2011). Individual spines on the same dendrite were functionally heterogeneous. Even neighboring spines were often tuned to different sound frequencies. In line with these observations, we found in the vibrissal cortex that stimulation of different whiskers activated distinct spines on the same dendrite (Varga et al., PNAS, 2012). Some spines were activated uniquely by single whiskers, but many spines were activated by multiple whiskers. These shared spines indicate the existence of presynaptic 'feeder' neurons that integrate and transmit activity arising from multiple whiskers. Together, our results suggest that in different cortices (visual, auditory, vibrissal), afferent sensory inputs to layer 2/3 neurons are widely distributed throughout the entire dendritic tree in a 'salt-and-pepper'-like manner and that the neurons use these complex input signals to compute their output signals in a cell-specific manner.

S10. Shedding light on the functional anatomy of presynaptic active zones

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The majority of rapid cell-to-cell communication mechanisms and information processing within the nervous system makes use of chemical synapses. Thereby, the molecular organization of presynaptic active zones, the places where neurotransmitter filled synaptic vesicles get released, is a focus of intense investigation. We recently identified two key scaffold proteins for presynaptic active zone organization, Bruchpilot (BRP) and Rim-binding protein (RBP) (1, 2), which are essential for structural organization and efficient neurotransmitter release at active zones in *Drosophila*. To overcome the resolution limit of standard light microscopy precluding the study of sub-synapse organization, we use super-resolution light microscopy (stimulated emission depletion microscopy, STED). Thus, functional molecular architectures connecting BRP and RBP with Ca²⁺ channels and release machinery could be studied. Our group moreover established protocols to directly visualize protein dynamics during synapse assembly and plasticity in living intact larvae over extended periods. Thus, we could characterize mechanisms of trans-synaptic signaling connecting assembly of the presynaptic active zone scaffold with postsynaptic assembly (3).

1. K. S. Liu *et al.*, *Science* **334**, 1565 (Dec 16, 2011).
2. R. J. Kittel *et al.*, *Science* **312**, 1051 (May 19, 2006).
3. D. Oswald *et al.*, *Nat Neurosci* **15**, 1219 (Aug 5, 2012).

S11. Imaging the plasticity of living synapses at the nanoscale by two-photon excitation STED microscopy

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Neuronal synapses are complex and dynamic structures ensheated by glia processes, forming elementary computational units for fast and flexible communication in the brain. Understanding how synapses are modified by neural activity and experience is a central challenge for neuroscience. Because they are so small and deeply embedded in dense brain tissue, they are a tough act to study by conventional light microscopy.

I will review our progress in developing and applying STED microscopy for uncovering and deciphering nanoscale neuro-anatomical rules for the function and plasticity of neurons and glia cells in living brain slices, specifically: 1) nanoscale imaging up to 100 μm below tissue surface in acute brain slices based on two-photon excitation STED microscopy (TPE-STED); 2) spine structure – synaptic plasticity analysis based on a combination of STED, FRAP experiments, electrophysiology and 2-photon glutamate uncaging; 3) plasticity of axon morphology during LTP and its impact on AP propagation.

S12. Postsynaptic molecular organization and dynamics at single living synapses resolved by PALM microscopy

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The internal organization of the postsynaptic density (PSD) critically determines the characteristics of excitatory transmission. A particularly important aspect of PSD organization is the subsynaptic positioning of AMPA-type glutamate receptors (AMPA-Rs), because the subset of receptors apposing a site of presynaptic release determines the strength of the synaptic response. Additionally, scaffold-mediated positioning of adhesion, signaling, and cytoskeletal proteins controls diverse aspects of synaptic function and plasticity. Measuring PSD structural organization in living neurons has been challenging because the small dimensions of the synapse essentially lie beyond the resolution of conventional imaging techniques. To gain direct insight to nanoscale architecture of living synapses, we have used live-cell photoactivated localization microscopy to map molecular families within single PSDs. Achieving a protein map resolution of ~25 nm, we find that scaffold proteins in individual PSDs are not homogeneously distributed, but instead accumulate in dense, subsynaptic clusters. These intrasynaptic protein ensembles undergo striking changes over time. Importantly, the dense subdomains of PSD-95 are enriched in both AMPA and NR2B-containing receptors. Chronic suppression of activity triggered changes in PSD interior architecture that may amplify homeostatic synaptic plasticity. Thus, modulation of subsynaptic alignment may directly regulate the strength of neurotransmission.

S13. Cell type- and synapse-specific molecular imaging in brain circuits by STORM microscopy

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Mechanistic understanding of biological processes requires integrated analysis of structural and functional parameters together with their underlying molecular dynamics. This is especially challenging to implement in complex brain circuits, where the density and position of signaling molecules varies at the nanoscale level in a compartmentalized manner. A prime example is retrograde endocannabinoid signaling, which is used by neurons to dynamically regulate their synaptic inputs. Although its strength is remarkably input-specific, the underlying mechanisms remain unresolved due to difficulties in measuring molecular and anatomical parameters in a cell type-, and synapse-specific manner. Therefore, we developed a rapid and versatile approach, which combines *in vitro* or *in vivo* physiological and anatomical characterization with quantitative molecular imaging at the nanoscale. We found both common and cell type-specific principles, which govern CB₁ cannabinoid receptor numbers and their nanoscale distribution on axon terminals of perisomatic and dendritic hippocampal GABAergic interneurons. Chronic Δ^9 -tetrahydrocannabinol administration, which reduces cannabinoid potency on GABA release, also evokes dramatic CB₁-downregulation and redistribution in identified boutons. Thus, nanoscale molecular organizational rules tune synaptic endocannabinoid signaling and contribute to the amnesic effects of cannabis use.

PL4. Molecular interrogation of endocannabinoid signaling in relation to cannabis sensitivity of the developing nervous system

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Besides acting as retrograde messengers in the adult nervous system, endocannabinoids have recently emerged as modulators of neuronal development. Available data furnish the hypothesis that a continuum of endocannabinoid actions overarches the differentiation and postnatal activity-dependent modulation of particular cortical synapses. We have recently dissected the anatomical organization of endocannabinoid signalling during nervous system development, characterized its roles in neurogenesis, cell migration, and axonal growth and guidance, and identified key molecular hubs for the upstream control of endocannabinoid signaling in vertebrate and invertebrate model systems. Moreover, we demonstrated that Δ^9 -tetrahydrocannabinol (THC) impacts a cannabinoid receptor-sensitive protein network to disrupt cytoskeletal organization in developing neurons, thus imparting permanent structural deficits in the cerebral cortex. Here, I will review available evidence from experimental models and human fetal brains (particularly weeks 18-22 of pregnancy) on a key role for endocannabinoid signaling in the developmental organization of the cerebral cortex, including neuronal diversification and network wiring. Next, I will emphasize the hierarchical interactions of endocannabinoid signaling with other “classical” signaling cassettes known to promote neuronal differentiation. Lastly, I will show that THC exposure erroneously times CB₁ cannabinoid receptor (CB₁R) activation to introduce wiring errors in the fetal cortical circuitry. I will characterize Superior Cervical Ganglion 10 (SCG10)/stathmin-2, a tubulin-binding protein in axons, as a molecular substrate, whose degradation *via* c-Jun N-terminal kinases-mediated phosphorylation limits microtubule instability. Overall, the data presented here will highlight novel roles for endocannabinoids in the nervous system, identify key sites of neuronal vulnerability to phytocannabinoids and synthetic ligands affecting endocannabinoid availability or CB₁R activity, and define the maintenance of cytoskeletal dynamics as a first-order molecular target for cannabis, impairing synaptic connectivity within cortical neuronal circuitries of affected offspring.

S14. HyaluronanBased ECM-Implications for Physiological Functions

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Background:

Specific types of neurons in the central nervous system are ensheathed by specialized extracellular matrix (ECM) components called perineuronal nets (PN) or perisynaptic matrix of axonal coats (AC). PNs and ACs are discussed to be involved in the modulation of synaptic activity. However, the function of this specialized ECM remains elusive. The medial nucleus of the trapezoid body (MNTB) is a promising nucleus for investigating the role of ECM in synaptic physiology for two reasons: (i) virtually all neurons in the MNTB are associated with perineuronal and perisynaptic ECM, and (ii) the principal neurons of the MNTB are targeted by huge axosomatic terminals, the calyces of Held, enabling experimental access to both the pre- and postsynaptic membrane. The ECM in the MNTB is characterized by a unique organization of the matrix component brevican, which forms a ring-like structure around presynaptic terminals and seems to seal the synaptic cleft, while other ECM components (like aggrecan) surround the entire cell body. Due to this prominent association with synaptic terminals, we assume that brevican is essential for the fast and reliable synaptic transmission at the calyx of Held.

We performed extracellular single-unit recordings at the calyx of Held in the MNTB *in vivo* in subadult wildtype mice and transgenic mice lacking the ECM component brevican. This approach enables simultaneous acquisition of pre- and postsynaptic activity and thus, allows for quantification of dynamics and reliability of synaptic transmission.

We found a significant change in the dynamics of synaptic transmission at the calyx of Held in brevican-deficient mice compared to wildtype animals. In detail, the pre- to postsynaptic AP transmission delay was prolonged and the postsynaptic AP was broader in brevican-deficient mice. The reliability of synaptic transmission was not changed in brevican-deficient mice.

Our results indicate that the ECM component brevican promotes the high speed of synaptic transmission at the calyx of Held, which is essential for the function of the MNTB within the sound localization circuit. These findings further support our general theory that perineuronal and perisynaptic matrix vitally modulates synaptic activity.

Fundings:

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S15. RAGE-mediated cell signalling in health and disease

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RAGE (receptor for advanced glycation end products) was initially isolated as a receptor for AGEs (advanced glycation end products) that accumulate in diabetes and during senescence. RAGE is a multi-ligand receptor that belongs to the immunoglobulin superfamily of transmembrane proteins. In addition to AGEs, RAGE binds HMGB1 (high-mobility group box-1; also designated as amphoterin), members of the S100 protein family, glycosaminoglycans and amyloid β peptides. Recent studies using tools of structural biology have started to unravel common molecular patterns in the diverse set of ligands recognized by RAGE. The distal Ig domain (V1 domain) of RAGE has a positively charged patch, the geometry of which fits to anionic surfaces displayed at least in a proportion of RAGE ligands. Association of RAGE to itself, to HSPGs (heparan sulfate proteoglycans) and to Toll-like receptors in the cell membrane plays a key role in cell signaling initiated by RAGE ligation. Ligation of RAGE activates cell signaling pathways that regulate migration of several cell types. Furthermore, RAGE ligation has profound effects on the transcriptional profile of cells. AMIGO (**a**mphoterin-induced **g**ene and **o**rf) was found as a major transcript upregulated by ECM-bound HMGB1 or by anti-RAGE antibodies. AMIGO associates to the potassium channel Kv2.1 and participates in regulation of neuronal excitability.

S16. Role of the Perisynaptic Extracellular Matrix in Synaptic Plasticity

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During brain maturation, the occurrence of the mature extracellular matrix (ECM) terminates juvenile plasticity. It consists of proteoglycans and glycoproteins of glial as well as neuronal origin that form a condensed meshwork around its backbone, hyaluronic acid. Interestingly, enzymatic removal of the ECM restores juvenile forms of plasticity, as for instance demonstrated by topographical re-connectivity in sensory pathways. Complementary, synaptic plasticity is fundamental for learning and memory formation in the adult. Henceforth, to which degree the mature ECM is a compromise between stability and flexibility for adult neuronal circuits and might thereby impact new learning and higher cognitive functions is virtually unknown. In our work we investigate the impact of the ECM on plasticity in the mature brain on the molecular, cellular and systemic level. We found that ECM is remodeled in response to synaptic activity. In addition, ECM removal lead to altered glutamate receptor mobility and trafficking. On the systemic level we found that ECM removal within sensory cortex increased cognitive flexibility required for reversal learning of previously acquired behavioral habits. Thus, our findings suggest experimental modulation of the cortical ECM open short-term windows of enhanced activity-dependent reorganization allowing for guided neuroplasticity.

S17. Compensation Following Vestibular Disorder: Possible Role of the Extracellular Matrix

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Vestibular disorders evoke static and dynamic deficits which are spontaneously restored during the vestibular compensation. Since the lesioned vestibular axons do not regenerate in mammals, the vestibular compensation is attributed to the plasticity of vestibular neuronal networks. On the other hand, morphological and functional regeneration is possible in lower vertebrates. Recent data showed the importance of extracellular matrix (ECM) in the regeneration and plasticity of CNS. However, the possible role of ECM in the normal function and disorders of vestibular system is still unknown. The aim of study was to examine whether (1) the vestibular lesion is accompanied by the changing of ECM expression in the vestibular nuclei and (2) the different regenerative capability of various species is associated with the different molecular composition of ECM. We have found modification in the ECM staining in the vestibular nuclei bilaterally and the perineuronal net (PNN) was not recognizable in the vestibular-lesioned animals on the first postoperative day. The ECM staining gradually reappeared and the time course correlated with the amelioration of postural deficits and restoration of the normal resting discharge of deafferented vestibular neurons. Our findings suggest that the HA-based PNN in frog versus the aggrecan-based PNN in rat may be associated with the high degree of plasticity and regenerative potential of non-mammalian species.

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P1. Functional Characterization of Genetically Identified Cholinergic Neurons in the Pedunculopontine Nucleus of Mice

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The pedunculopontine nucleus (PPN) is one of the cholinergic nuclei of the reticular activating system. The nucleus is thought to have a role in transitions of slow wave sleep and REM sleep or wakefulness. The cholinergic cells of the PPN do not form a heterogeneous population: previous studies defined several subgroups. In this study we characterize functional properties of genetically identified cholinergic cells and group them according to their spontaneous action potential firing frequency.

Whole-cell patch clamp recordings were done on slices from mice which express tdTomato fluorescent protein in a choline acetyltransferase (ChAT) dependent way.

All cholinergic cells had A-current (transient potassium current) and displayed high threshold oscillations. 66% of the neurons fired action potentials spontaneously, with a frequency between 0.07 and 3.12 Hz and with regular, irregular or clustered firing pattern.

Neurons were divided into two subgroups due to their spontaneous action potential firing: the ones between 0-1 Hz and the ones above 1 Hz. Cells with higher firing rate have significantly lower resting membrane potential (-59 ± 1.1 vs. -55 ± 1.2 mV), higher amplitude of depolarizational sag to hyperpolarizing current injections (-30.5 ± 3 vs. -14.6 ± 6 mV) and the amplitude of the A-current (674 ± 90 vs. 407 ± 99 pA).

The two subgroups described here might correlate with groups of cholinergic cells based on their in vivo firing properties.

P2. Modulation of Action Potential-Triggered Dendritic Calcium Signals by Local Membrane Parameters in Dentate Gyrus Granule Cells

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Local dendritic calcium signals are fundamental for dendritic functions and are subject to several modulatory mechanisms. Using a combined electrophysiological and confocal imaging approach we found that activation of dendritic GIRK channels surprisingly increased (instead of inhibition) backpropagating action potential (bAP)-associated the local Ca^{2+} signals in the dendrites of dentate gyrus granule cells (GCs). In addition, somatic hyperpolarization significantly increased bAP-associated Ca^{2+} signals in the proximal dendrites largely independently of T-type Ca^{2+} channel activation. Dual somato-dendritic recordings showed that somatic hyperpolarization accelerated the repolarization phase of proximal dendritic bAPs, whereas the peak amplitude was not affected. We hypothesized that the larger driving force for calcium ions during the faster repolarization is responsible for the increased calcium signals. Indeed, the accelerated perisomatic bAP waveforms increased the evoked calcium current in nucleated patches, supporting the above hypothesis. Introducing an A-type potassium conductance by somatic dynamic clamp resulted in faster repolarization and increased AP related calcium signals. In conclusion, our results revealed that activation of potassium currents can profoundly enhance dendritic bAP-related Ca^{2+} signals in GC dendrites, thus providing a previously unknown modulatory mechanism in dendritic signalization.

P3. Hippocampal sharp waves associated dendritic calcium transients and high frequency oscillations in parvalbumin interneurons

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Parvalbumin expressing, fast spiking (PV-FS) interneurons are one of the key members in feedforward and feedback inhibition during sharp wave (SPW) activity, however their excitatory dendritic input pattern and signaling during SPW has remained elusive. We used fast, two-photon acousto-optical (3D-AO) imaging and glutamate uncaging to measure the integration of excitatory inputs in PV-FS interneurons during SPW oscillations. We found SPW associated dendritic Ca^{2+} signals accompanied by somatically recorded supra- and subthreshold responses. SPW associated Ca^{2+} transients were initiated in dendritic hot spots and propagated along the apical dendritic segments both in the presence and absence of somatic action potentials while the basal dendrite of the interneuron remained nearly inactive. The amplitude of the 3D Ca^{2+} responses increased as a function of the distance from the soma. These Ca^{2+} signals were mediated mainly by L-type voltage dependent Ca^{2+} channels. In addition these Ca^{2+} signals were well related with membrane potential oscillations which were mediated voltage-gated Na^+ channels. These oscillations showed the same frequency as the field potential oscillations associated with SPW-ripples. Our results indicate that SPW oscillations can actively induce Ca^{2+} transients in distal apical dendritic segments of PV-FS interneurons and a segment of a dendrite can generate ripple-frequency oscillations.

P4. Synthesis and *in vitro* pharmacological characterization of opiate-cannabinoid bivalent ligands

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Morphine and its substitutes are still one of the primary choices for clinical treatment of severe acute and chronic pain. However, sustained opiate-treatment leads to increased sensitivity to pain and reduced analgesic efficacy. Recent data have shown that sustained opiate treatment activates spinal glia and inhibition of spinal glial activation seems to attenuate opiate-induced hyperalgesia, allodynia and antinociceptive tolerance. Also, it has been demonstrated that CB2-receptor selective cannabinoid agonists acting at glial CB2 receptors, are able to suppress chronic opiate administration-induced neuropathic and inflammatory pain sensitization and spinal glial activation in animal models of chronic pain.

It is known that cannabinoids, especially the CB1 agonists have analgesic activity and they can potentiate the analgesic effects of opiates when given together. It has also been shown that co-administration of opiates and low doses of cannabinoids can prevent or delay the development of antinociceptive tolerance.

These facts that CB2 selective agonists can attenuate the aforementioned side-effects, and that CB1 agonists at low concentration can potentiate the analgesic effects of opiates, prompted us to introduce a novel approach by which we may be able to further improve the analgesic efficacy of opiates and minimize their side-effect profile. Herein, we present the design, synthesis of opiate-cannabinoid bivalent ligands and the results of their ongoing *in vitro* pharmacological characterization.

This work was supported by the NKTH-OTKA "Outgoing Mobility" Grant (OTKA ID: 84459) and the National Institute of Health (NIH).

P5. Astrocytic activation, interleukin-1 receptor type 1 expression and interleukin-1 beta production upon glutamate and ATP treatment

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Accumulating evidence shows that activation of spinal glial cells have important contribution to the onset and maintenance of nerve injury or inflammation-induced persistent pain. Upon activation spinal glia produce inflammatory cytokines (e.g. interleukin-1 beta /IL-1 β /) and other factors which influence nerve cell functions leading to central sensitization and enhanced pain states. Our earlier data shows that the spinal astrocytes are the main source of IL-1 β in the chronic phase of inflammatory pain.

In order to further study astrocytic activation and IL-1 β production upon different stimuli we generated primary astrocyte cultures from spinal cord of Wistar rats. As in inflammatory situations astrocytes are exposed to increased levels of ATP and glutamate (released by neurons) we studied the effect of these molecules on the astrocyte cultures. Increasing amounts of glutamate or ATP induced the activation of astrocytes shown by the elevated GFAP expression of the cultures. IL-1 β production was also increased by ATP or glutamate treatment but the active form was detected in the supernatants only if LPS pretreatment was done or if ATP and glutamate was added to the cultures together. Spinal astrocytes express IL-1 receptor type1 in moderate amount which is increased upon ATP treatment.

Although the exact mechanism of astrocytic activation and IL-1 β production is not yet known our results suggest that both glutamatergic and purinergic mechanisms are involved in the process.

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P6. Subcellular distribution of voltage-gated potassium channels on CA1 pyramidal cells

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Voltage-gated K⁺ channels are key determinants of the neuronal firing. Understanding their functional impact requires the knowledge of the precise subcellular location and density of each K⁺ channel subunit on the axo-somato-dendritic surface of nerve cells. Here we aimed to identify the location and determine the density of two delayed rectifier K⁺ channel subunits (Kv1.1 and Kv2.1) in 18 axo-somato-dendritic compartments of hippocampal CA1 pyramidal cells (PCs). Using the highly sensitive SDS-digested freeze-fracture replica-labelling (SDS-FRL) method, significant densities of gold particles could be detected for the Kv1.1 subunit in axon initial segments (AISs) and axon terminals, with 8-times higher density in AISs compared to axon terminals. Gold particles labelling the Kv2.1 subunit were detected on the somata, proximal apical dendrites and AISs of CA1 PCs at the same density. Within these compartments the distribution of the Kv2.1 subunit was not uniform; a population of gold particles were associated with clusters of intramembrane particles. These Kv2.1 subunit clusters never overlapped with postsynaptic densities of GABAergic synapses. Our results reveal unique cell surface distributions for two K⁺ channel subunits and predicts their role in increasing the computational power of nerve cells.

P7. KCNQ4 is the Major M-Current Subunit of Cholinergic Neurons in the Pedunculopontine Nucleus (PPN)

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KCNQ4 is an M-current subunit with special distribution within the brain: it is located in the brainstem nuclei of the auditory system and the reticular activating system. Within the reticular activating system, its presence in the raphe nuclei and the ventral tegmental area was shown.

Immunohistochemical labeling revealed that KCNQ4 subunit is present in the PPN. Using whole-cell patch-clamp recording on coronal midbrain slices from KCNQ4 knockout mice and wild type littermates, we showed that cholinergic cells from the PPN of wild type animals possess M-current, whereas non-cholinergic ones lack it. M-current was never found in KCNQ4 knockout mice.

On those cells where M-current was detected, afterhyperpolarization current was also present, whereas cells lacking M-current lacked afterhyperpolarization current as well. The action potential morphology also showed differences between wild type animals having or lacking M-current and KCNQ4 knockout animals.

When depolarization and action potential firing was elicited by increasing steps of current injections, cells lacking M-current displayed more depolarization and higher frequency for the same current injection.

Taken together, KCNQ4 subunits present on the cholinergic cells of the PPN and can not be found on non-cholinergic cells. Furthermore, all KCNQ4 knockout mice lack M-current, indicating that KCNQ4 subunit is the major M-current subunit of the PPN.

P8. Endocannabinoid Signaling Modulates Neurons of the Pedunculopontine Nucleus (PPN) via Actions on the Neuron-Glia Communication

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The pedunculopontine nucleus (PPN) is known as the cholinergic part of the reticular activating system (RAS) and it plays an important role in transitions of slow wave sleep to REM sleep and wakefulness. Although both exogenous and endocannabinoids affect sleep, the endocannabinoid neuromodulation has not been characterized at cellular level in the PPN.

In our study we observed that both neurons and glial cells from the PPN possess CB1 receptor and respond to cannabinoid type 1 (CB1) receptor agonists. The response was absent in CB1 gene deficient mice. Responding neurons can be depolarized or hyperpolarized by this drug. The effect depended on initial neuronal and glial activity: i. e., higher action potential firing of the recorded neuron or more frequent calcium waves of the surrounding astrocytes led to smaller responses by CB1 receptor agonists. Blockade of the fast synaptic neurotransmission or application of tetrodotoxin did not significantly change the effect on the neuronal membrane potential, but preincubation with metabotropic glutamate receptor blockers or inhibition of astrocytic calcium waves by thapsigargin diminished the response.

Cells respond to cannabinoid receptor stimulation in the PPN. Neuronal activity can elicit calcium waves on astrocytes, which possibly leads to glutamate release from them. This glutamate, in turn, activates different metabotropic glutamate receptors of neurons and may contribute to regulation of the PPN cells.

P9. Morphological analysis of electrophysiologically characterized glutamatergic cells in the Median Raphe

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The hippocampus receives serotonergic modulatory input from the raphe nuclei. The serotonergic system plays a key role in several normal and pathological conditions of the brain. It has been shown that raphe neurons, even serotonergic ones, contain the third subtype of the vesicular glutamate transporter (VGluT3), which raises the possibility of glutamate transmitted modulation. Our group previously showed that VGluT3-containing raphe cells are involved in the modulation of hippocampal circuits. However, the function of these cells in the regulation of cortical networks is unknown. The aim of this study was the electrophysiological and morphological characterization of median raphe neurons, especially those contain VGluT3. In urethane-anesthetized male Wistar rats, median raphe cells were juxtacellularly recorded simultaneously with local field potential of the dorsal hippocampus and the ventromedial prefrontal cortex. Anatomical characterization requires strong labeling, therefore we filled the electrode with 2% Neurobiotin solved in 0.5 M NaCl. After the recording, we attempted to label the cells applying 200 ms ON-OFF cycles of positive current pulses for 5 to 10 minutes. The transport time of Neurobiotin is approximately 1 mm/hour, therefore in order to visualize the distal dendrites also, the animals were perfused 3 to 5 hours after successful labeling. The presence of serotonin and VGluT3 in the labeled cells was immunohistochemically tested whereas the Neurobiotin was converted by DAB-Ni for light microscopic reconstruction. The cells were reconstructed in three dimensions using the Neurolucida Stereo Investigator setup. Finally, the cells were clustered based on their firing patterns, morphology and neurochemical properties.

P10. Similarities and differences in presynaptic Ca^{2+} transients of hippocampal interneurons

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There is an agreement that changes in Ca^{2+} transient in the nerve terminal make a significant contribution to the short- and long-term plasticity of the synapse. Using femtosecond two-photon laser microscopy, we recorded Ca^{2+} transients and calculated $[\text{Ca}^{2+}]_i$ evoked by somatic stimulation in boutons of various hippocampal GABAergic interneurons on a millisecond time scale with high resolution in acute slice preparations that maintain anatomical and functional integrity (Kisfali, Lőrincz and Vizi, J. Physiol DOI 101113, 2013). High- (OGB-1) and low- (OGB-6F) affinity dyes were applied.

The interneurons were *post hoc* identified (immunocytochemistry in NIKON laboratory). In non-fast-spiking CB1+ GABAergic interneurons we have found that $[\text{Ca}^{2+}]_i$ at rest (53.8 ± 4 nM, $n_b = 26$) was increased by 335 ± 21 nM ($n_b = 113$) in response to single somatic stimulation.

In all the various types of interneurons, the action potentials (AP) propagated without failures from the axon hillock into the axonal arbour crossing several branching points and resulted in Ca^{2+} transients. The origin of $[\text{Ca}^{2+}]_i$ increased by somatic stimulation was exclusively extracellular.

According to Katz & Miledi (J. Physiology 195: 481-492, 1968) the peak amplitude of Ca^{2+} transient by an AP acts at presynaptic site to trigger phasic ACh release, while residual Ca^{2+} summates with the $\Delta[\text{Ca}^{2+}]_i$ and induces short-term potentiation (Zucker and Regehr, 2002). At GABAergic nerve terminal this is also the case.

P11. GABA_A Beta3_Alpha5 Concatenate: Utility for Ligand-Binding and Functional Assays

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Variable subunit composition underlies the differences in the pharmacologic properties of GABA_A receptors (GABA_A-R). However, as GABA_A-R consist of five integral membrane protein subunits, the study of GABA_A-R of defined composition is difficult to accomplish. Concatenation of GABA_A-R subunits by genetic engineering has been used to express 1 GABA_A-R of defined composition. The present study examined the properties of concatenated human GABA_A $\alpha 5$ and $\beta 3$ subunits ($\alpha 5\beta 3\alpha 5\beta 3$) stably transfected into human embryonic kidney (HEK293) cells. Cells expressing $\alpha 5\beta 3\alpha 5\beta 3$ contained a ~100K membrane protein reacting with GABA_A-R $\alpha 5$ and $\beta 3$ subunit antibodies. High-affinity binding sites for ³H-muscimol were present moreover, GABA-activated Cl⁻ currents could be monitored by patch-clamp electrophysiology as well as voltage-sensitive fluorescence in cells cultured in 96-well microtitre plates. In the latter system the EC₅₀ for GABA was $0.1 \pm 0.04 \mu\text{M}$ (mean $\pm$ S.E.M., n=6). When cells were co-transfected with the GABA_A $\alpha 5$ subunit, ³H-flumazenil binding-sites were also evident. Cl⁻ currents resistant to picrotoxin could be measured when a mutant version (T309F) of the $\alpha 5$ subunit was transfected. High-affinity ³H-muscimol binding by $\alpha 5\beta 3\alpha 5\beta 3$ was retained upon detergent-solubilisation. In summary, the $\alpha 5\beta 3\alpha 5\beta 3$ concatenate appears suitable to investigate the properties of GABA_A-R $\alpha 5$ receptors in isolation and could provide a tool for determining the structure of a mammalian GABA ligand-binding site.

P12. Effect of resolvins on Transient Receptor Potential ion channel activation

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Transient Receptor Potential ion channels, such as TRP Vanilloid 1 and Ankyrin repeat domain 1 (TRPV1 and TRPA1) are expressed in nociceptive primary sensory neurons and sensory nerve endings. Resolvins are endogenous lipid mediators. Resolvin D1 (RvD1) is described as a selective inhibitor of TRPA1. RvD2 has been reported as a potent inhibitor of TRPV1 and TRPA1 in dorsal root ganglion cells. Our aim was to analyse the effects of RvD1 and RvD2 on TRPV1 (by capsaicin) and TRPA1 (by mustard oil (MO)) receptor activation on trigeminal sensory neurons and nerve endings.

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

Significant decrease in the percent of capsaicin- and MO-sensitive cells was observed after 10 nM RvD2 incubation on trigeminal neurons. RvD1 (10 nM) inhibited the MO- induced Ca^{2+} -influx but had no effect on capsaicin-evoked TRPV1 activation. Both RvD1 (100 nM) and RvD2 (10 nM) decreased the capsaicin-evoked CGRP release from nerve terminals.

We demonstrated the inhibitory effects of resolvins on sensory neurons and sensory nerve terminals, but further investigations are needed to understand the mechanisms of these effects.

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P13. A GABAergic communication pathway from individual neurons to glial cells

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Neurogliaform cells (NGFCs) in the neocortex reach GABA_A and GABA_B receptors on target cells through a unitary form of volume transmission by flooding the area covered by the extremely dense axonal arborization with GABA. Based on this phenomenon we hypothesize that NGFCs are able to act on neural as well as glial elements of the cortex within the axonal arborization of the NGFCs.

We applied paired whole cell recordings to investigate the potential effect of layer I. interneurons on neighbouring mature astrocytes by triggering single presynaptic action potentials in anatomically identified NGFCs and non NGFCs. The effect of single presynaptic spikes in non NGFCs was undetectable in astrocytes, however, unitary action potentials of NGFCs elicited small inward currents with a fast and slow component. Our initial analysis indicates that the slow component of this effect was blocked with extracellularly delivered CGP-35348, a GABA_B receptor antagonist. Intracellular application of GDP-β-S in astrocytes also reduced the average amplitude of the inward current suggesting astrocytic GABA_B receptors involved in the slow component of the current. Two-photon calcium imaging experiments did not detect changes in the intracellular concentration of Ca²⁺ in astrocytes following single action potentials in NGFCs.

Our experiments identify a presynaptic cell type specific, GABA mediated communication pathway from individual neurons to glial cells and suggest that unitary volume transmission driven by NGFCs effectively targets neural and glial elements.

P14. Role of semicarbazide-sensitive amine oxidase (SSAO) inhibitors on TRP ion channel activation

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A newly developed SSAO inhibitor, SZV-1287 was described to inhibit both acute and chronic inflammation. Transient Receptor Potential ion channels, such as TRP Vanilloid 1 and Ankyrin repeat domain 1 (TRPV1 and TRPA1) are expressed on nociceptive primary sensory neurons and regulate inflammation. Since the chemical structure of SZV-1287 is similar to the selective TRPA1 antagonist HC030031, we investigated the effects of SZV-1287 on the TRPV1 (by capsaicin) and TRPA1 (by mustard oil (MO)) receptor activation of trigeminal ganglion neurons in comparison with SZV-1911, the reference SSAO inhibitor with different structure.

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

SZV-1287 (1 μ M) decreased the capsaicin- and MO-induced Ca^{2+} -influx in trigeminal neurons, and MO-induced Ca^{2+} -influx in sensory nerve endings. SZV-1911 had no effect in either model. Neither compound had any effect on KCl-evoked Ca^{2+} -influx.

These are the first evidence for an antagonistic action of SZV-1287 on TRPV1 and TRPA1 ion channels on the cell bodies of sensory neurons and peripheral sensory nerve terminals. This effect is independent of its SSAO inhibitory action.

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P15. Effect of Enkephalin-Conotoxin Hybrid Peptides on Opioid Receptors

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Enkephalin is known as an endogenous ligand peptide for opioid receptors and conotoxin is a neurotoxic peptide isolated from the venom of the marine *cone snail*, genus *Conus* with Ca²⁺ ion channel blocker characteristic.

Our objective was to investigate the specific binding of two different enkephalin-conotoxin hybrid peptide (AMRC5 and AMRC6) and two type of conotoxin derivative (AMRC7 and AMRC8) towards mu, kappa and delta opioid receptors (MOR, KOR and DOR respectively) in competition binding experiments with opioid receptor specific radioactive ligands in rat brain membranes. Additionally the opioid G-protein efficacy of the observed peptides were investigated in functional [³⁵S]GTPγS binding assays in presence and absence of different opioid receptor specific antagonists, again in rat brain membranes.

AMRC5 and AMRC6 effectively inhibited all three opioid receptors specific binding, where AMRC5 show the higher affinity towards KOR. AMRC7 decreased the specific binding of KOR only in higher concentrations, while AMRC8 did not show any effect on either opioid receptor specific ligand binding. AMRC5 and AMRC6 stimulated G-protein activation which was inhibited by opioid receptor antagonists, indicating these two hybrid peptides have opioid receptor activity. On the other hand conotoxin derivatives did not alter G-protein activation.

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P16. The role of state-dependent affinity and accessibility in sodium channel inhibitor effects

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Sodium channel inhibitors (e.g. local anesthetics, class I antiarrhythmics, and certain anticonvulsants) share some essential properties of inhibition: it is voltage-dependent, frequency-dependent, and is accompanied by a shift of the “steady-state” availability curve. The mechanism of this specific form of inhibition has been studied for decades, but the essential mechanism is still not fully agreed on. The two main theories explaining drug-induced behavior of the channel are the modulated receptor hypothesis (MRH), which is based on state-dependent affinity; and the guarded receptor hypothesis (GRH), which supposes state-dependent accessibility.

We believe that the two hypotheses are not mutually exclusive, and we suggest a relatively simple experimental method to test the contribution of these two hypotheses. Affinity is primarily reflected by the equilibrium of inhibition, while accessibility is expected to determine mainly the rate of onset (and offset). By observing to what extent depolarization affects equilibria and binding kinetics, one could conclude to what extent the inhibition by an individual SCI can be explained by the MRH and the GRH.

We tested five sodium channel inhibitor drugs: the local anesthetic and class Ib antiarrhythmic lidocaine, the local anesthetic bupivacaine, the class Ic antiarrhythmic flecainide, the antiepileptic phenytoin, and the neuroprotective agent riluzole.

Lidocaine and bupivacaine showed both state-dependent affinity and accessibility. For phenytoin the state-dependence of affinity was more prominent. Neither the affinity, nor the accessibility was state-dependent for flecainide, which is consistent with its open channel blocker mechanism. The behavior of riluzole was found inconsistent: Using onset data the drug appeared to be operating predominantly by the GRH, while offset data indicated predominantly MRH mechanism. These data indicate a specific, more complicated mechanism of action, for which this simple model is not appropriate.

A simple kinetic model of the channel was used to test hypotheses regarding the mode of action. Simulated drug effects confirmed that the supposed differences in mode of action can cause the observed experimental behavior.

P17. Three-dimensional population Ca^{2+} measurement from *in toto* preparation during rhythmic oscillations reveals cell type specific firing properties

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Neurons receive thousands of inputs from other neighboring and distant neurons, and it has long been a question how these connections form assemblies or neuronal networks of synchronously working units. This assembled function has long been proposed of being a key component in how neurons compute in the neuronal networks. GABAergic interneurons are presumed to control the synchronization of pyramidal cell activity. In this study, we aimed to investigate network activity during spontaneous rhythmic oscillation in complete (*in toto*) hippocampal preparations.

We have developed a high-resolution, acousto-optic (AO) two-photon microscope with continuous three-dimensional (3D) trajectory and volumetric random-access scanning modes that reaches near-cubic-millimeter scan range (up to $700 \times 700 \times 1,400 \mu\text{m}^3$) which allows calcium imaging of spontaneous activity from hundreds of neurons with sub-millisecond temporal resolution.

We saw correlated LFP and Ca^{2+} activity in area CA1 during spontaneous rhythmic activity, and found distinct spatial and temporal firing characteristic between interneurons and pyramidal during network oscillations. Nevertheless, whether interneurons are driven by local network activity or their long-range inputs is still unclear. What's more, their connection to the local network is still to be investigated and their connectivity patterns are to be elucidated.

P17a. Retinal aging in the diurnal Chilean rodent, *Octodon degus*

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The retina is sensitive to age-dependent degeneration. To find suitable animal models to understand and map this process has particular importance. The degu (*Octodon degus*) is a diurnal rodent with dichromatic color vision. Its retinal structure is similar to that in humans in many respects, therefore, it is well suited to study retinal aging. Histological, cell type-specific and ultrastructural alterations were examined at the ages of 6, 12 and 36 months.

The characteristic layers of the retina are present at all ages, but slightly loosened tissue structure could be observed in 36-month-old animals both at light and electron microscopic levels. Elevated GFAP expression was observed in Müller glial cells in aging retinas. The number of rod bipolar cells and the ganglion cells were reduced in the aged specimens. Other age-related differences were detected at ultrastructural level: alteration of the retinal pigment epithelium and degenerated photoreceptor cells were evident. Ribbon synapses were sparse and often differed in morphology from those in the young animals. These results support our hypothesis that degu retina maybe a suitable model for studying retinal aging and with a few restrictions the results may be translated to humans. However, future observations must consider ages even older than 36 months.

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P18. Molecular dynamics study of the structural basis of dysfunction and the modulation of reactive oxygen species generation by pathogenic mutants of human dihydrolipoamide dehydrogenase

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Human dihydrolipoamide dehydrogenase (LADH, E3) is a component in the pyruvate-, alpha-ketoglutarate- and branched-chain ketoacid dehydrogenase complexes and in the glycine cleavage system. The pathogenic mutations of LADH cause severe metabolic disturbances, called E3 deficiency that often involve cardiological and neurological symptoms and premature death. Our laboratory has recently shown that some of the known pathogenic mutations augment the reactive oxygen species (ROS) generation capacity of LADH, which may contribute to the clinical presentations. A recent report concluded that elevated oxidative stress generated by the above mutants turns the lipoic acid cofactor on the E2 subunits dysfunctional. In the present contribution we generated by molecular dynamics (MD) simulation the conformation of LADH that is proposed to be compatible with ROS generation. We propose here for the first time the structural changes, which are likely to turn the physiological LADH conformation to its ROS-generating conformation. We also created nine of the pathogenic mutants of the ROS-generating conformation and again used MD simulation to detect structural changes that the mutations induced in this LADH conformation. We propose the structural changes that may lead to the modulation in ROS generation of LADH by the pathogenic mutations.

P19. Projection Neurons in the LSN and Lateral Spinal Lamina I Are Subject to Propriospinal Ascending Mono- and Polysynaptic Excitation

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The role of spinal dorsal horn propriospinal connections in nociceptive processing is not yet established. Rostrocaudally oriented axon collaterals of lamina I projection- and local circuit neurons (PNs and LCNs) in the dorsolateral funiculus may be the anatomical basis for intersegmental processing. Putative targets of these axons include lateral dendrites of superficial dorsal horn neurons, including PNs, and also neurons in the lateral spinal nucleus (LSN). We used an intact spinal cord preparation to determine whether lateral lamina I and LSN neurons receive synaptic input from neighbouring spinal cord segments. Despite the low connectivity rate, we detected monosynaptic input to lamina I and LSN neurons from neurons located caudally, some of which were located in the neighbouring spinal cord segment. In other cases we observed polysynaptic long lasting excitation of the recorded postsynaptic neuron that was clearly connected to the activation of the presynaptic cell. Our findings indicate that certain neurons in the lateral part of lamina I and in the LSN integrate sensory information from more caudal segments with segmental peripheral input before relaying to supraspinal centers. The relatively low number of monosynaptic connections indicates that axon collaterals of lamina I PNs and LCNs in the DLF mostly target neurons in other laminae. These findings emphasize the role of propriospinal connections in spinal cord function besides the classical segmental processing.

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P20. The role of different regimens of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment in neurochemical changes of mice striatum

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The magnitude and the mode of dopaminergic cell death induced by MPTP depend on its regimen of administration. Here we examined the neurochemical changes induced by sub-acute (20 mg/kg/day for 4 consecutive days) and acute regimen (4x20 mg/kg with 2h i.p. injections) of MPTP in the striatum. Experiments were performed using wild-type and P2X7 receptor deficient (KO) mice and on-line column-switching LC method. Acute MPTP treatment provoked a massive reduction (92.9 ± 0.09 ; 94.1 ± 1.4 %, $p < 0.001$) in dopamine (DA) content of striatum. However, the sub-acute treatment caused (73.5 ± 2.3 ; 72.7 ± 3.1 %, $p < 0.001$) a moderate DA decreasing in both types of mice. The glutamate (Glu) content was higher after sub-acute treatment (24.1 ± 2.9 vs. 15.2 ± 1.5 nmol/mg protein, $p < 0.05$) in KO mice. Acute treatment increased both of Glu (33.4 ± 1.6 vs. 10.5 ± 0.7 nmol/mg protein, $p < 0.05$) and GABA (49.1 ± 3.5 vs. 21.5 ± 2.0 nmol/mg protein, $p < 0.001$) levels in wild-type mice. The 2-arachidonoylglycerol content elevated (44.5 ± 5.5 vs. 16.7 ± 1.5 pmol/mg protein, $p < 0.05$) after acute MPTP treatment in KO mice. The sub-acute MPTP treatment significantly raised the anandamide (AEA) levels (19.0 ± 2.4 ; 14.2 ± 1.9 vs. 1.3 ± 0.2 ; 0.8 ± 0.1 pmol/mg protein, $p < 0.001$) both type of mice. Here we provide the evidence for direct protective effects of AEA against MPTP toxicity. These data may suggest potential therapeutic benefits of elevating endocannabinoids in preventing degeneration of nigrostriatal dopaminergic neurons in PD.

P21. Localization of CD26/DPP4 enzyme and the effects of its inhibitors in the rat spinal cord

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CD26/DPP4 is a moonlighting protein being present within and in the membrane of several cell types and acts as a receptor, a costimulatory protein and a proteolytic enzyme. It also has a role in immune response and is involved in adhesion and apoptosis. We have demonstrated that DPP4 inhibitors (vildagliptin and diprotin A) 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 astrocytes. 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(psi), while the mu-selective CTAP and kappa-selective gNTI abolished only the 40% and 50% of this effect, respectively. However, diprotin A-induced antihyperalgesia was fully blocked by CTAP, while TIPP(psi) 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.

P22. TNF-alpha pretreatment improves the neuronal differentiation and integration of grafted embryonic spinal cord: a morphological study

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Avulsion injury to one or more ventral roots of the spinal cord induces motoneuron death. A promising way to replace the missing neurons is to use embryonic postmitotic motoneuron grafts. In this study we investigated the effect of tumor necrosis factor-alpha (TNF- α) pretreatment on grafted embryonic cells and their integration process into the host tissue.

In all animals the left lumbar 4 ventral root was avulsed and then reimplanted immediately along with motoneuron-rich neural grafts, derived from 12,5-day-old embryos of SD-eGFP rats. The embryonic cells were treated with rhTNF- α prior to grafting. Control animals received embryonic tissue without any treatment.

In the untreated grafts the majority of grafted cells differentiated into astrocytes and a very small proportion of them reached near full neuronal maturity. Following TNF- α pretreatment the vast majority of the grafted cells differentiated into neurons. In this group we found some motoneurone-like cells with large cell bodies, strong ChAT expression and a reinnervation capacity to the denervated hindlimb muscles. These cells resided in the periphery of the graft, which was densely innervated by 5-HT fibres. The host-derived cells did not migrate into the grafted area, however, vessels of host origin were present in the graft, supporting the view of close contact between grafted cells and host cord.

These results suggest that TNF- α pretreatment increases the integration and maturation of grafted embryonic neurons.

P23. Flickering Light Stimulation Affects Global Form Detection, but not Global Motion Detection

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Ever since the description of residual vision after damage to the primary visual cortex (V1) (Riddoch, 1917), the question of how the functionality of V1 influences the different elements of visual consciousness has been around. In this study we applied a simple, noninvasive method to examine how V1-selective visual noise (generated by flickering light stimulation) influences global form and global motion perception thresholds. Stimuli were presented on a computer screen. Flickering light stimulation was applied simultaneously. Signal (coherently arranged or coherently moving dots) was embedded in noise (randomly arranged or randomly moving dots), and absolute perception threshold was determined by varying the signal-to-noise ratio. Absolute perception threshold was defined as the lowest signal-to-noise ratio at which subjects performed over chance level. Global motion thresholds were almost unaltered by flickering stimulation, while it caused a significant threshold elevation in the global form perception task. Our results support the assumption that V1 is not crucial for the conscious processing of the dynamic aspects of visual stimuli, but form identification does depend on its functioning.

P24. Lamotrigine is more effective than riluzole in rescuing motor unit numbers and muscle properties after sciatic nerve crush in neonatal rats

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A large proportion of motoneurons die after injury to their axons shortly after birth. Here we reduced excitability of motoneurons using lamotrigine or riluzole, agents that inhibit glutaminergic excitation. After a crush injury to the sciatic nerve 3 hours after birth the rat pups were divided into 3 groups, one was treated with daily intraperitoneal injection of saline, the second received lamotrigine (5mg/kg for 7 days) and the third received riluzole (16mg/kg for 14 days). The number of surviving motoneurons to extensor digitorum longus (EDL) and soleus muscles was assessed three months later by counting the number of motor units in individual muscles. The recovery of muscle function was established by recording isometric twitch and tetanic tensions of each muscle. Lamotrigine and riluzole treatment prevented the reduction in motor unit numbers produced by nerve injury. Following nerve crush at birth soleus muscles in the saline treated animals produced only 16% and EDL 10% of force produced by control muscles during a single twitch, while in the lamotrigine treated animals the force produced by soleus was 84% and by EDL 88%. After riluzole the force produced by soleus was 32% and by EDL 30% of control. Our results demonstrate that lamotrigine treatment is superior to riluzole for promoting functional recovery and improved muscle performance following sciatic nerve crush in rats.

P25. The influence of acoustic stimuli ranging from 81-120dB on the startle reflex in rat: determination of acoustic startle response threshold level

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Acoustic startle response (ASR) is a fast behavioral response to sudden, intense stimuli. ASR has been used as a behavioral tool to model neuropathological dysfunctions of sensorimotor information processing. Moreover, children and adults with migraine may have an exaggerated ASR. The objective of the current experiments was to establish a new experimental method to measure the threshold level of ASR.

The rats were placed in the startle chamber and they were exposed to a startle session that contained trials of sound bursts ranging from 81 dB to 120 dB in 3 dB increments with a background noise level of 65 dB. Each sound intensity was presented 3 times. We have used visual detection of the startle reflex: the ASR pattern consists of eyelid-closure and a contraction of facial, neck and skeletal muscles. The observed average threshold level was 99 ± 2.6 . When we plotted sigmoidal curve on the basis of recorded ASR data, the calculated EI_{50} value was defined as ASR threshold (99.9 dB) which is in accordance with observed average threshold level.

We established a new, *quantitative* experimental method which may be a suitable tool to define the ASR threshold and which may contribute to the face and predictive validity of animal models of migraine.

P26. Endogenous vanilloids are putative activators of the trigeminal nocisensor complex: relevance to headache mechanisms

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In the rat dura mater, the activation of TRPV1 receptors by capsaicin results in vasodilatation and release of CGRP, hallmarks of neurogenic inflammation implicated in the pathomechanism of migraine. The aim of the present study was to reveal possible activation of meningeal TRPV1 receptors by endogenous vanilloid compounds. Laser Doppler flowmetry was utilized to measure changes in meningeal blood flow in a rat open cranial window preparation following local applications of anandamide and N-arachidonoyl-dopamine (NADA). ELISA was used to measure vanilloid-induced CGRP release in an ex vivo dura mater preparation. Topical applications of anandamide and NADA induced modest and significant increases in meningeal blood flow, respectively. NADA-induced increases in blood flow were markedly inhibited by pretreatments with the TRPV1 antagonist capsazepine, the CGRP antagonist CGRP8-37, or by prior systemic capsaicin desensitization. In the ex vivo dura mater preparation NADA evoked a significant increase in CGRP release. Administration of AM251, a CB1 receptor antagonist significantly increased the vasodilatory effect of anandamide.

The present findings suggest that endogenous vanilloids are potential activators of meningeal TRPV1 receptors and, consequently the trigeminal nocisensor complex. The results also suggest that prejunctional CB1 receptors may modulate meningeal vascular responses.

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P27. Spinal expression of interleukin-1 receptor type 1 in an animal model of chronic inflammatory pain

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Nociceptive nerve impulses generated in peripheral tissues are conducted by sensory fibers to the superficial spinal dorsal horn. The intense and long-term noxious stimuli that are associated with chronic inflammation lead to neuronal plasticity and central sensitization. The effects of IL-1 are mediated by the type I receptor. Our earlier data showed IL-1R1 RNA is elevated in the spinal dorsal horn during the course of Freund adjuvant-induced inflammatory pain.

Mechanical and thermal behavioural tests were performed in IL-1R1 knock out mice after administration of Freund adjuvant. According to our results mice lacking proper IL-1R1, showed a significantly reduced mechanical and thermal nociceptive sensitivity.

Other goal was to study the expression and cellular localisation of the IL-1R1 in the same model by immunohistochemistry. Double immunostaining followed by quantitative analysis of the slides showed that the IL-1R1 is mainly expressed and by astrocytes, and neurons. Colocalisation with the PSD-95 marker suggests a postsynaptic location, and role in modulating glutamatergic neurotransmission.

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P28. Kanamycin-induced changes in calcium-binding protein expression in the inner ear of wild type, heterozygous and homozygous pituitary adenylate cyclase activating polypeptide (PACAP)-deficient mice

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PACAP is a neuropeptide with diverse neuro- and cytoprotective effects. It protects cochlear cells against H₂O₂-induced apoptosis *in vitro*. In this experiment we investigated the role of endogenous PACAP in aminoglycoside (kanamycin) induced ototoxicity *in vivo*.

We examined the effect of kanamycin treatment on Ca²⁺-binding protein (CBP) expression in hair cells of wild type (WT), heterozygous (HZ) and homozygous PACAP-deficient (KO) mice. We treated 5-day-old mice with kanamycin and 2 days later examined the expression of CBPs of the hair cells with immunohistochemistry. Control animals received physiological saline.

We found stronger expression of CBPs in hair cells of control HZ and KO mice compared to wild-type animals. Kanamycin induced a significant increase in CBP expression in WT and HZ mice, but the baseline higher expression in KO mice did not show further changes after the treatment.

Elevated endolymphatic Ca²⁺ is deleterious for the cochlea, against which the high concentration of CBPs in hair cells may protect. We suggest that the lack of PACAP is a pathological condition for hair cells, leading to a compensatory increase in CBP expression. That provides evidence for the important protective role of PACAP in the inner ear.

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P29. Effect of unilateral labyrinthectomy on brevicane expression in the vestibular nuclear complex of the rat

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Unilateral labyrinthectomy (UL), results in disturbances of the vestibular function. Symptoms settle during the process of vestibular compensation that requires plasticity of the central nervous system (CNS). The extracellular matrix (ECM) molecules promote or block neural plasticity. In the CNS, ECM molecules are in condensed forms called perineuronal net (PNN), or distributed in the neuropil. The PNN is formed by hyaluronan, chondroitin sulfate proteoglycans, tenascin-R and link proteins. Data lacks on the contribution of various ECM molecules to the vestibular compensation. Here, we observed the expression of *brevican* in the superior (SVN), lateral (LVN), medial (MVN) and descending vestibular nuclei of the rat.

After UL, rats produce uncoordinated motion on limbs, trunks and head. After 1, 3, 7 and 14 days of survival the brainstem was removed, fixed and embedded. Immunohistochemical reaction was visualized by DAB reaction.

On postoperative day 1 brevicane staining decreased in the SVN, LVN, and MVNm on the affected side. Day 3 showed the SVN PNN to be fainter than the unoperated side. On day 7 and 14 SVN PNN was restored.

Our results showed that the UL is accompanied by the modification of brevicane staining mostly in the PNNs of vestibular nuclei engaged in the vestibulo-ocular reflex. The time course of PNN re-establishment is parallel to the improvement of postural deficits.

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P30. Integration of grafted embryonic motoneurons into the host spinal cord circuitry after lumbar 4 ventral root avulsion and reimplantation

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The aim of this study was to investigate the morphological and functional integration of grafted embryonic motoneurons into the host spinal cord circuits. The lumbar 4 ventral root of all animals was avulsed and reimplanted. Treated animals received an embryonic graft derived from SD-eGFP donor rat embryos, control rats had their ventral root avulsed and reimplanted without an embryonic graft. Six months later EMG recordings were performed from the hind limb ankle flexors and extensors on the intact and operated sides. During locomotion all the recorded motoneuron activity was related to rhythmic locomotor limb movement in grafted animals, but to a far less extent in controls. Two populations of motor unit action potentials (MUAPs) were distinguished in the grafted animals. The first population of MUAPs was recruited for a long period of time (firing below 50 Hz) in sitting position. The second group of MUAPs was detected during walking in a range between 50 and 200 Hz firing for short periods of time (<200 ms) with relatively higher amplitude. Retrograde labelling from the reinnervated muscles proved that both the grafted and host neurons contributed to the reinnervation of the denervated hind limb muscles. Anterograde tracing with Phaseolus vulgaris leucoagglutinin revealed substantial reciprocal connections between the graft and the host spinal cord. These results suggest that grafted embryonic motoneurons are able to integrate into the host circuitry.

P31. The relationship between the internal clock and reaction time

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Many studies have shown that perception of time changes as a result of rapid auditory stimulation (RAS), which can be explained by the temporary change in the pace of the internal clock. As a result of the prospective distorting effect, the perceived time interval is different from the actual one. Our hypothesis is that the internal clock, which can be modified by the RAS, may affect reaction time (RT). The objective of the present study was to examine the relationship between the RAS frequencies and RTs. The predictive low-frequency RAS stimuli were presented at 4 different paces (3.33, 2, 1 and 0.67 Hz), each of which was paired with a target that appeared after one of 5 different, randomly chosen foreperiods (200, 400, 900, 1400, 1600 ms). The measured RTs were inversely related to the length of the foreperiod. Even though the correlation between the frequencies of sound stimuli and the foreperiod was not significant ($p > 0.05$), their correlation diagrams show a similar pattern. RTs to targets with foreperiods of 1400 and 1600ms stimulus delays are significantly longer compared to RTs to 200 and 400ms delays. In conclusion, the RT and foreperiod lengths have a consistent inverse relationship. Based on our results, we cannot unequivocally conclude that the low RAS frequencies employed during the measurements are capable of changing the rhythm of the internal clock, therefore further measurements are needed. Supported by Campus Hungary and OTKA 108747.

P32. The nature of cortical layer 5 input to the basal ganglia recipient motor thalamus

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Higher order thalamic nuclei are known to receive large, multisynaptic inputs from the cortical layer 5 (L5). Basal ganglia (BG) recipient thalamic nuclei are also considered as higher order but the nature of their L5 input is unclear. Since the BG recipient thalamus is pivotal in motor execution and planning and is involved in Parkinson's disease its cortical control is of significant interest. Here we investigated the L5 input to the mouse BG recipient thalamic nucleus (VM).

Neither anterograde tracing nor immunocytochemical labeling revealed large cortical terminals in the VM, questioning its higher order status. Retrograde tracing however, clearly showed that VM receives L5 input. Selective labeling of L5 axons in Rbp4-Cre mice revealed large (4-6 μ m) L5 terminals in the well-known higher order nucleus Po (as described before) and much smaller (1-2 μ m) terminals in the VM. L5 terminals in the VM mainly targeted dendritic appendages via long, frequently branching synapses. This significantly differed from the ultrastructure of corticothalamic terminals following conventional anterograde tracing (which mainly labeled L6 terminals). The vast majority of L6 terminals targeted thin ($\leq 0,5 \mu$ m) dendrites via single small synapses. Photoactivation of L5 input could reliably drive VM neuron firing up to 20Hz.

According to our data L5 input to the (BG) recipient thalamic nucleus and to Po are significantly different. Within VM L5 input also differs from the L6 input. Despite the small bouton size L5 input seems to be efficient in driving VM cells. Our results suggest strong cortical control over the BG recipient thalamus and contribute to the growing evidence that the rules of operation can fundamentally differ between different thalamic regions.

P33. Evoked responses elicited by acoustic and MGB electrical stimulation 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 stimulation 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 of cats 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 unit and multiunit activity (MUA) were recorded and analyzed. Position of the MGB electrodes was verified by recording tonotopic responses and post mortem histology. We presented separated acoustic and MGB stimulations. Mostly early multiunit responses and locally generated potential fields appeared in the MGB. The configuration of the auditory cortical evoked potentials induced by MGB microstimulation was similar to those elicited by click stimuli but with shorter latency. In slow wave sleep the amplitude of the early components of the click evoked cortical response was not suppressed and the late phase was prevailed by large amplitude positive wave and decreased unit firing. Similarly, late components of the cortical responses evoked by MGB microstimulation displayed increase both in amplitude and latency. The characteristics of these components depended on the stimulation location in the MGB. Simultaneous presentation of acoustic and electric stimuli with different delays revealed mutual modulation of both early and late cortical evoked components.

P34. Thermoregulatory and motor disturbances after intrathecal injections in freely moving rats

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The goal of this study was investigate the effects of intrathecally (IT) administered vehicles on core temperature and motor activity in freely moving condition.

Methods: Male Wistar rats were catheterized IT, and Minimitter transmitters were implanted intraperitoneally (IP). The effects of handling procedure, IT injections of saline in 5 and 15 μ l volumes, and IP saline and paracetamol (40 or 80 mg/kg) were determined.

Results: Body temperature and locomotor activity showed clear-cut daily rhythm. Handling, IP injection of saline or paracetamol caused a temporary enhancement in the motor activity accompanied by slight, but significant hyperthermia. The IT injection of 15 μ l saline produced a prolonged hyperthermia and it caused decreased motor activity during the active phase. Saline in lower volume (5 μ l) produced blunted changes compared to the effects of 15 μ l. Regarding acute effects of these interventions, the changes were similar to the handling procedure at the first hour. Paracetamol in the higher dose blunted the 15 μ l saline-induced hyperthermia, but it did not influence the disturbed motor activity.

Our data suggest that the telemetry together with chronic intrathecal cannulation may be a valuable tool for investigating the effects of drugs at spinal level, but the prolonged effects of intrathecal vehicle injections should be considered.

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P35. The Analgesic Action of P2Y₁₂ Receptor Antagonists in Animal Models of Inflammatory and Neuropathic Pain

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P2Y₁₂ receptor (P2Y₁₂R) belongs to metabotropic purine receptors and is the molecular target of widely used antithrombotic drugs, such as clopidogrel. Our aim was to assess the effects of P2Y₁₂R antagonists in rodent models of inflammatory, neuropathic pain and on acute thermal nociception.

To examine neuropathic pain behavior we ligated the nervus ischiadicus partially and mechanical allodynia was measured 7 days later in rats.

In the inflammatory pain model Complete Freund adjuvant (CFA) was injected into the right hindpaw and mechanical allodynia was tested 48h after the injection.

The hot-plate test was applied to investigate acute thermal nociception.

In addition to clinically used P2Y₁₂ blockers, the effect of the potent and selective P2Y₁₂R antagonist PSB-0739 and P2Y₁₂R knockout mice were also examined.

All the tested P2Y₁₂R antagonists had analgesic action in inflammatory pain with the following rank order of potency (mED): PSB-0739=cangrelor>reactive blue 2> MRS2395>ticlopidine=clopidogrel.

Similar findings were obtained in the neuropathic pain model: PSB-0739=cangrelor>MRS2395>clopidogrel>ticlopidine.

P2Y₁₂R^{-/-} mice showed attenuated mechanical hyperalgesia following CFA injection compared to wild-type mice.

In the hot plate test the following rank order potency was obtained: PSB-0739>MRS2395>clopidogrel>ticlopidine.

Our data suggest that P2Y₁₂R can be a potential target for treating neuropathic and inflammatory pain.

P36. Differential expression patterns of K⁺/Cl⁻ co-transporter (KCC2) in neurons within the superficial spinal dorsal horn of rats

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GABA_A receptor mediated inhibition is associated with a chloride-influx that depends on inwardly directed chloride electrochemical gradient, which is regulated by potassium-chloride co-transporter, KCC2. Here, we investigated the cellular distribution of KCC2 in the superficial spinal dorsal horn of rats by using immunocytochemical methods. We demonstrated that perikarya and dendrites widely expressed KCC2, but axon terminals proved to be negative for KCC2. Studying the somato-dendritic distribution of KCC2, high and low levels of KCC2 expression were equally recovered. In single ultrathin sections we also observed dendritic segments that were negative for KCC2. Investigating KCC2 expression on neurons immunoreactive for NK1 receptor, which allowed us to study a large part of the somato-dendritic compartment of some neurons we found that KCC2 presented a quite heterogeneous distribution along the dendritic membrane. Measuring the distances between gephyrin-IR and KCC2-IR spots on NK1-R-IR dendrites we found that some putative inhibitory postsynaptic membranes keep larger distances from KCC2 than others. In addition, we found that postsynaptic membranes of putative inhibitory synaptic contacts establishing loose association with KCC2 transporters are arranged in clusters along the dendritic membrane. The results suggest that GABA_A receptor mediated synaptic mechanisms may vary at different sites of the somato-dendritic membrane of neurons in the superficial spinal dorsal horn.

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P37. Contribution of nitric oxide to odor-elicited behavior in the snail, *Helix pomatia*. An *in vitro* and *in vivo* study

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Nitric oxide (NO) is necessary to induce oscillatory activity in the snail olfactory center (procerebrum, PC) during odor processing. Recently, we have described the distribution of NO synthase (NOS), and characterized downstream protein kinases, through which NO acts potentially in the PC. In the present study further details of NO signaling are provided, (i) by the stimulation of NO release with enzyme regulators and neurotransmitters; (ii) by the localization of the NO-receptor soluble guanylyl cyclase (sGC), and determination of the level of cGMP after pharmacological and odor stimulation; and (iii) by the detection of Akt-kinase phosphorylated proteins under *in vitro* and *in vivo* conditions. Flow cytometry of the NO-scavenger DAF-2 positive cells showed that the NOS inhibitor L-NAME decreased the number of both bursting and non-bursting PC neurons, whereas the co-factor NADPH and glutamate increased the NO production of non-bursting cells. sGC and cGMP showed co-localization with NOS. Exposition of PC to the NO-donor, PAPANONOate, the NO-independent sGC activator YC-1, and glutamate induced cGMP synthesis. Attractive food stimuli increased cGMP synthesis and evoked the phosphorylation of three, yet unidentified proteins in the PC. These phenomena were decreased during start of feeding and stopped by aversive odor stimuli. The results suggest that the glutamate-NO-cGMP-protein phosphorylation signal cascade plays an important role in odor-evoked behavior of snails.

P38. TNAP Activity is localized in critical Sites of retinal Neurotransmission across various vertebrate Species

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Evidence is emerging regarding tissue non-specific alkaline phosphatase's (TNAP) role in neural functions. As an ectophosphatase this enzyme could influence neural activity and synaptic transmission via diverse ways. The localization of the enzyme in known neural circuits, such as the retina, could significantly advance the understanding of its role in normal and pathological functioning. However, the presence of TNAP in the retina is scarcely investigated. In our multispecies comparative study using enzyme histochemistry and Western blots, we found that TNAP activity was present in the retina of several mammalian species including humans. Although TNAP activity pattern varied across species, we observed the following trends: 1 In all investigated species (except golden hamster) retinal vessels displayed TNAP activity. 2 We consistently observed TNAP activity in the photoreceptor layer. 3 In the majority of the investigated species, marked TNAP activity was found in the outer and inner plexiform layers. In some species (zebrafish, frog, chicken, guinea pig and rat) TNAP histochemistry revealed several sublayers of the inner plexiform layer. In frog, golden hamster, guinea pig, mouse and humans, a subpopulation of amacrine cells stained positive for TNAP activity. The expression of TNAP in critical sites of retinal neurotransmission across species suggests its fundamental, evolutionally conserved role in acquiring visual information.

P39. Terminations of different primary afferents on the hypoglossal motoneurons in the frog, *Rana esculenta*

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The hypoglossal nucleus is one of the components of neuronal network underlying prey-catching behavior of the frog, responsible for the contraction of tongue muscles. The activity of the neuronal network is modified by various inputs, for example vestibular system conveys the information from the position of the head and body, whereas vagal afferents carry the information from the alimentary canal, and internal organs. The prey object stimulates the hypoglossal afferent terminals, which initiates the contraction of tongue muscles and a small division of fibers enter the brainstem accompanying the second spinal nerve. The aim of our experiments was to study whether various afferent fibers establish direct connections with the motoneurons of the hypoglossal nerve.

In the animals the vestibular, vagus, second cervical spinal nerves, or hypoglossal sensory root and hypoglossal nerve were simultaneously labeled with neuronal tracers.

Close appositions between afferent fibers and motoneurons were detected with a confocal laser scanning microscope. Neurolucida reconstruction of the brainstem was applied to show the spatial distribution of connections in different subdivisions of the hypoglossal nucleus.

These possible direct connections may serve as one of the morphological substrates of the fast response during feeding movements of amphibians that gives the reflex-like ability of the prey-catching behavior. This work was supported by MTA-TKI 11008 and TÁMOP 4.2.4. A/2-11-1-2012-0001.

P40. Activity and thermoregulatory changes in a new substrain of rats

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The effects of gene-environment interactions in psychiatric disorders can be modeled by using selective breeding after applying multiple treatments. The aim of our study was to characterize the changes in motor activity and intraabdominal core temperature in selectively bred rats after chronic NMDA receptor antagonist ketamine treatment and social isolation.

Two groups of male Wistar rats were studied: naive rats without any treatment and the 18th generation of the new substrain. At the age of between 5 and 6 month MiniMitter® transmitters were implanted into the abdominal cavity to monitor these parameters continuously in freely moving animals.

Both groups showed circadian rhythm of the observed parameters. Regarding the locomotor activity, a tendency for a decreased activity was observed during the light phase in treated animals accompanied by a significantly lower core temperature. The core temperature was also significantly lower during the night. The change of bedding immediately induced an increase in both observed parameters, however, the occurrence of core temperature change significantly delayed in the new substrain. The duration of changes was longer in case of core temperature than in motor activity independently of the group.

In summary, our results showed disturbed thermoregulation in a new substrain, suggesting that this model can simulate this phenomenon of schizophrenia.

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P41. PACAP enhances muscle contraction at an excitatory synapse of the snail

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Pituitary adenylate cyclase activating polypeptide (PACAP) can modulate synaptic output both in vertebrates and invertebrates. We have previously found that in muscles of the snail, *Helix pomatia* tentacles, acetylcholine (ACh) is the main excitatory neurotransmitter. The aim of the present study was to investigate the effect of PACAP on the excitatory transmission at the muscle and to identify and localize the receptor of PACAP. We also wanted to find out if there is connection between PACAP and the NO system. It was observed that PACAP27 increased both the nerve stimulation and ACh evoked contractions. This effect could be blocked by PACAP6-27 and the specific PAC1 receptor (PAC1R) antagonist M65 suggesting the presence and postsynaptic localization of PAC1R. The NOS inhibitor L-NAME and the soluble guanylate cyclase inhibitor ODQ increased the stimulatory effect of PACAP. Forskolin increased the nerve stimulation induced contractions, which was proportional to the enhancement of contractions triggered by PACAP. It is concluded that PACAP may have a synaptic activity increasing property obtaining this effect acting via PAC1R and the adenylate cyclase-dependent transduction cascade. The receptors are located postsynaptically, although the presence of presynaptic receptors cannot be excluded. The effect of PACAP is antagonistic with NO, since blocking the NO system enhances the action of PACAP.

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P42. Temporal frequency tuning and response phase of blue-ON cells in the lateral geniculate nucleus of the cat

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We have described a neurone population from the lateral geniculate nucleus of cats, which showed opponent input from "blue" (S) and "green" (ML) cones (blue-ON cells). Here, we were interested in their temporal frequency (TF) response properties and their optimal chromaticity. Responses of achromatic and blue-ON single units were recorded from seven anaesthetised, paralysed and artificially ventilated adult cats. TF tuning curves were measured for sinusoidal modulation of S- or ML-cone-contrast. Optimal chromaticity was determined from the phase of the response to quadrature modulation of ML- and S-cone contrast in a uniform field. Blue-ON cells showed band-pass TF tuning with a maximum of 3.4 ± 2.1 Hz. Tuning curves for S- and ML-cones were similar in shape suggesting adaptation of the afferent retinal networks for chromatic processing. The chromaticity optimum of blue-ON cells was consistent with equal but opposite responses from the opponent cones (S-cone weight 0.53 ± 0.06). Among achromatic cells, we distinguished high-pass cells with increasing response throughout the frequency range tested (1-48 Hz) and band-pass cells with an optimal stimulus frequency of 10.7 ± 1.8 Hz. Only weak S-cone input was detected (S-cone weight 0.10 ± 0.06). Thus, the chromatic properties of cat blue-ON cells were essentially identical to their primate counterparts but their tuning to lower frequencies suggests different behavioural role of the colour channel in cats.

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P43. Motoneuron survival and elongative axonal growth induced by spatiotemporally restricted ex vivo BDNF/GDNF gene therapy

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Axonal injury close to cell bodies of motoneurons induces the death of the vast majority of affected cells. Neurotrophic factors, such as brain derived neurotrophic factor (BDNF) and glial cell derived neurotrophic factor (GDNF) produced in situ in a non-regulated manner induce good survival of damaged motoneurons and sprouting of axons without functional reinnervation. Therefore, we devised an ex vivo gene therapy to restrict expression of the neurotrophic factors to approx. two weeks using in vitro transfected rat adipose tissue-derived stem cells applied in vivo periradicularly, i.e. around the reimplanted ventral root, embedded in collagen gel. In vivo results suggest, that axons of injured motoneurons were able to enter the reimplanted root following reimplantation and BDNF treatment and produce morphological and functional reinnervation. The axons of surviving motoneurons showed elongative growth typical of regenerative axons, without the presence of aberrant or sprouting axonal growth. These findings provide evidence that damaged motoneurons require limited and spatially directed amounts of BDNF or GDNF to support their survival and regeneration. Moreover, neurotrophic support appears to be needed only for a critical period of time not longer than two weeks after injury.

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P44. Functional restoration after spinal cord injury from a new aspect: synchronous analyses of footprint pattern and limb motion

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Spinal cord injury often results in irreversible impairment of locomotor function implying inadequate limb movement or even paralysis. Though the restoration of the motor function is severely hindered after the injury successful experimental treatments require decent methods enabling us to assess the extent of functional restoration. Description of motor abilities reclines mainly on analysis of walking tract (footprint) providing relatively little information. To achieve a more reliable assessment we combined the use of video-based footprint and hind limb motion analyses after avulsion injury resulting in critical loss of motoneurons. Sprague-Dawley rats that underwent a lumbar 4 ventral root avulsion and reimplantation received embryonic spinal cord grafts or riluzole in order to prevent motoneuron loss. Control animals received no treatment. Functional deficits were estimated in a single video frames by measuring specific joint angles, footprints and gait parameters. Altogether 6 joint-related parameters of the hind limb and two angles of the paw along with the classical parameters of the footprint and gait were determined. The functional examination was followed by retrograde tracing of the reinnervating motoneurons employing Fast Blue. The numerical kinematic records were compared with the numbers of the reinnervating motoneurons. These preliminary results confirmed the strong relationship between functional restoration and morphological data.

P45. Movement related cortical potentials on corticography

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Knowledge of sensorimotor integration is essential to plan brain computer interfaces (BCI). Movement related cortical potentials (MRCP) have been studied for long, but intracranial sources have not been evaluated in details.

One epilepsy patient implanted with subdural macro electrodes (MAE) over the left central region was included in this study. Self-paced finger tapping (FT), and n. medianus somatosensory evoked potentials (SSEP) were recorded. Surface voltage maps were drawn. MRCP was averaged to the button press event.

FT induced peri-movement motor potential (MP) was visualized on the MAE array. The negativity lasted from -10ms to 50ms, and had the peak negativity postcentrally. The surface dipole configuration resembled the N20 component of intracranial SSEP.

MP negativity seems to localize to the postcentral region despite previous data provided in the literature. We plan to further investigate the sensory-motor integration, and try to compare it to preoperative high-density EEG results and time frequency analysis. The findings could be translated for BCI applications as the investigated MP can be used as a control signal.

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P46. Effects of tachykinin receptor activation on prefrontal cortex neuronal activity

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Tachykinins are neuromodulatory peptides found in the mammalian brain and high levels of tachykinin receptors are expressed on pyramidal neurons of the prefrontal cortex (PFC). The present study used the whole cell patch clamp technique and a mathematical model to investigate the ionic mechanisms underlying the responses of guinea pig PFC neurons to senktide, a tachykinin receptor agonist. Senktide (500nM) depolarized neurons, and at -70 mV in voltage-clamp, induced an inward shift of the holding current, a change that was accompanied by a decrease in membrane conductance. Current-voltage (IV) relationships showed that the senktide-sensitive current reversed at -91.6 mV, very near the calculated potassium equilibrium potential (E_K) of -93 mV. Elevations of extracellular potassium shifted this reversal potential in accordance with the Nernst equation. The response to senktide was similar to the response seen with extracellular cesium (5mM) or barium (200 μ M), known inhibitors of the inward rectifier potassium current ($I_{K_{ir}}$). Simulations using a biophysically-based neuronal model consisting of twelve differential equations showed that decreased $I_{K_{ir}}$ conductance yielded responses consistent with our biological data. We thus conclude that tachykinin receptor activation on PFC neurons may result in inhibition of the potassium current, $I_{K_{ir}}$.

P47. Neuronal responses to stationary and dynamic visual stimuli of the caudate nucleus in behaving cat.

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The caudate nucleus (CN), one of the input structures of the basal ganglia, is considered to play an important role in the control of oculomotor and skeletomotor processes. The CN neurons receive sensory information, which may represent the surrounding environment and integrate it with the motor inputs to refine the movements during motor tasks. The major novelty of the present study is that we developed an awake, behaving, head restrained, eye-movement controlled cat model where we could investigate the effect of stationary visual stimulus and optic flow as a dynamic visual stimulus on the activity of the CN.

Extracellular multielectrode recordings were carried out with 8 implanted platinum-iridium electrodes in the CN. The cat performed fixation on the center of the monitor and we applied during this visual fixation 'center in' and 'center out' optic flow stimulation. We categorized the CN neurons upon their electrophysiological properties (interspike intervals, autocorrelation of firing, firing rate and post-spike suppression) and investigated their activity during different epochs of the behavioural paradigm.

Altogether 53 putative medium spiny projection neurons (MSNs), 167 fast-firing neurons (FFNs) and 8 tonically-firing neurons (TFNs) were recorded from the CN. Neurons in each group possessed paradigm dependent activity changes. MSNs, FFNs and TFNs responded to stationary visual stimulation as well as to optic flow (dynamic) stimulation. Twenty (6 MSNs, 14 FFNs) of the 124 optic flow sensitive CN neurons (16,1%) were sensitive to the direction (center-in or center-out) of the optic flow. Furthermore the majority of the recorded CN neurons showed beside the visual activity responses during the reward period after the correct completion of the task.

We suggest that the visually active CN neurons may contribute to the control of initiation of reflexive saccades. CN neurons with increased activity enhance while CN neurons with decreased activity discontinue the tonic inhibition of the superior colliculus originating from the substantia nigra pars reticulata and thereby they may help keeping fixation or triggering a saccade.

P48. Areal and laminar distribution of Interneurons targeted by somatosensory cortical Afferents in the non-human Primate *Samiri sciureus*

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In areas 3b and 1 the precise somatotopic representation of the fingers is a major factor of the neural mechanisms of tactile functions. Neighboring distal finger pad representations form strong neuronal connectivity within areas 3b and 1. However, connections between areas 3b and 1 are largely confined to the homotopic fingertip representations. It is also known that inhibition is essential for optimal cortical functioning. However, the role of cortical inhibition in shaping tactile perception is not known. We used laser scanning confocal microscopy and neuronal tract tracing combined with multiple immunofluorescent labeling to examine the prevalence of close appositions between labeled afferents and different types of GABAergic interneurons. Of all labeled boutons identified 3.9% targeted immunolabeled interneurons. Parvalbumin (PV) positive interneurons were the most prevalent target of the labeled afferents. Somatostatin (SOM) and calretinin (CR) positive neurons were targeted less frequently. For horizontal connections within and between areas, SOM targeted afferents appeared more often in the supragranular layers. Only a slight supragranular dominance was found for intrinsic PV targeting afferents; however, inter-areal fibers were observed more frequently in the infragranular layers. These findings point to the intricate, regional and laminar specific role of GABAergic interneurons in the cortical processing of tactile information.

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P49. Effect of an AMPA receptor antagonist, Talampanel, on the calcium level of the motor nerve terminals of G93A SOD1 mutant mice

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Increased intracellular calcium 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). Earlier we demonstrated that the non-competitive AMPA-receptor antagonist (Talampanel; Ta), applied to transgenic mice with mutant Cu/Zn superoxide dismutase (SOD1 G93A) modeling familial ALS has significantly reduced intracellular calcium of spinal motor neurons in Ta treated presymptomatic transgenic group. To determine the effect of Ta in peripheral nervous system, the nerve terminals innervating interosseus muscle (nerve endings of vulnerable cell types in ALS) have been examined. Six and five G93A SOD1 mice were treated with Ta or Vehicle (Ve), respectively, both in early and late stage of the disease. Non-transgenic (NTG, C57Bl/6) mice treated either with Ta (n=6) or with Ve (n=5) served as control. Intracellular calcium levels in the neuromuscular junctions were estimated electron microscopically using the oxalate-pyroantimonate technique and were expressed as volume percent (mean $\pm$ s.e.m.) of the precipitates. Our preliminary results, obtained in presymptomatic mice show no differences in Ca-levels in different treatment groups (SOD1-Ta: 10.69 ± 1.77 ; SOD1-Ve: 14.12 ± 1.89 ; NTG-Ta: 12.27 ± 1.77). Studies analyzing the late-stage changes of motor nerve terminals of the vulnerable type are in progress.

P50. Response Characteristics of the Striatal Neurons to Visual Motion in Freely Moving Rats

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Earlier electrophysiological studies proved that neurons located in the dorsocaudal subfield of the caudate nucleus respond to moving visual stimuli; however these experiments were performed in anaesthetized animals. These results do not reflect the naturally occurring top down response amplification and filtering mechanisms of the intact brain, nor the interference arising from the sensorimotor feedback in the basal ganglia during self-motion. Moreover the population measures (e.g. simultaneous phase locking, monosynaptic interactions of single units, etc.) were not investigated due to the lack of simultaneous multichannel recordings. To overcome these challenges, in our study we trained a rat to run for water reward in a linear maze, where the walls and the floor were covered intermittently either with a homogenous gray painting, or with a black and white sinewave pattern. We use a dynamically changing environment, where the visual stimuli were projected onto an acrylic glass maze by the help of multiple mirrors. The grey colored wall was serving as a control condition, while the latter was perceived as a moving sinusoidal grating during the run along the track, resembling the stimulations used in the previous anesthetized experiments. The neuronal activity in the caudoputamen was recorded using either 4 movable tetrodes, or a 64 channel silicon probe. The neuronal responses were correlated with the temporal frequency of the perceived motion of the visual stimulus. The recorded cells were classified according to their putative cell types and the modulation of their firing rate by the oscillation of the local field potential. Our results suggest that 25 out of the 169 recorded cells were sensitive to the visual motion percept, and four of them were successfully encoding the phase of the motion percept in their firing pattern. In our currently running study in order to investigate the local striatal microcircuits, we are aiming to reconstruct the local networks of monosynaptically connected neuron pairs, and we study how the response properties are ‘inherited’ within these networks.

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P51. The investigation of visual categorization with low and high spatial frequency modified pictures

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Visual categorization is a basic function of cognition which allows us, people, to process the every-day information more effectively. Despite of the large amount of experimental results, many questions remain unclear about the neural basis of visual categorization. The main aim of this research is to get to know the impacts of the low and high spatial frequency modified pictures to the event-related potentials which appear between 0-200 milliseconds.

We had asked 10 people to make an animal-vehicle categorization task. We have shown them high and low spatial frequency pictures besides the original ones. We know that the magnocellular pathway is sensitive to the low spatial frequency and the parvocellular pathway is sensitive to the high spatial frequency so these modified pictures, which were used by us, enable us to investigate the subcortical visual pathways. We recorded a 32-channel EEG and assessed changes of the P1 and N1 visual event-related components at channels O1, O2, P7 and P8.

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P52. Effect of chronic restraint stress on nociception and behaviour in mice

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Experimental and clinical data suggest that chronic stress enhances pain sensation and plays an etiological role in some conditions characterized by clinically significant pain (e.g. fibromyalgia, irritable bowel syndrome). Changes in the central pain processing were extensively examined in patients with stress-related pain disorders, but the mechanisms and the role of peripheral nervous system is unclear. Thus, we studied the effect of chronic restraint stress (6 hours/day, 4 weeks) on nociception and behaviour of CD1 mice. Mechanonociceptive threshold and noxious heat sensitivity were measured with dynamic plantar aesthesiometer and hot plate test, respectively. Cold tolerance was determined by the measurement of paw withdrawal latency from 0°C water. Behavioural effects of chronic stress were examined in open field-, light-dark box- and tail suspension tests. Weight of the thymus and adrenal glands were also assessed. The role of capsaicin sensitive nerve endings was investigated with desensitization with resiniferatoxin (RTX), an ultrapotent capsaicin analogue. Chronic stress caused a significant mechanical hyperalgesia and cold allodynia. The decrease of thymus and increase of adrenal weight were accompanied by significant changes in acute stress-related behaviour. Very interestingly, pretreatment with RTX significantly increased the effect of chronic stress on nociception, suggesting a protective role of capsaicine-sensitive nerve endings in this phenomenon.

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P53. Sinusoidal signal propagation in layer II/III pyramidal neurons of the somatosensory cortex in wild type and Alzheimer disease related transgenic (Tg2576) mice

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Morphological changes of principal cells have been reported in human amyloid beta precursor protein overexpressing mutant mice, a widespread animal model of human Alzheimer disease. We aimed to investigate and compare the dendritic impulse propagation in mutant (TG) and wild type (WT) principal cells.

We used morphologically detailed passive segmental cable models of these cells (n=58) within the NEURON simulation environment. Sinusoidal (f=50Hz) current was injected to dendritic points of modelled TG and WT neurons to simulate local activity of synapses and the dendritic current and voltage transfers to the soma were investigated.

No difference was found in the rates of voltage and current transfers as a function of path distance from the soma in the apical dendrites of TG and WT cells. In the basal dendrites more differences (Mann-Whitney test, $p < 0.05$) were found in the rates of current transfers than in voltage transfers. When the distribution of dendritic surface (an approximation of the number of synapses received) of WT and TG cells was analyzed as a function of current and voltage transfers we found minimal but significant difference in the rates of voltage transfers both in apical and basal dendrites but current transfers were identical in the mutant and control principal cells.

The authors are grateful to Dr. Alán Alpár for the files containing the reconstructed morphology of neurons and for the OTKA K67747 and TÁMOP 4.2.4.A/2-11-1-2012-0001 for the financial support.

P54. Distinct Patterns of Synaptic Integration in the Dendrites of Tonic Spinal Lamina I and Lateral Spinal Nucleus Neurons

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Electrophysiological studies of lamina I show a high prevalence of ‘tonic neurons’. This classification refers to the periodic action potential firing of the neurons upon injection of a constant current in the soma. This artificial form of stimulation is unlikely in neuronal networks where synaptic activation exhibits complex temporal properties. In this work we question the functional homogeneity of the neurons in this class and show that ‘tonic’ neurons can display distinct features in terms of synaptic integration.

This work uses neuronal reconstructions to build computer models with detailed morphologies. Electrophysiological data is used to calibrate the membrane dynamics allowing the models to fit the firing-frequency response curves recorded experimentally. Assuming uniform passive properties in the dendrites, we showed important functional differences within the ‘tonic’ population. 1) spines (markers of potential synaptic contacts) show different spatial organizations suggesting differences in input sources. 2) analyses of voltage attenuation associated with the location of each spine (as a measure of synaptic efficacy) showed that while some neurons exhibit hot-spots with high efficacy spines, others have spines with a uniform spectrum of efficacies. Finally, computer simulations show that, distinct tonic neurons can respond differently to similar (same statistical properties) patterns of synaptic activations modeled as Poisson processes.

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P55. Low-Threshold Afferent Driven Inhibition to Spinal Lamina I Neurons

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Spinal lamina I is a key element of the pain processing system relaying primary afferent input to supraspinal areas. However, little is known about how the signal is modulated by its intrinsic network including anterolateral-tract projection neurons (ALT-PNs) and more numerous local-circuit neurons (LCNs). Here, we used whole-cell patch-clamp recordings in an isolated spinal cord preparation to examine properties of identified ALT-PNs and LCNs in their functionally preserved local networks. About a half of LCNs fired rhythmically (2-7 Hz) at zero current injection, and this firing persisted in the presence of blockers of fast synaptic transmission. In remaining cases, most ALT-PNs and LCNs fired tonically in response to depolarizing current injections. We also identified a subpopulation of ALT-PNs and LCNs receiving a low-threshold primary afferent driven inhibitory input, which in many cases was disynaptic. This direct inhibitory link between the low-threshold afferents and ALT-PNs can function as a postsynaptic gate controlling the nociceptive information flow in the spinal cord. We hypothesize that there may be dedicated inhibitory interneurons reliably excited by low-threshold primary afferents and possessing axon collaterals in lamina I. Our results provide new insights into how the superficial dorsal horn network controls the nociceptive information flow.

The work was supported by the grant from the Portuguese Foundation for Science and Technology funded by COMPETE and FEDER (BS), the Ciência-2007 and POPH-QREN programs (PSz) and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (PSz).

P56. Connexin-36 Distribution in the Cat Retina Follows the General Scheme of Other Mammalian Species

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Connexin-36 (Cx36) is the major constituent of mammalian retinal gap junctions (GJs) positioned in key signal pathways. Here, we were interested in the distribution of Cx36 punctate immunoreactions (IR) in the retina of the cat, a classical model of the visual system. Calretinin (CaR)-IR cell populations served to outline the nuclear and plexiform layers. In the outer plexiform layer, small Cx36-IR plaques were seen at the level of photoreceptor terminals that are likely sites of cone-cone and/or cone-rod GJs. Unlike in other species, strong IR was found along horizontal cell (HC) dendrites as well. In the inner nuclear layer, cell bodies of two amacrine cell (AC) populations were CaR-IR, the one with smaller diameters were identified as AII ACs. Interestingly, AII ACs showed diffuse Cx36-IR in their cytoplasm and punctate labels linked to the plasma membrane of the cell body. Although, Cx36 puncta were seen throughout the entire inner plexiform layer they showed significantly higher density and bigger average diameters in the ON sublamina. In particular, puncta that co-localized with varicose AII AC transversal processes showed uniquely large size and strong staining. These results are comparable with descriptions of Cx36-IR in both rodents and primates indicating its fundamental role in gap junction pathways of the mammalian retina.

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P56a. Monoacylglycerol lipase, a key regulator of nociceptive signaling, is localized within the superficial dorsal horn of the mouse spinal cord

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Monoacylglycerol lipase (MGL) is a serine hydrolase responsible for the metabolism of the major endocannabinoid 2-arachidonoyl glycerol (2-AG). Systemic or spinal inhibition of MGL is anti-nociceptive in models of acute and chronic pain via elevation of 2-AG. Recent evidence suggests that this also depletes arachidonic acid levels, blocking the synthesis of pro-nociceptive prostaglandins. Despite the dual analgesic potential of this approach, the regional, cellular, and sub-cellular distribution of MGL in spinal nociceptive circuits remains unknown. Here, we utilized immunohistochemical techniques to address this issue in mouse spinal cord tissue.

Immunoperoxidase staining revealed a dense accumulation of MGL within the superficial layers of the dorsal horn. Subsequent electron microscopic analyses revealed this staining to be within excitatory and inhibitory axon terminals. Double immunofluorescence staining was then performed for MGL and neurochemical markers of peptidergic (CGRP) and non-peptidergic (IB4) nociceptive terminals, and with markers of local excitatory interneuron (vGluT2) or inhibitory (VIAAT) terminals. The ratio and intensity of MGL-immunolabelling was highest in CGRP-positive peptidergic primary afferents, whilst little colocalization was observed with the other markers. The intensity of both CGRP-, and IB4-immunostaining was significantly reduced in MGL-knockout mice, suggesting a compensatory mechanism following chronic absence of MGL activity. These observations highlight the spinal nociceptor synapse as a potential anatomical site for the analgesic effects of MGL blockade.

P57. Differential involvement of fast spiking cell populations to distinct stages of sleep spindles in unanaesthetized, freely moving rats.

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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 stages and 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 labeling of cortical neurons in freely moving rodents. This technique allows stable recordings for up to 60 minutes from principal cells and up to 42 minutes for FS interneurons in layers 2 to 3 of the parietal cortex of Wistar rats. Anatomically identified pyramidal neurons fired sporadically at low rates during spindles. Firing patterns of fast spiking 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 with infrequent 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. Some FS cell could not be classified into these two groups. In general, FS interneurons showed no phase locking preference to theta cycles during REM sleep. Anatomical analysis of FS confirmed the presence of parvalbumin in the first and second group, but reconstruction of one example from each group indicates heterogeneity in the distribution of axons. Our results identify K-complex associated and unassociated groups of FS interneurons suggesting preferential involvement in corticothalamic/spindle inducing and corticocortical/spindle maintaining interactions, respectively.

P58. Caloric-Restriction and the Hippocampus: Ultrastructural Link Between Food-Intake and Synaptic Architecture

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Several lines of research is being devoted to reveal the neuronal mechanisms regulating food-intake. Behavioral patterns of food intake involves learning and memory functions. The hippocampus plays an important role in encoding relationships between internal states (e.g. hunger) and action, providing a mechanism by which motivation and memory are coordinated to guide behavior. Intestinal hormones (e.g. ghrelin) or fasting can generate synaptogenesis and physiological changes in the hippocampus. Learning can induce plasticity, however, it is obscure, whether metabolic conditions are able to cause such changes at the synaptic level. As activity-dependent ‘morphing’ of spines are linked to changes in synaptic efficacy, we studied spine rich areas (CA1 and dentate gyrus) known to be crucial in long term synaptic changes. Using quantitative electron-microscopy we analyzed the effect of caloric restriction (CR) on synapses. We found, that short-term CR induces significant ultrastructural changes in the architecture of synapses: the average length of postsynaptic densities and the ratio of perforations are significantly elevated. We also observed changes in the architecture of the spinoskeleton. Support: OTKA#K83830 and János Bolyai Research Fellowship

P59. Optogenetic stimulation of the median and dorsal raphe: effects on social behavior

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Stimulation of serotonergic (5-HT) neurotransmission elevates social interaction and decreases aggression. 5-HT neurons are located mainly in median (MR) and dorsal raphe (DR), but it is difficult to separate their functions. Our aim is to dissect their role in social behavior with optogenetic technique.

We injected channelrhodopsin to MR or DR with a help of adeno-associated virus (5nl). Two weeks later an optic fiber was implanted above the region to control and virus injected mice. On the next week the animals' behavior was examined in social interaction test, namely an intruder (20-day-old CD1 male) was introduced for 9 min to the operated resident (B16 male). During the intermediate 3 min mice were stimulated by 20 Hz at 473 nm (blue) and social and aggressive contacts were measured during the whole 9 min. Location of the virus and optical fiber was determined by immunocytochemistry.

Two groups was separated: „low anxiety” animals did not attack before stimulation, showed more social interactions and later occurring aggression. The „high anxiety” group attacked before stimulation, were less social and highly aggressive. Both MR and DR stimulation increased social interaction and diminished aggressive behavior in both groups. However, in the „high anxiety” group the reduction of aggression lasted shorter in DR than in MR stimulated animals.

Thus, we can conclude that both MR and DR has an anxiolytic-like effect on social interaction, however, the MR-induced changes last longer.

P60. Cognitive Enhancement Through Stabilization of the Hippocampal Network: The Role of Alpha7 Nicotinic Receptor Mediated Mechanisms as Indexed with *in vivo* Extracellular Electrophysiology

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One of the possible new strategies in drug development for Alzheimer's disease is the targeting of neuronal alpha7 nicotinic acetylcholine receptors (nAChR) with highly selective agonists. In our laboratory, a parallel behavioral study has confirmed that the alpha7 nAChR agonist PHA-543613 (PHA) exerts significant memory enhancer potential in a spontaneous alternation task in scopolamine-induced memory impairment in rats. However the underlying cellular mechanisms of such action still need to be investigated. As the hippocampus is one of the crucial structures in memory formation and retrieval and it is primarily affected in early stages of Alzheimer's disease, here we aimed to study the effects of PHA on maintained and NMDA-evoked firing of CA1 hippocampal neurons. Extracellular recordings combined with microiontophoresis were performed in anesthetized Wistar rats and the neurons were separated on the basis of their electrophysiological properties into "single spiking" and "complex spiking" groups. We have found that the effect of PHA administration on complex spiking neurons was dependent on the level of excitation prior to treatment, while single spiking neurons have responded mainly with firing rate decrease independently from their previous state. These results suggest that alpha7 nAChRs may play a crucial role in the physiological functioning of the hippocampal cellular network by maintaining a stable and optimal level of excitability for information processing.

P61. The role of arginine vasopressin in the regulation of impulsive behavior in virgin and lactating Brattleboro rats

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Recent studies suggest that arginine vasopressin (AVP) –besides its important physiological effects– plays a role in the modulation of behavior. AVP deficiency is associated with a specific behavioral profile, which is reported to be affected by the current neuroendocrine state of the animal. In the present study we investigated a novel element in this profile, impulsive behavior. Both virgin and lactating female AVP deficient Brattleboro rats were used to evaluate the impact of the neuroendocrine state during lactation on putative AVP-effects. The delay discounting paradigm was used to assess AVP effects on the ration and number of impulsive choice. We also observed whether AVP deficiency alters the known impulsivity-increasing effects of a benzodiazepine treatment (chlordiazepoxide, CDP). We showed that AVP deficiency did not alter impulsivity in virgin, but reduced impulsivity in lactating rats. We also found that CDP increased the level of impulsivity in virgins independently of AVP, but it only exerted such effects in AVP deficient dams. Based on these results we can conclude that AVP plays a role in the regulation of impulsivity of lactating females, but it does not have such effects in virgin rats. Moreover, AVP desensitized dams to CDP-effects on impulsivity, suggesting that AVP –at least partially– mediates dam impulsivity *via* tuning GABAergic mechanisms. Our results contribute to the knowledge on AVP regulation of impulsive behavior.

P62. Structural and functional alterations of the prefrontal cortex in post-weaning social isolation induced abnormal aggression

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Early-life social adversities (neglect, abuse) are profound risk factors for the development of abnormal aggression-related psychopathologies in adulthood. Post-weaning social isolation is a valid laboratory model to mimic early-life aversive environment. The prefrontal cortex is considered important in the regulation of aggression and exhibits severe functional and structural deficits in psychopathologies associated with aggressive behavior. In the present study we investigated the structural and functional alterations of the medial prefrontal cortex (mPFC) of male Wistar rats submitted to resident–intruder conflicts. Our results show that rats reared in social isolation delivered substantially more attacks which were poorly signaled and preferentially aimed at vulnerable body parts of opponents (head, throat and belly). We showed with immunohistochemistry that social isolation induced a decrease in the volume of the right hemispheric prefrontal cortex, while it decreased left hemispheric prefrontal glial density as well. While basal activation was significantly lower, aggressive encounters increased neuronal activation (indicated by c-Fos-immunolabeling) in the infralimbic and prelimbic subregions of the mPFC of isolation-reared rats. These regions send afferents to the mediobasal hypothalamus, the crucial point in the control of aggression. In summary, both childhood neglect in humans and social isolation in rats cause prefrontal deficits in conjunction with abnormal aggression.

P63. Hemokinin-1 plays an important role in anxiety and depression-like behaviours of mice

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The Tac1 gene-encoded Substance P (SP) acting at tachykinin NK1 receptor have been described to be involved in psychiatric disorders but NK1 antagonists failed in clinical trials. The Tac4 gene-derived hemokinin-1 (HK-1) binds also to NK1 receptors and is present in the brain. We investigated the roles of SP, HK-1 and NK1 receptors in anxiety, stress and depression-like behaviours in Tac1^{-/-} (lacking NK1 receptors), Tac1^{-/-} and Tac4^{-/-} gene-deleted mice compared to C57Bl/6 wildtypes (WT).

Anxiety and locomotor activity was evaluated in the light-dark box (LDB) and open field test (OFT) for 20 and 5 mins, respectively. In the forced swim test (FST) and tail suspension test (TST) immobility was assessed for 4 mins. Sucrose preference test (SPT) was performed for 2 days.

Tac4^{-/-} mice spent significantly less time in the light compartment of LDB, showed decreased motility in OFT, longer immobility in FST and TST and consumed less sucrose, while Tac1^{-/-} animals showed no difference to WTs, except for OFT. Tac1^{-/-} animals spent significantly more time in the light compartment of the LDB, spent more time with moving in OFT, were less immobile in FST and TST and consumed more sucrose compared to the WTs.

We provided the first evidence for the anti-anxiety and stress-coping effects of HK-1. These are not NK1 receptor-mediated, and a putative hemokinin receptor can be proposed. Identification of these targets might open new perspectives for anti-anxiety and anti-depressant therapy.

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P64. Juxtacellular recording and labelling of prefrontal interneurons during a rule switching task in freely moving rats.

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The medial prefrontal cortex is implicated in complex cognitive functions including decision making, rule switching and goal-directed behaviours. For this, the processing of information by the principal cells is thought to be controlled in time by GABAergic interneurons. The role of interneurons during network oscillations and temporal control has been studied in detail, but the contribution of fully identified interneurons during complex cognitive processing remains largely unknown.

To address this question, we recorded neuronal activity in the medial prefrontal cortex in freely-moving rats performing a repetitive decision making task with rule switching using juxtacellular recording and filling method. The neurons were identified based on their molecular expression profile, dendritic and axonal arborisations, and firing patterns during the task performance.

Distinct types of interneurons, including parvalbumin-expressing basket and axo-axonic cells were recorded and identified. The firing activity of these interneurons often correlated with the actual phase of every trial and in addition, dynamic changes in these patterns were observed across the trials when a strategy change was necessary to successfully complete the new rule.

Our results support the hypothesis that the information flow through the medial prefrontal cortex can be temporally and differentially modulated by distinct types of GABAergic interneuron.

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P65. Maternal motivation and lactation are influenced by thalamic tuberoinfundibular peptide 39

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Extensive maternal caring is critically important for the survival of a newborn offspring. In this study, we describe novel neuronal pathways containing a thalamic neuropeptide that are involved in the regulation of maternal responses.

We demonstrated that suckling activated TIP39 neurons in the PIL. Indeed, neurons in the PIL receive ascending information from the spinal cord and project to maternal brain centers including the preoptic area and the arcuate nucleus. For functional analysis of the projections, genetically modified lentivirus expressing an antagonist of the receptor of TIP39, the parathyroid hormone 2 receptor (PTH2R) was injected into the mediobasal hypothalamus of female rats. Infected cells around the injection site permanently express and release the PTH2R antagonist HYWY-TIP39. We observed a lower basal prolactin level and a significant reduction in suckling-induced prolactin levels in the PTH2R antagonist expressing dams. The same virus was injected into the preoptic area and conditioned place preference test was performed in virus injected mothers. PTH2R antagonist expressing mothers showed no preference for pup-associated chamber as did control virus injected dams suggesting reduced maternal motivation following blockade of endogenous TIP39 action.

In conclusion, TIP39 neurons are ideally positioned in the PIL to convey suckling information towards maternal brain centers to regulate different maternal responses in the postpartum period.

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P66. Feeding and metabolic consequences of IL-1 β microinjection into the cingulate cortex in the rat

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The cingulate cortex (cctx) has an important role in the maintenance of homeostasis. Single neuron recordings verified that interleukin-1 β (IL-1 β) responsive neurons exist in this cortical area. IL-1 β is a primary cytokine involved in the homeostatic regulation partly via cyclooxygenase (COX) mediated mechanism.

The present experiments were designed to investigate IL-1 β mediated regulatory mechanisms in the cingulate cortex. To do so, 1) blood glucose levels in glucose tolerance test, and 2) body temperatures, food and water intakes were measured (with or without the COX inhibitor paracetamol pretreatment) after administration of IL-1 β into the cctx of male Wistar rats.

The IL-1 β microinjection resulted in a significant increase of body temperature ($p < 0.05$, ANOVA), and the paracetamol pretreatment eliminated this hyperthermic effect. Blood glucose levels, food and water intakes did not differ significantly in the cytokine-treated and control groups.

Our findings indicate homeostatic significance of IL-1 β mediated control mechanisms in the cctx.

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P67. The role of vasopressin released from the paraventricular nucleus of hypothalamus in stress-related behavior: studies in Brattleboro rats

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Arginine-vasopressin (AVP) is a well-known regulator of the stress response. The secretion of adrenocorticotropin (ACTH) and corticosterone (CS) depend on its level and AVP also contributes to anxiety and depression. Brattleboro rats have general AVP deficiency resulting in reduced stress reactivity and depression-like behavior. The role of the AVP released from the paraventricular nucleus of hypothalamus (PVN) were studied by bilateral stereotaxic injection of an AVP-containing adeno-associated virus (AAV) vector to AVP-deficient rats. Body weight and water consumption were followed and behavior tests were conducted as follows: open field (OF; general locomotor activity); object recognition (OR; short term memory); marble burying (MB; novelty-induced anxiety) and forced swim test (FST; depression-like behavior). Finally, ACTH and CS level changes to urethane anesthesia were examined. AVP restoration in the PVN tended to normalize body weight and water consumption. In the AVP-treated group the number of line crossing increased, the memory disturbance was restored and the enhanced struggling were diminished compared to the alteration in AVP-deficient animals. Reduced ACTH level of AVP-deficient rats were also normalized without changes in anxiety and CS levels. Our results show that AVP released from PVN stimulate ACTH secretion and - in connection with this - has an impact on the development of depressive-like behavior. On the other hand it is a cognitive enhancer.

P68. Fate of Neurons in various Stages of Neurogenesis after Trauma

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Traumatic events disrupt hippocampal neurogenesis which is believed to underlie the long term learning deficits that develop in trauma-exposed animals and humans.

At the same time, however, the memory of the shock-associated environment is life-long; animals re-exposed to the shock-associated context readily shows fear-indicating behaviour, when tested at any interval after the traumatic event. In humans, traumatic experiences induce post-traumatic disorder, which persists for decades. The mechanisms underlying this life-long, imprinting-like memory process is basically unknown.

We hypothesize that (i) newly formed hippocampal neurons play a role in the consolidation and storage of trauma-related spatial cues and, consequently, (ii) they are highly preserved, enabling the retrieval of trauma-associated memories.

The final aim of this project is to establish the survival rate of neurons that are in various stages of neurogenesis at the moment of shock exposure. We expect that (i) the division rate of progenitor cells decreases after shocks, (ii) the survival rate of newborn neurons that are in early stages of development decreases, while (iii) the survival rate of neurons that are close to, or around the period of integration into hippocampal circuits increases. Our preliminary findings suggest that survival of the cells in these developmental stages depends from the rostro-caudal position in the dentate gyrus.

P69. Examination of the Role of Reward in Consumption of Highly Palatable Food in Intrauterin Underfed Rats

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Intrauterin undernutrition is a risk factor of obesity formed in adulthood. We assumed that hedonic component of food intake may be one aspect of the phenotype disposed to obesity. Intrauterin undernutrition was achieved by feeding the dams on a diet containing reduced protein (PR) from the conception to the delivery. Hedonic food intake was investigated by presenting sweetened condensed milk solution to control and PR rats at 10 weeks of age after a prior training. Drinking was allowed for 10 min, milk consumption was measured, parameters of drinking behavior were analysed. At the end, the animals were sacrificed and brains were processed for Fos immunohistochemistry. The number of activated cells in the subdivisions of the accumbens nucleus, a key center of reward was determined. Birth weights of PR rats were lower, but they gained more rapidly than controls. During the experiment PR rats drank more milk than controls, but duration of drinking and frequency of licking did not differ. However, quantity consumed correlated to drinking duration within both groups and to licking frequency in controls. The number of Fos+ cells was similar in the groups both in the accumbens core and shell regions. Still, there was a correlation between the drunken quantity and the number of Fos+ cells in the medial shell of nucleus accumbens within both groups and in the lateral core in PR rats. Data show that hedonic component plays a complex role in altered nutritional behavior of PR rats.

P70. 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. HFOs may serve as biomarker for epilepsy, also being capable to localize the epileptogenic zone.

We used laminar microelectrodes (MIE) to record potential gradient, current source density (CSD) and multi-unit activity (MUA) in wakefulness in 14 cases. Time frequency analysis was used to detect ongoing or event related spectral perturbations. ANOVA analysis was used for statistical analysis.

The majority of HFO events (over 90%) were coincident with interictal epileptiform discharges (IED). Such HFO bursts were maximal after the onset of the IID. Conversely, HFO was present only in 30% of all IEDs. When present, HFOs with a central frequency of 80-150 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 lower cortical layers. In general, this faster, deeper activity preceded the slower activity seen in superficial layers (10-30 ms). In addition, we often observed a long lasting (100-300ms) de-activation before the IIDs.

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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P71. Sleep spindles are modulated by network state

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Sleep spindles are 1-3 s long 7-15 Hz oscillations occurring mainly during stage II. sleep. They are generated by the interplay of excitatory thalamocortical (TC) and inhibitory thalamic reticular (nRT) neurons, though the finer mechanisms are still debated.

Our previous results show that the recruitment level of nRT population at spindle onset negatively correlates with the length of the oscillation. This may indicate that the nRT recruitment level directly determines spindle duration, or alternatively, that both factors are a function of the overall network level.

We tested these hypotheses by evoking spindles in PV-ChR or vGAT-ChR by optical stimulation of the nRT. The stimulus intensity/duration was varied in order to activate different size nRT populations. The experiments were done under urethane anesthesia, in which the level of network activity fluctuates between desynchronized and synchronized states, mimicking natural sleep.

The success rate of spindle induction varied with the level of anesthesia. No spindles could be evoked during desynchronized or highly synchronized states, only during weakly synchronized periods, also characterized by the presence of spontaneously occurring spindles. Within these epochs, the length of both spontaneous and evoked spindles covaried, but showed no correlation with the strength of the stimulus.

We conclude that spindle duration is not determined by nRT recruitment *per se*, but both depend on the general excitability of the network.

P72. Effects of chronically injected neuroprotective compound on spatial memory and hippocampal dendritic spine density in APP/PS1 transgenic mice

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The Alzheimer's disease (AD) is a neurodegenerative disorder characterized by amyloid-beta (A β) deposits and neurofibrillary tangles (NFT). Recently the common used AD transgenic (tg) mice strain is APP/PS1.

The mutations of the amyloid precursor protein (APP) and the presenilin 1 (PS1) are related with the production of elevated A β levels.

The aims of study to investigate the effects of a new neuroprotective compound (BRF) against the toxic effect of A β in spatial memory test and measure the density of the dendritic spines.

In this study we used 9 months old male APP/PS1 tg mice, in four experimental groups. Controls: wild- and tg-treated with physiological saline. Treated groups: wild- and tg-injected with BRF i.p at dose of 5mg/bwkg.

To measure the impaired spatial memory we used Morris water maze (MWM). The test conducted during 6 days: 5 training days and probe trial.

Significant different was found between the experimental groups in the MWM: particularly within the transgenic groups we found stronger neuroprotective effect. These transgenic mice learned significantly better in the MWM task than physiological saline treated tg group. Additional dendritic spine density was examined in hippocampus CA1 region. Significant different was found in the dendritic spine number within compound treated tg mice compared to tg control group.

Our results reflect that our developed chronically injected BRF protect against A β toxicity and memory disfunction.

P73. Nootropics Prevent the Effect of Scopolamine in an phMRI Provocation Model

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The effective treatment of cognitive decline is still a major challenge of the drug research. Scopolamine based small animal phMRI provocation model can be a suitable translational method for testing new compounds. First we studied the effect of scopolamine on BOLD responses in rat brain and the reversibility of its effect by nootropic agents.

Male Wistar rats weighing 240-260 g anaesthetized with isoflurane were used in the experiments. Test drugs were administered one hour before scopolamine. ROI analysis was performed comparing the effect of scopolamine on BOLD responses in drug pretreated or in vehicle treated animals in various brain regions.

Scopolamine strongly decreased the BOLD responses in the prefrontal cortex (PFC) but had no visible effect in other brain areas. Scopolamine's effect could be fully prevented with the donepezil pretreatment, whereas vinpocetin and piracetam caused a partial reversal of the BOLD responses only. The new vinpocetin derivative RG compound (compound X) was effective at much lower dose than that of the reference compound donepezil.

Functionally, the prefrontal cortex (PFC) of rats is implicated in working-memory, attention, response initiation and management of autonomic control and emotion. The strong negative effect of scopolamine on PFC well explains its memory disturbing effect. It could be prevented with nootropics in varying degrees. Further validation of the model with various nootropic and memory disturbing agents is in progress.

P74. Streptozotocin microinjection into the cingulate cortex alters glucose tolerance in the rat

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The cingulate cortex (cctx) has intimate interconnections with several glucose-monitoring (GM) neuron containing limbic forebrain structures already known for their involvement in the central feeding control. The main goal of the present experiment was to investigate whether streptozotocin (STZ) induced selective destruction of the GM cells in the cctx cause alteration of carbohydrate metabolism.

Glucose tolerance test (GTT) was performed in male Wistar rats 20 minutes following bilateral microinjection of STZ into the cctx (acute GTT), and 4 weeks later (subacute GTT).

The dynamics of the blood glucose level curves of the acute GTT proved to be different in the STZ treated and control groups. There was, however, no significant difference between the two groups in the blood glucose concentrations at the beginning and at the end of both GTTs. Our results support that the GM neurons of the cctx are involved in homeostatic control mechanisms.

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P75. Cognitive enhancer effects of an alpha7 nicotinic receptor agonist (PHA-543613) in pharmacologically induced transient amnesia models in the rat

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PHA-543613 is a potent and selective alpha7 neuronal nicotinic acetylcholine receptor (nAChR) agonist with good bioavailability and efficacy. It has been initially developed for the potential treatment of cognitive deficits in schizophrenia. However, its efficacy has not been directly addressed in models of chemically induced transient memory impairment. Here we aimed to test the memory enhancer potential of PHA-543613 in muscarinic receptor antagonist scopolamine or NMDA receptor antagonist MK-801 induced amnesia in Wistar albino rats. Spontaneous alternation in T-maze was used as it is considered as a reliable measure of spatial working memory and functioning of the hippocampus in rodents. Scopolamine (0.5 mg/kg) and MK-801 (0.1 mg/kg) caused similar decrease of alternation rate and both impaired overall maze performance. Prior administration of PHA-543613 (1 or 3 mg/kg) dose dependently improved symptoms and diminished scopolamine but not MK-801 induced impairment. The present results suggest that alpha7 nAChR agonist has good efficacy to alleviate working memory deficits caused by cholinergic receptor dysfunction but not those which may directly inactivate NMDA receptors of the rat hippocampus. In conclusion, as PHA-543613 effectively improved cholinergic deficits of spatial memory function it may be further explored as a potential specific reference molecule in animal models of dementia.

P76. Does intra-amygdaloid oxytocin have a pro-social effect in rhesus macaques?

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Mosher et al. have shown that the primate amygdala contains neurons that respond to looking at the eyes and to eye contact. Eye contact is enhanced (in both humans and non-human primates) by intranasal administration of oxytocin (OT). To determine whether the effect of OT is mediated by the amygdala, we injected bilaterally in the basal nucleus 100 ng OT and compared the time the monkeys spend looking at the eyes of other monkeys before and after OT administration. OT doubled and tripled eye contact and gaze following respectively. Further investigations are ongoing to confirm these pilot data.

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P77. The MAM-E17 rat model of schizophrenia: Behavioral examinations

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The purpose of our experiments was to establish and investigate the methylazoxymethanol acetate (MAM)-E17 rat model of schizophrenia with behavioral methods. Treatment of the pregnant rats on the 17th embryonic day (E17) results in schizophrenic symptoms in the offsprings. Various behavioral tests at three different ages were performed. For investigating the overall locomotor activity open field test (OPF) was used, examination of anxiety was studied in elevated plus maze (EPM), and for motor coordination skills the rotarod test was carried out.

As compared with the controls the MAM-E17 rats displayed significant hyperactivity in the OPF which was only detected after puberty. In EPM no differences were observed between the two groups. Performances in rotarod test exhibited significant increase in the MAM-E17 rats compared to controls depending on the age. Histological analyses showed decreasing in the volume and cell disorganization in the hippocampus.

The age dependent hyperlocomotion can be an evidence for validation of the schizophrenic model. The absence of anxiogenic or anxiolytic effects is in agreement with studies of others. The data received by the rotarod test, however, are in contradiction to previous findings. Here we report an increased performance which can be considered to show perseveration. Our results show that the MAM treatment was successful and it is supported by our preliminary histological analyses.

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P78. Simultaneous Electrophysiology and Ca-imaging of human cortical synchronous population activity *in vitro*

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Spontaneous synchronous population activity (SPA) emerges from the cortical slices of epileptic and non-epileptic patients maintained in physiological medium *in vitro*. SPA was recorded until now using sharp intracellular and laminar extracellular methods to analyze the neural mechanisms giving rise to population synaptic/trans-membrane and spiking activity.

In order to gain additional spatial information about the network mechanisms involved in the SPA generation, we introduced the two-photon Ca-imaging technique on human *in vitro* slice preparations. The excellent spatial coverage and resolution of this technique supplements the lower spatial resolution but higher temporal resolution of laminar extracellular, sharp intracellular and whole cell patch recording techniques.

Human slices were maintained in a dual superfusion chamber of high flow rate physiological incubation medium and otherwise conventional submerged technique to elicit SPA in a two-photon microscope. The population activity was recorded by laminar extracellular electrodes and with extracellular patch electrode. After identifying the active regions of the slice using electrophysiology techniques, bolus loading of OGB-1 and SR101 was applied on the tissue. The neuronal and glial cells took up these dyes, thus we were able to image the SPA related Ca-transients in pyramidal cells with two-photon technique, simultaneously with extracellular and whole cell patch measurements.

Combining high spatial resolution two-photon Ca-imaging technique and high temporal resolution extra- and intracellular electrophysiology techniques may permit a deeper understanding about the network properties of SPA in the human cortex.

P79. Conductance measurements of electrical fields generated by transcranial electrical stimulation (TES) in human cadavers

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Transcranial electrical stimulation can modulate the ongoing brain activity. The efficacy 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 homogeneity of the tissues and thickness of the skull, etc. In principle, TES can be also spatially selective 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.

We recorded TES-generated field potentials in 20 human cadavers. We used 4 independently isolated stimulation pairs to deliver sinusoidally modulated TES with various parameters (1 to 200 Hz, +1 to +7 V and phase shifts between the stimulation pairs) and with two different arrangements (linear and crossed alignment). 38 multiple-site electrodes were used to sample the field potentials in the brain.

Our results confirm that the brain itself is electrically inhomogeneous because the ventricles can shunt the current spread. We found that the high stimulation frequencies generated the largest intracerebral field gradients; however, the low frequencies generated currents could mainly spread up to the midline of the brain which indicates that the high frequencies have more local effect. The spatially focused patterns were achieved by only the phase-shifted and not by the crossed stimulation protocol.

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P80. Application of the three hit theory in PACAP heterozygote mice: Maternal separation and chronic stress influence BNST CRF and cpEW Ucn1 in an inverse manner

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According to the three hit theory genetic, epigenetic and stress factors lead to major depression. To model this, litters from pituitary adenylate cyclase-activating polypeptide (PACAP) heterozygote pairs were exposed to maternal separation (MS) vs. controls. Half of adult offspring was subjected to chronic variable mild stress (CVMS). The model's validity was determined by the measurement of hypothalamus-pituitary-adrenal (HPA) axis activity. The contribution of corticotropin releasing factor (CRF) producing neurons in the oval nucleus of bed nucleus of the stria terminalis (BNSTov), and central amygdala (CeA) moreover that of urocortin1 (Ucn1) neurons in the central projecting Edinger-Westphal nucleus (cpEW) was studied.

We hypothesized that mice carrying all risk factors will show maladaptation in the HPA axis, CRF and Ucn1 systems. Results indicate that CVMS most effectively increased adrenal weights and corticosterone titers in MS mice. MS mice upon CVMS had increased CRF-cell counts and specific signal density (SSD) in the (BNSTov). In the CeA, the CVMS-induced rise in CRF SSD was observed only in non-MS mice. In MS, the CVMS induced rise of FosB in Ucn1 neurons was abolished. The increased BNSTov-CRF and decreased npEW-Ucn1 neuronal activity suggests that the inverse alterations may contribute to the psychopathology. The three hit theory of depression seems to be applicable in PACAP heterozygote mice to study the pathophysiology of stress-related mood disorders.

P81. Effects of intraamygdaloid microinjections of RFRP peptides on passive avoidance learning in rats

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RFRP-1 and RFRP-3 (RFRPs) are members of the RF-amide peptide family. RFRP-positive nerve cells were detected in the rat hypothalamus and RFRP immunoreactive fibers were identified in the central amygdaloid nucleus (CeA) by immunohistochemical analyses. RFRP analogues bind with relatively high affinity to the NPFF1 and NPFF2 receptors. RFRPs have potent activity for NPFF-1 that is expressed in the CeA. The CeA, part of the limbic system, plays an important role in learning and memory processes. The aim of our study was to examine in the CeA the possible effects of RFRPs on passive avoidance learning. Male Wistar rats were microinjected bilaterally with 50 ng or 100 ng RFRPs. Other animals have received 20 ng NPFF-receptor antagonist RF9 alone, or RF9 15 min before 50 ng RFRP-1 treatment or vehicle solution into the CeA. Results showed that 50 ng RFRP-1 significantly enhanced passive avoidance learning, while 100 ng RFRP-1 was ineffective. Prior treatment with RF9 could block the RFRP-1 effects on passive avoidance paradigm. 50 ng or 100 ng doses of RFRP-3 had no effects on negative reinforcement. Our results suggest that intraamygdaloid RFRP-1 enhances learning processes and memory in aversive situations, and this effect can specifically be prevented by ANT pretreatment. Our results are the first to demonstrate that RFRP-1 plays important role in learning and memory processes in the CeA.

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P82. Cholinergic basal forebrain structures are not essential for the mediation of the arousing action of glutamate

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Cholinergic basal forebrain (BF) structures contribute to cortical activation and receive rich innervations from the ascending activating system. They are involved in the mediation of the arousing action of noradrenaline and histamine. Glutamatergic stimulation in the BF results in cortical acetylcholine release and suppression of sleep. However, it is not known to what extent the cholinergic vs. noncholinergic BF projection neurons contribute to the arousing effects of glutamate (GLUT). To elucidate the roles of cholinergic BF structures, we administered *N*-methyl-D-aspartate (NMDA), a GLUT agonist into the BF in intact rats and after destruction of the BF cholinergic cells by 192 IgG-saporin (SAP).

In 7 male Han-Wistar rats SAP was injected into the BF, the 7 control animals received artificial cerebrospinal fluid (CSF). Two weeks later a microdialysis probe targeted into the BF was perfused with CSF on the baseline day and with NMDA on the subsequent day. Sleep-wake activity was recorded for 24 h on both days.

NMDA exhibited a robust arousing effect both in the intact and the lesioned rats.

Thus, cholinergic BF structures are not essential for the mediation of the arousing action of GLUT.

P83. Brain serotonergic mechanisms underlying abnormal aggression resulting from early social adversities

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Here we studied the role of serotonergic mechanisms on postweaning social isolation-induced abnormal aggression in rats. The model was based on human observations linking early social neglect, exacerbated stress-responses and aggression-related psychopathologies in adulthood. We have earlier demonstrated that postweaning social isolation results in escalated and abnormal forms of aggressiveness and marked autonomic hyperarousal. Using qRT-PCR, we found profound changes in mRNA expression levels of the serotonin metabolizing enzyme MAOA in two key brain regions of aggression control, the medial prefrontal cortex (mPFC) and the medial amygdala: while MAOA mRNA levels were significantly higher in socially isolated rats under resting conditions, aggressive interaction-induced MAOA increase of socially reared rats was reversed in isolated rats in both brain regions. There were no differences in 5-HT1a receptor mRNA levels between groups, however, there was a significant negative correlation between 5-HT1a mRNA levels in the mPFC and medial amygdala that was accompanied by inverse correlations between plasma corticosterone levels and 5-HT1a mRNA levels in these regions in isolated rats. The 5-HT1a receptor partial agonist buspirone dose-dependently increased plasma glucocorticoid levels and decreased abnormal aggression of isolated rats. Our findings may help to explain the complex interaction between the serotonergic system, childhood neglect and adult violent behavior in humans.

P84. Abnormal aggression induced by early-life social adversity with a focus on acute and long-term epigenetic regulation in the medial amygdala and the medial prefrontal cortex.

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Social disturbances experienced during childhood often lead to psychopathologies accompanied by abnormal aggression in adulthood. Epigenetic mechanisms may play a fundamental role in conducting the effects of early-life social aversion but the issue has been poorly studied regarding abnormal aggression. Previously, we have developed an animal model where rats are subjected to post-weaning isolation, causing marked social deficit and aggression with increased attacks on vulnerable targets (head, neck, belly). We examined two enzymes that play a key role in epigenetic mechanisms, DNA methyltransferase-1 (DNMT1) and DNMT3a in two brain areas that are relevant in regulating aggression: the medial amygdala (MeA) and the medial prefrontal cortex (mPFC) using qRT-PCR. Results: in the mPFC the level of DNMT1 mRNA has decreased due to social isolation. The level of DNMT3a mRNA has not changed neither in the baseline state nor in animals that have fought. In the MeA the level of DNMT3a mRNA measured 1 hour after the fight correlated significantly with the level of aggression displayed and with the number of attacks aimed at vulnerable targets. In summary, early-life social isolation produces long-term epigenetic changes, in turn the aggression resulting from the isolation brings forth acute changes, supporting the claim that epigenetic mechanisms may play an important role in abnormal aggression caused by early social disturbances.

P85. Metabolic Effects of Local Microinjection of Streptozotocin into the Mediodorsal Prefrontal Cortex

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Intracerebral microinjection of streptozotocin selectively destroys the local GM neurons causing deficits of feeding and metabolism.

The aim of our study was to examine metabolic changes after STZ microinjection into the mediodorsal prefrontal cortex.

The glucose tolerance test was performed in 29 male Wistar rats, in the acute condition 20 min after the intracerebral microinjection of STZ or saline (acute GTT) and then, 4 weeks later (subacute GTT). Plasma total cholesterol, HDL, LDH, triglycerides, uric acid were determined 30 min after the microinjection.

Glucose intolerance of the STZ treated animals was found in the acute GTT. There was no significant difference between the two groups in the subacute GTT. Concerning the metabolites, significant decrease of the level of triglycerides was detected in the STZ group.

The present findings support our hypothesis that damage to the GM neurons elicits and maintains complex homeostatic disturbances.

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P86. The Effect of Different Hypoxic/Ischemic Stress Models on the Cortical Microcirculation – a Laser Speckle Imaging Study in Piglets.

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The newborn piglet is an accepted model to observe the effects of hypoxic/ischemic (HI) stress on cortical blood flow (CoBF) and neuronal injury. However, multiple HI stress models exist in the literature, but critical comparison among these models is lacking. The aim of the study was to examine different HI insults on the cerebral cortical microcirculation.

Anesthetized piglets (n=7) were equipped with remotely controlled vascular occlusion cuffs on both common carotid arteries. Using the closed cranial window with laser speckle imaging technique, we assessed the CoBF and pial arteriolar diameter changes during transient normoxic bilateral carotid artery occlusion (BCAO), hypoxia ($\text{FiO}_2=0,1$), hypoxia with BCAO, and asphyxia/reventilation.

Hypoxia ($\text{pO}_2=22\pm 2$ mmHg) elicited marked cortical hyperemia, robust vasodilation and increased flow velocity in the pial arterioles. Asphyxia ($\text{pCO}_2=87\pm 6$ mmHg, $\text{pO}_2=14\pm 3$ mmHg) resulted in significant reduction in CoBF. BCAO did not affect cortical perfusion or arteriolar diameters either during normoxia or hypoxia.

BCAO *per se* did not elicit brain ischemia and did not reduce hypoxia-induced hyperemia in the cortical microcirculation.

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P87. Effects of neurotensin microinjection in the ventral pallidum on anxiety

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Neurotensin (NT) is a neuromodulator in the central nervous system, which plays a role in learning and reinforcement and regulation of anxiety. The ventral pallidum (VP), innervated by the mesolimbic dopaminergic system, is also important in motivation, memory and reward related processes. In the VP neurotensin receptor 1 (NTS1) has been detected in high density. Our earlier experiments showed that NT in the VP has positive reinforcing effects, but its possible role on anxiety in this structure has not been examined yet.

In our present experiments the possible effects of NT in the VP were investigated in elevated plus maze test. Male Wistar rats were microinjected bilaterally into the VP with 100 ng or 250 ng NT in a volume of 0.4 µl. Control animals received vehicle.

While 250 ng NT was ineffective, NT in 100 ng dose significantly increased the time spent on the open arms and decreased the time spent on the closed arms. There was no significant difference, however, in the frequencies of visits of the different compartments. Our results prove that 100 ng dose of NT injected into the VP has an anxiolytic effect.

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P88. The role of D2 dopamine receptors of the ventral pallidum in motivational and learning processes

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The ventral pallidum (VP) is innervated by the mesolimbic dopaminergic system. It has been shown that VP dopamine receptors regulate the locomotor activity, however, less is known about the role of these receptors in rewarding and learning processes. The VP contains both D1 and D2 dopamine receptor subtypes.

In our present experiments the possible effects of D2 dopamine receptor agonist quinpirol administered into the VP on learning and rewarding processes were investigated. The following paradigms were applied: Morris water maze test (MWM), passive avoidance test (PAV) and place preference test (CPP). Male Wistar rats were microinjected bilaterally into the VP with 0.1, 1.0 or 5.0 µg quinpirol in a volume of 0.4 µl. Control group received vehicle.

In MWM the 1.0 µg dose of the agonist enhanced memory consolidation, but it did not influence the resistance of the consolidated memory trace against extinction. In PAV the 0.1 µg dose of the agonist improved both learning and retention. In CPP the agonist did not evoke place preference.

Our results demonstrate that VP dopamine receptors play an important role in learning processes.

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P89. Region-specific adenosinergic modulation of the slow cortical rhythm in urethane-anesthetized rats

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Slow cortical rhythm (SCR) is a rhythmic alternation of active (UP) and silent (DOWN) states during natural sleep and anesthesia. SCR-associated slow waves (“delta waves”) may be related to the homeostatic function of sleep. Adenosine is an endogenous sleep-inducing factor accumulating during prolonged wakefulness and sleep deprivation (SD). It may play a role in the delta power increment seen during recovery sleep following SD. In the present study, adenosine was administered topically to the surface of frontal and somatosensory cortices, respectively, of urethane-anesthetized rats to examine the direct effect of adenosine on UP and DOWN states.

Cortical local field potentials (LFPs) were recorded by a 16-pole vertical electrode array inserted near by the location of adenosine application. Multiple unit activity (MUA) was measured from layer V-VI in close proximity of the recording array.

In the somatosensory cortex, adenosine modulated the SCR with slow kinetics on the LFP level while MUA remained unaffected. Delta power increased in conjunction with beta activity superimposed on slow LFP waves characteristic to UP states. Delta power increment was based on increased frequency of state transitions as well as on increased height of UP-state associated slow waves. Adenosine did not affect SCR and MUA parameters in the frontal cortex.

These results show that adenosine may directly modulate SCR in a complex manner and its effect may show region-specificity.

P90. An Ascending Inhibitory Pathway from the Brainstem Synchronizes Thalamocortical Activity

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The co-operation of the brainstem, thalamus and the cortex is crucial both in consciousness and sleep. The well described brainstem reticular activating system (RAS) is responsible for making the thalamocortical system sensitive to incoming information by depolarizing its postsynaptic targets in the intralaminar (IL) thalamus and in the cortex. In a glycine transporter type 2 (GlyT2)- eGFP transgenic mouse line however, we found an inhibitory glycinergic-GABAergic pathway originating in the reticular pontine nucleus (PnO) of the brainstem. This ascending reticular inhibitory system (RIS) innervated the IL thalamus in both mice and humans indicating the evolutionary conserved nature of the pathway. RIS fibers exerted strong non-depressing synaptic inhibition on IL neurons via large multisynaptic terminals. Under ketamine-xylazine anesthesia GlyT2 positive PnO neurons fired rhythmic clusters of action potentials locked to frontal cortical (FC) slow oscillation and could be faithfully activated by cortical stimulation. Photoactivation of PnO terminals in the IL were able to induce a change from tonic to rhythmic firing in IL cells and synchronize thalamic and cortical activity. In freely moving experiments photostimulation of RIS fibers in IL in awake animals disrupted the actual behavior and resulted in motionless inactive period with the appearance of a synchronous 3Hz oscillation in the cortical local field potential. During sleep the same stimulation increased the power of slow oscillation (1-4Hz) frequency range in the signal. Together these data indicate the existence of a powerful cortico-brainstem-thalamus loop, which exerts strong synchronizing effect on forebrain activity both during awake and sleep states.

P91. Expression of primary ciliary markers in satiety centers of the brain

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Primary cilia are specific organelles of mammalian cells, they are called the antenna of the cell. Besides their multiple roles, they have been implicated in the regulation of central nervous system development and maintaining physiological neuronal function. Recently it has been suggested that primary cilia are required for the hypothalamic control of energy homeostasis and food intake. The aim of this study was to characterize the distribution of appetite related primary ciliary proteins in the feeding centers of the hypothalamus.

C56Bl/6 mice were processed for immunohistochemistry, the distribution of appetite related primary ciliary markers AC3, Sstr3 and Mchr1 in the hypothalamus was analyzed.

AC3 was the most abundant ciliary marker in the hypothalamus, it was present in the paraventricular nucleus, in the arcuate nucleus, in the ventromedial and dorsomedial nucleus of the hypothalamus. Sstr3 was strongly expressed in the ventromedial nucleus, only few neurons had Sstr3 positive primary cilia in the paraventricular and arcuate nucleus. The expression of Mchr1 was high in the paraventricular nucleus.

Neurons of hypothalamic feeding centers widely express appetite related primary ciliary markers in mice further supporting the role of primary cilia in the regulation of food intake. Their potential role in the development of pathologic obesity in human needs to be further investigated.

P92. Circadian and homeostatic regulation of slow wave activity under ultra-short light-dark conditions in rats

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Sleep is regulated by homeostatic and circadian factors. Increased need for sleep after sleep deprivation demonstrates *homeostatic* regulation. Suprachiasmatic nucleus (SCN), which is adjusted to the light information from the retina, provides *circadian* regulation, though light also affects sleep directly via fibers originating from the retinal melanopsin containing ganglion cells and terminating in VLPO (ventrolateral pre-optic area).

In our experiment, male Wistar rats were kept in LD1: 1 (1:1 light-dark) conditions. After two weeks, the daily rhythm of sleep time and EEG delta power was maintained, but the amplitude of their fluctuations decreased. It seems that the homeostatic sleep drive was weakened because of the evenly distributed sleep time during the 24 hour recording period.

Delta power (DP) during periods of deep sleep is determined by the incidence and magnitude of cortical slow waves. We have shown that light affects directly the appearance of slow waves. Light increased the incidence of slow waves and thus delta-power during deep sleep, even if the time spent in deep sleep decreased. In contrast, the amplitude and duration of slow waves did not vary significantly. The characteristic changes of cortical neuronal activity (MUA) during slow waves also were not significantly affected by the lighting conditions.

P93. Application of fear conditioning and fear extinction assay on anxious (Ax) and non-anxious (nAx) mice

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Patients suffering from anxiety disorder often show increased fear when encounter a new context. This phenomenon is also observable in mice. Two inbred mice strains having extremes in anxiety-dependent behaviour were tested in the context-dependent fear conditioning and fear extinction assay. This behavioural test is used for determining the basal anxiety level (measured as freezing) in two environmental contexts and for assessing the coping style of the animals upon introducing a cue dependent foot-shock.

It has shown earlier, that these mice employ different coping strategies in novel environmental context. Anxious mice (Ax) exhibited more freezing in the novel context compared to non-anxious mice (nAx).

Our trial is based on the well known Pavlovian conditioning protocol, complemented by a context change using different wallpaper and odours, fear conditioning, extinction and evaluation phases. Measures of anxiety were augmented by a clinically used anxiolytic, buspirone, which successfully decreased the fear response in anxious mice compared to untreated animals.

P94. Gut microbiota determines the level of anxiety in two inbred mouse strains

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Recent data have begun to shed some light on the ability of gut microbiota (GM) to influence behavior in mice and humans. Modification of microbiota by a combination of probiotics has been shown to reduce anxiety in animals and bring beneficial psychological effects with a decrease in serum cortisol in patients. We have developed two inbred mouse strains having differences in anxious phenotype as was determined by using elevated plus maze and open field. These mice also employ different coping strategies in novel environments. The gut microbiome of the two strains, determined by next-generation 16S rRNA gene sequencing of fecal metagenome extracts, showed significant differences, which was influenced by chronic anxiolytic treatment applied intraperitoneally. Gut microbiome was eliminated from another cohort of mice by gavage of antibiotics, and mice were recolonised with uncultured fecal microbiota from the other strain. Elimination and recolonisation was also verified by next-generation sequencing technique. AX mice with nAX GM showed decreased anxiety and active coping strategy. In contrast, nAX mice with AX GM exhibited increased anxiety and passive coping style. Control mice having received the GM of their strain showed no difference compared to untreated animals. Bioinformatic analysis revealed that the relative abundance of some bacterial species correlate well with the level of anxiety. These results shed light on the connection between gut-microbiome and mental health.

P95. Single unit analysis of auditory sensory gating in the medial prefrontal cortex (mPFC) in freely moving rats

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Prepulse inhibition (PPI) is an operational paradigm of sensorimotor gating, in which a weak preceding stimulus inhibits the motor response to an intense startling stimulus. By means of electrophysiological methods this phenomenon was studied to unravel the neural basis of PPI deficits which appear in several neuropsychiatric disorders such as schizophrenia. Single unit activities were recorded from chronically implanted tetrode electrodes in the mPFC. The digitalized wideband data were obtained and saved by a 64-channel ADC converter. The startle response was recorded by means of a 3-dimensional accelerometer and a microphone attached to the headstage and circuit board (PCB) microdrive. The acoustic stimuli were generated by open source audio editor software, sound card of a PC and amplified by a power amplifier. Data analyses of single unit (SU) activity were performed off-line using softwares. SUs were simultaneously recorded from the same electrodes used to record auditory evoked potentials (AEP) in awake behaving rats during PPI paradigm. SUs were grouped according to classes of their response. Data showed a tone- and phasic- related increase or decrease in firing rate. To our knowledge no other studies have examined the effects of PPI on SU level in the mPFC. SUs responded with different types of activations. Sensory gating contained both excitatory and inhibitory components. This evidence supports that the mPFC has crucial role in the inhibitory circuitry.

P96. Spatial variability of cortical ripples in humans

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Based on animal and human research ripple oscillations (80-500Hz) participate in epileptiform transformations. The cortical generators of such fast oscillations were demonstrated to be limited to a small area.

Two epilepsy patients undergoing invasive preoperative evaluation were selected in this study. Ripple oscillations were detected using automated threshold cutting method. Histograms of the involved cortical area were plotted. Seizure onset zone was determined by expert clinician, and was correlated with the ripple area. The purpose of this study was to describe the spatial variability of ripple oscillations and demonstrate that wide cortical area can contribute to their generation.

The majority of PRs appeared on 1 or 2 channels, but there was a significant proportion of the events presented on various number of channels, in some cases almost all channels. Wide range ripples were found more often in the proximity of the seizures. In this case long-tailed distribution was observed in the involved channel number histogram. Patient with wider ripple area had wide SOZ preventing resective surgery.

We demonstrated that relatively large cortical areas may contribute to the genesis of ripple oscillations, that area might vary significantly. Two population of small and wide range ripples were demonstrated. We think that the cortical extent of the ripples may be linked to their epileptogenic nature.

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P97. Effect of calcium-permeable AMPA receptor alterations on expression and extinction of conditioned fear in rats

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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 (Ca-AMPA receptors) by IEM-1460 might be involved in fear extinction.

First we compared four different extinction training protocols. Rats underwent extinction trials 24-h (I) or 28 days (II) after footshock once a day for 7 days (A) or 5 times a day for 3 days (B). Extinction occurred earlier when trials were more frequent; a significant decrease in freezing was detected at the beginning of the 2nd day of training at groups B and on the 5th day at groups A. Spontaneous recovery was observed at group I/A at 28 days after extinction.

For studying the effect of IEM-1460 (1 and 3 mg/kg i.p. 60 min before the first session), trials were conducted 24-h (I) or 28 days (II) after trauma, 5 times a day on the 1st day and one trial was given on the 2nd day. 1 mg/kg IEM-1460 reduced the expression of freezing at group I, and accelerated extinction at group II, which effect lasted at least 28 days, but was ineffective on freezing expression at group II and on extinction at group I. Moreover, the dose of 3 mg/kg had no effect at all.

Our in vivo data partly confirmed the involvement of Ca-AMPA receptors in the extinction of conditioned fear with a bell-shaped dose-response curve.

P98. Nesfatin-1/NUCB2: a potential new Regulator in the Sleep-Wake Cycle of Rat

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Nesfatin-1, the nucleobindin2 (NUCB2)-derived satiety peptide has been involved in the regulation of stress, emotions, and also in pathologies like depression, that is often accompanied by appetite and rapid eye movement sleep (REMS) abnormalities. In the dorsolateral hypothalamus (DLH), a key area of vigilance regulation, nesfatin-1/NUCB (nesfatin)-containing neurons are co-expressed with melanin-concentrating hormone (MCH), a neuropeptide also involved in food intake regulation and depression. As MCH is a known hypnogenic factor, we investigated the effect of nesfatin-1 on vigilance. Rats were subjected to a 72h REMS deprivation, followed by a 3h 'rebound sleep'. Nesfatin-1 mRNA and protein expressions as well as neuronal activity (Fos) were measured by *in situ* hybridization technique, ELISA and immunohistochemistry, respectively, in 'deprived' and 'rebound' groups compared to controls. We also analyzed vigilance by EEG in rats injected centrally with nesfatin-1 or saline. REMS deprivation reduced nesfatin-1 mRNA and protein levels; however, REMS 'rebound' reversed these to control levels. 'Rebound' caused an increase of transcriptional activity (Fos) in nesfatin+ neurons. Nesfatin-1 injected icv at light on reduced REMS, while increased passive wake for several hours and caused a short-term increase in light slow wave sleep. The potential role of nesfatin in sleep regulation may shed new light on the better understanding of nesfatin-1 in the pathomechanism of depression.

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P99. Twenty-Minute UMTS Mobile Phone Exposure does not Affect Cognitive Performance of Young Healthy Volunteers Evaluated on the Basis of the Stroop Color Word Test

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Universal Mobile Telecommunications System (UMTS) is a widely used mobile phone technology. Epidemiological studies revealed longer completion time for several cognitive tasks such as the Stroop task for those reporting higher number of daily mobile phone (MP) voice calls. Therefore, the aim of the present study was to examine the possible effects of UMTS radiofrequency (RF) exposure on cognitive performance of human volunteers in a direct manner using a provocative study. In two separate sessions the right side of the head of 27 (15 females) young healthy volunteers was exposed to either real or sham MP-like UMTS signal characterized by 1947 MHz carrier frequency, 5MHz bandwidth and maximum 1.75 W/kg specific absorption rate (SAR). In two experimental sessions, volunteers performed a computerized Stroop color word test before and after real or sham RF irradiation. Differences in performance were analyzed with repeated measures of ANOVAs with median RTs (ms) as the dependent variable. Reaction times differed statistically in task-related factors as *condition* (congruent/incongruent/neutral), *order* (pre/post exposure) and *task* (word-naming/color-naming). However, no main effect of the factor *exposure type* (real/sham) was found. We conclude that a single 20 min UMTS exposure does not affect selective attention and executive function of healthy young volunteers measured by RTs of Stroop test in a double blind, counterbalanced within-subject experimental design.

P100. Learning and memory in patients with temporal lobe epilepsy and hippocampal sclerosis

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Implicit sequence learning has been associated with activation of the fronto-striatal brain circuitries. In contrast, recent results suggest that the Medial Temporal Lobe (MTL) is also involved in this fundamental learning mechanism – though this area is generally linked to the encoding and consolidation of explicit memories.

The aim of the present study is to investigate the role of the MTL in implicit sequence learning, furthermore to have a better understanding on how different brain networks work and interact with each other. We administered the Alternating Serial Reaction Time (ASRT) Task in the evening and after a twelve hour retention period in order to measure two components of implicit learning: sequence specific and general skill learning. Here we show the results of nine patients with temporal lobe epilepsy whom all had been implanted with electrodes, and were under a 24 hour video EEG.

We found significant sequence specific and general skill learning in the learning phase. After the offline period, they showed significant retention of the sequence specific knowledge. In contrast, the general skills declined in the testing phase.

Results until this point indicate that the different aspects of implicit sequence learning are differently affected by temporal lobe epilepsy. Our ongoing study can lead us to a better understanding of the interactions between brain networks underlying memory formation and consolidation.

P101. The effects of chronic stress on the composition of the gut microbiome

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Bidirectional communication between the central nervous system and the gut is essential to maintain homeostasis. Chronic stress results in long lasting alterations in neuroendocrine and behavioral regulation similar to those seen in depression. Depression is often associated with various gastrointestinal symptoms implicating central effects on the gut microflora. The aim of this study was to reveal chronic stress-induced changes on the gut microbiome.

C57BL/6 mice were exposed to early maternal separation stress and to a 3-week chronic variable stress paradigm (CVS) in the adulthood. Chronic stress resulted in anhedonia and increased anxiety-related behavior. CVS increased adrenal weight, but did not change plasma corticosterone levels and CRH mRNA expression in stress-related brain areas.

Four dominant intestinal bacterial phyla were quantified in cecum and colon samples of control and stressed mice by real time PCR measurement of phylotype specific 16S rRNA primers. Chronic stress resulted in a significant increase of Bacteroides, Firmicutes and Coliforms in the cecum, however the abundance of Bifidobacteria decreased.

These data demonstrate that chronic stress specifically targets intestinal microbiota by increasing the relative number harmful bacteria and decreasing probiotic phyla.

P102. Orexigenic effect of QRFP-26 in the medial hypothalamus is caused by interaction with NPFF receptors.

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RFamide-related peptides are known to be involved in the regulation of feeding and body weight in rodents. The recently identified QRF neuropeptide (QRFP-26), member of the RFamide peptide family, acts as an endogenous ligand of the orphan G protein-coupled receptor GPR103 and possibly interacts with NPFF2 receptors as well. Available data suggest that QRFP-26, administered i.c.v., has a tendency to increase food consumption, especially intake of the food with high fat content. By means of immunohistochemical methods QRFP-26 binding sites were identified in the hypothalamus, an important feeding regulation center.

Male Wistar rats received unilateral microinjections of vehicle or QRFP-26 (100 ng/side) or equimolar dose of NPFF receptor antagonist BIBP 3226 into the ventromedial hypothalamic nucleus (VMH). After peptide administration milk with normal fat content was introduced to the animals, and liquid food intake was measured over 60 minutes period. QRFP-26 administration into the VMH increased liquid food intake. At the same time receptor antagonist BIBP 3226 prevented orexigenic effect caused by the peptide.

This study indicates that QRFP-26 directly acts on hypothalamic NPFF receptors to modify feeding and mechanisms of hunger and satiety. Further research regarding GPR103 receptor antagonists is necessary to unravel detailed receptorial mechanisms of QRFP-26.

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P102a. Spatial and temporal processing of cooperator and defector facial expressions – Event-related EEG and fMRI experiments

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Our aim was to investigate whether specialized cortical brain regions are activated when participants evaluate complex social facial expressions of co-operation or deception.

Twenty-nine healthy volunteers (16 females) participated in an event-related fMRI experiment, and twenty-two volunteers (11 females) participated in an event-related EEG experiment, where standardized grayscale co-operator and defector facial images (taken previously in socioeconomic game) were briefly presented as visual stimuli. In both experiments, brain responses (BOLD signal and canonical ERPs) evoked by face stimuli were analyzed.

Results of the experiments revealed that the *occipital face area* (OFA) showed higher bilateral activation and the *fusiform face area* (FFA) showed higher activation with left-sided dominance to co-operator faces, and that co-operator faces evoked more negative N170 component, indicating that cooperator faces were processed as a preferred, natural perceptual category. In contrast, defector faces evoked higher ERPs compared to neutral ones approximately 200 ms after stimulus presentation over fronto-central electrode sites, and also induced higher activation in the *prefrontal cortex* (BA9), which may reflect the greater covert attention allocated to them as a non-salient perceptual category.

Results, for the first time, suggest the existence of distinct cooperator and cheater-detector modules in the human neocortex.

P102b. Chronic stress affects the structural integrity of specific GABAergic neuronal subpopulations in the hippocampus of rats

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We studied the stress-induced morphological changes in the hippocampal GABAergic network using the chronic mild stress model, which is one of the best validated animal models of depression. Rats were subjected to six weeks of daily stress and behaviorally characterized into anhedonic and resilient animals, based on their response to stress in the sucrose consumption test. Different subtypes of GABAergic interneurons were visualized by immunohistochemistry using antibodies for parvalbumin (PV), cholecystokinin (CCK) and calretinin (CR). We quantified these neurons in the different subareas of the dorsal and ventral hippocampus respectively. Compared to control animals, chronic stress significantly reduced the number of PV+ and CR+ neurons, mainly in the dorsal hippocampus, whereas CCK+ neuronal numbers remained unaffected.

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P102c. Changes in PACAP Levels following Environmental Enrichment in the Central Nervous System of Rats

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a pleiotropic and multifunctional neuropeptide. Numerous studies have shown that PACAP is involved in the development of the central nervous system, and has neuroprotective effects. Environmental enrichment has also demonstrated to be protective in various injuries. A few studies have suggested that trophic factors are involved in the protective mechanisms exerted by environmental enrichment. The interaction between PACAP levels in the brain and environmental effects has not yet been studied.

Wistar rats were divided into two groups: control group and environmental enrichment group. PACAP27 and 38– like immunoreactivity was measured with a specific and sensitive radioimmunoassay in brain samples. Enriched environment has the most beneficial effects in new born animals, and these rats have the highest rate of neuroplasticity. We did not know whether enrichment has any effects in adulthood. The second part of the experiment was to investigate two groups of adult rats: newborn enriched and adulthood enriched animals, both group were examined in adulthood.

Environmental enrichment started at birth led to decreased levels of PACAP in several areas of the brain (brain stem, cerebellum and different areas of the telencephalon). When animals were kept in enriched environment after birth, then put them back under regular circumstances, and 3-6 months later checked their PACAP levels, we have found it also decreased. But when the rats were kept under regular circumstances and then in adult age we put them into environmental enrichment for a week, their samples showed higher PACAP levels.

Environmental enrichment causes changes in the PACAP levels of the central nervous system. The childhood effect of environmental enrichment seems to decrease the level of PACAP, and it shows the same pattern in adulthood as well, but the only adult exposure of enrichment leads to increases in PACAP immunoreactivity.

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P102d. The effect of caffeine on delay discounting in rats

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In the delay discounting paradigm, rats learn to choose between two reinforcers that differ in amount and delay. They can choose either a small immediate or a large but delayed reward. Impulsive choice is defined as the preference for the small immediate over the delayed reward. The decrease in subjective value with the increasing delay is named as temporal/delay discounting. Impulsivity as a cognitive domain is a substantial component of ADHD, pathological gambling, substance abuse. To alleviate symptoms in ADHD pharmacologically, methylphenidate is a first-choice treatment. Psychostimulants increase the choice of the delayed reinforcer in delay discounting procedures in rats as well. Recently we started the establishment of this paradigm in the Behavioural Pharmacology Laboratory.

We tested d-amphetamine, methylphenidate and atomoxetine at various literature-based doses, but we only obtained controversial results. Then we analyzed the effect of the most widely used legal psychostimulant in the world, caffeine, on temporal discounting. In accordance with earlier findings, caffeine significantly increased the choice of the delayed reward, i.e. decreased impulsive decision making.

Delay discounting is a useful cognitive model to screen drugs affecting cost-benefit decision making. We demonstrated that caffeine decreases impulsivity in delay discounting paradigm in rats, which result can be used as reference in further studies.

P102e. Responding to social and non-social challenges: the involvement of the hypothalamus-pituitary-adrenal-axis in 2-arachidonoyl-glycerol effects

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The behavioral effects of enhanced 2-arachidonoyl-glycerol (2-AG) signaling *via* JZL184-induced monoacylglycerol-lipase (MAGL) blockade highly depend on environmental conditions. As the hypothalamus-pituitary-adrenal-axis (HPA) is differentially activated in diverse contexts, we studied its possible involvement in MAGL blockade-induced behavioral changes in CD1 mice facing social or non-social challenges, respectively, which situations differentially alter the activity of the HPA-axis. We showed that enhanced 2-AG activity caused anxiolytic effects when subjects faced non-social challenges (i.e. the elevated plus-maze) and these effects were partially caused by enhanced basal HPA-axis activity induced by MAGL blockade. When animals faced social challenges (i.e. an “intruder” in their home cage or a “resident” in its home cage, respectively), enhanced 2-AG signaling remarkably decreased aggressive behavior and increased HPA-axis activity during aggressive conflict. The behavioral effects were independent of the basal HPA-axis activity-enhancing effects of 2-AG. These results demonstrate that the HPA-axis is differentially involved in the effects of enhanced 2-AG signaling on responses to social and non-social challenges, respectively. In the former, 2-AG alters the response by an intrinsic mechanism, and abolishes aggressive behavior, while it affects responses to the latter *via* enhanced HPA-axis activity as a secondary effect and causes anxiolysis.

P102f. Nucleus Accumbens and Orbitofrontal Cortex: Endogenous and Exogenous Chemical Responsiveness of Neurons in the Rat

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The nucleus accumbens (NAcc) and the orbitofrontal cortex (OBF) play integrative roles in the central regulation of feeding. Since previous studies demonstrated the importance of glucose-monitoring (GM) neurons in these processes, our main goal was to characterize the endogenous and exogenous chemical sensitivities of these chemosensory cells.

In the present study, extracellular single neuron activity was recorded in the medial (mOBF) and lateral OBF (lOBF) as well as in the NAcc of adult male rats by means of tungsten wire multibarreled glass microelectrodes, during 1) microelectrophoretic administration of various chemicals, 2) intraoral gustatory stimulations, and 3) intragastric infusion of monosodium-L-glutamate, D-glucose and NaCl.

In all three examined areas, differential neurochemical sensitivity of neurons, and their distinct taste responsiveness to intraoral and intragastrical stimuli were observed.

Our data demonstrate that the chemosensory cells in the NAcc and OBF monitor and integrate signals from the internal and external environments. The GM neurons here are suggested to be crucially important in adaptive mechanisms of the organization of feeding.

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P102g. Schizophrenia test battery in Brattleboro rats

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Schizophrenia is a complex psychiatric disorder with 0.3–2.7% prevalence worldwide. It has many subtypes with different levels of the symptoms, like a spectrum disorder. We need animal models to understand its neurobiology and develop new effective drugs. Most rodent models have behavioral phenotype changes that resemble positive-like symptoms, but fewer models show altered social interaction, and learning and memory impairment, analogous to negative and cognitive symptoms.

Our aim was to establish a schizophrenia test battery in our laboratory representing wide range of symptoms and to validate the tests with the Brattleboro strain and antipsychotic (Clozapine) treatment (subcutaneous or intraperitoneal injection before the tests). Previous studies suggested this strain (with diabetes insipidus phenotype) as a good schizophrenic model based on the prepulse inhibition of startle (PPI) test.

We compared AVP-deficient (-/-) Brattleboro rats to +/- or +/+ ones. The following tests were used: open field, separation-induced ultrasonic vocalization of pups (USV), social avoidance, social interaction and discrimination, object recognition, PPI.

The AVP-deficient animals were hyperactive, vocalized significantly less, showed less sociability, worse learning and social memory and significantly lower PPI. These deficits were normalized with Clozapine treatment. The pups' USV test can be a good measurement for strain models and for new drug testing (low weight-small amount of drugs).

**P102h. The role of prefrontal cortex to lateral hypothalamus projections in abnormal aggression
fotoactivating the axon terminals of PFC in lateral hypothalamus**

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Previous studies from our laboratory indicated that prefrontal cortex (PFC) projects to lateral hypothalamus (LH) and we hypothesize that this pathway plays role in the regulation of aggressive behaviour. Although aggression has been investigated in a variety of models, optogenetic provide novel approach to study the role of prefrontal cortex to lateral hypothalamus (LH) projection in aggressive behaviour. Light-sensitive membrane proteins labelled adenovirus are able to activate neural activity over behaviorally-relevant timescales. Using these light-responsive tool, the actions of PFC neurons can be dissociated from other inputs of LH.

We investigated our hypothesis in the paradigm, where the virus was injected to the PFC and the optrod was implanted to the LH. The axon terminals of PFC was stimulated on 20Hz by laser light through the LH during a resident-intruder test. The length of the test was 10 min with a 3 min long stimulation in the beginning.

Interestingly, the number of attacks did not change, but the ratio of bites aimed at vulnerable targets (head and throat) increased by stimulating the axon terminals of prefrontal cortex in LH. These results are in line with some previous findings, which highlight the PFC to LH pathway as an important component of aggression. Moreover suggest marcant role of LH in predatory aggression, where the attacks also tend to these targets.

P102i. Blockade of CB₁ receptors and 5-HT_{2C} receptors causes opposing explorative behavior and locomotor activity effects in rats

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Background Antagonists of cannabinoid-1 (CB₁) and serotonin 2C (5-HT_{2C}) receptors have anxiogenic and anxiolytic properties, respectively. Accordingly, effects of these drugs on behavioral tests of anxiety including also social interaction test (SIT) are the opposite. This test is used to assess anxiety-related behavior in rodents by using their natural tendency to interact with an unfamiliar partner. The locomotor response to novelty is suggested to be linked to the mechanisms of stress. Beyond horizontal locomotion, the response to novelty is also characterized by vertical activation, namely rearing, which is considered to reflect exploratory activity and emotionality. Our aim was to establish the effects of the CB₁ receptor antagonist AM-251 and the 5-HT_{2C} receptor antagonist SB-242084 on line-crossing and rearing in the SIT.

Methods Male Wistar rats were used in SIT applying low light-familiar arena conditions. Pairs of rats were injected i.p. with the same drug: SB-242084 (1mg/kg) or AM-251 (5mg/kg) or vehicle. Behaviors of rats were recorded, and the duration of rearing and the number of line-crossing were evaluated.

Results The number of line-crossing was significantly enhanced by SB-242084 and the duration of rearing was markedly attenuated by AM-251 compared to the control.

Conclusion Our results support the opposite effects of the CB₁ and the 5-HT_{2C} receptor antagonists on anxiety, although the differences in the two tests cannot be neglected.

P103. Cell-type specific feed-back excitation of parvalbumin and cholecystokinin expressing inhibitory cells in the basolateral amygdala

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Amygdala is a brain region, which has a key role in fear and extinction memory formation. During information processing in the basolateral amygdala (BLA), local inhibitory cells provide an effective control of principal cell (PC) function, thereby shaping the output of the amygdalar network. However, the features of the excitatory synaptic inputs onto interneurons are largely unknown. To characterize the local synaptic excitation onto the two main types of inhibitory cells in the BLA, the parvalbumin (PV) expressing, fast spiking and the cholecystokinin (CCK) expressing, regular spiking GABAergic cells, we obtained recordings in the monosynaptically-coupled PC-PV and PC-CCK pairs in acute slices prepared from PV/eGFP and CCK/DsRed transgenic mice, respectively. Using whole-cell patch-clamp technique, three or five action potentials were evoked in a presynaptic PC, while the excitatory postsynaptic currents were recorded in the postsynaptic interneuron. Our preliminary results showed that the magnitude of the postsynaptic events recorded in PV cells (n=5) was larger and the failure rate was lower compared to those connections observed between PCs and CCK cells (n=6). The probability of the connectivity between PCs and PV cells was higher than that found between PCs and CCK cells. Our observations propose that these two distinct types of inhibitory cells are differently embedded into the local feed-back loop of BLA networks.

P104. Horizontal Spread of Low Frequency (<1Hz) Activity Induced in *in vitro* Slice Preparation of Entorhinal and Perirhinal Cortices

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During slow wave sleep the membrane potential spontaneously fluctuates between two states (<1Hz): up state is the active phase, when the plasma membrane is depolarized and action potential can be elicited; down state is the inactive phase. The goal of our study was to define the area which triggers the low frequency oscillation and to study the spread of activity in connected cortical regions of rat brain.

We investigated this slow oscillation in *in vitro* limbic cortical slice preparations measuring field activity with glass micropipettes. Up states registered *in vitro* have similar characteristics as those registered *in vivo*.

We studied the relationship between the field activity of entorhinal and perirhinal cortices. Multi- and single unit activity was identified in the field recordings, and correlation between field events and single cell firing was evaluated. According to our findings the frequency of up states is the same in both brain areas investigated. Additionally, up states registered from the perirhinal cortex generally followed the activity generated in the entorhinal cortex. Beside this, single unit activity was strictly correlated with field events.

Our results indicate that these up states generated *in vitro* correlate with action potentials. We suppose that the entorhinal cortex controls the activity of limbic structures, where the spontaneous activity is generated and which in our horizontal slices spread toward the perirhinal cortex or further in whole brain.

P105. Observation of the correlated ganglion cell activity in the mouse retina by using Ca^{++} imaging.

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Retinal ganglion cells display spontaneous activity even in the absence of light stimulation. Individual ganglion cells, however do not present this activity in isolation but correlate it to their ganglion cell neighbors. We investigated the temporal precision of this correlated activity as well as the spatial extent of correlated ganglion cell assemblies by imaging the Ca^{++} dynamics of several dozens of neurons simultaneously. We found that ganglion cells often displayed Ca^{++} -transients that are presumably corresponded to action potential bursts. These transient $[\text{Ca}^{++}]$ changes were shown among nearby cells and also between ganglion cell somata as far as 500 microns. Transient Ca^{++} signals of nearby ganglion cells often showed precise synchronization to similar events of neighbor neurons. Such synchronous activation of neighbor cells suggested the presence of common synaptic inputs that synched the activity of cells. The putative presynaptic cell could be an excitatory bipolar cell and/or an inhibitory amacrine cell. In addition to precise temporal synchronization we often observed sequential activation of ganglion cells with time delays of 200-400 msec. Interestingly, sometimes the somata of these sequentially activated ganglion cells lined up in the same direction suggesting that the activating input was carried by a long radially running dendrite of a presynaptic wide field amacrine cell. The observed correlated ganglion cell activity indicates that retinal ganglion cells are not individual encoders but certain features of the visual scene are coded by population activity.

P106. Morphological evidence for direct glycinergic input to cholinergic neurons in the mouse basal forebrain

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Glycine is a major inhibitory neurotransmitter in the mammalian central nervous system and an important regulator of glutamate neurotransmission; its extracellular concentration is regulated by glycine transporters (GlyT-1 and GlyT-2).

The aim of the present study was to reveal whether cholinergic neurons in the basal forebrain are targets of glycinergic neurotransmission. Double-label immunohistochemical approaches were used to reveal by confocal or electron microscopic analyses, whether cholinergic neurons are in morphological relationship with GLYT1- and/or GLYT2-immunoreactive (IR) cells or immunoreactive for glycine receptors (GlyRs).

Immunoreactivity for GLYT-2 (the marker protein of glycinergic neurons) was detected in varicose axons. The appositions on ChAT-IR neurons in the HDB were shown to establish either symmetric or asymmetric synapses. Presence of GlyRs in HDB cholinergic neurons was confirmed. At the ultrastructural level, the GLYT-1-immunoreactivity was detected in glial elements; in some cases these GLYT-1-IR structures were found in close proximity to dendrites and cell bodies of the cholinergic neurons receiving synaptic inputs.

Our results suggest that glycine has a direct influence on basal forebrain cholinergic neurons by demonstrating (1) synaptic connections between glycinergic and cholinergic neurons (2) GlyRs in cholinergic neurons and (3) strong presence of glial and neuronal transporters in the vicinity of cholinergic neurons.

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P107. Effect of median-raphé-manipulation on the hippocampal network

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The median raphe (MR) is a major source of the ascending serotonergic system involved in neuromodulation related to eg. mood and stress. Previous studies have shown a fast and vigorous glutamatergic component of the MR-hippocampal pathway. The aim of this study was to decipher the role of MR-manipulation in the regulation of the hippocampus in acute and freely moving animals.

Adeno-associated viral vectors, containing channelrhodopsin-2 tagged with mCherry or YFP, were injected into the MR of C57Bl/6 mice. After 3-4 weeks, a 32-channel linear or multishank probe was implanted into the dorsal hippocampus for sampling the network response of the MR neuron light activation through an optic fiber. One week after, the MR was illuminated by blue light at different frequencies in freely moving animals. Photostimulation was performed 1 day, 1 week and 2 weeks after first MR stimulation.

Preliminary data show that all stimulation frequencies evoked layer-dependent response in the hippocampus composed of two or three components. However, the stimulation failed to induce state transitions. In chronic recordings, the power of theta oscillations dramatically decreased by 20 Hz light pulses and weak entrainment of the hippocampal network was detected by 8 Hz stimulation. The amplitude and duration of evoked potentials were affected in linear track recordings.

In conclusion, light-activation of the raphe-hippocampal connection exerted a state-dependent modulatory influence on the hippocampus.

P108. Frequency and firing pattern dependent short-term plasticity of IPSCs on perisomatic region targeting interneurons (PTIs)

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Perisomatic region targeting interneurons (PTIs) control the activity of principal cells and are themselves under GABAergic influence. They control the output of their target cells and contribute to network activity generation. Moreover, the properties of inhibition conducted by PTIs are target-cell specific.

To characterize the synaptic dynamics of PTI-PTI pairs, we performed paired recordings among PV+ and among CCK+PTIs in the CA3 region of the mouse hippocampus. In both cases, presynaptic cells were stimulated by a train of 10 action potentials with frequencies varying between 1 and 320 Hz, and properties of inhibitory currents (IPSCs) and short-term plasticity were calculated.

We found, that PV+BC mediated IPSCs on PV+BCs had shorter latency and T50 value of decay time compared to PV+BC-pyramidal cell pairs. IPSCs among PV+BC pairs displayed frequency-dependent short-term depression.

Next, we compared the synapses between CCK+BCs-pyramidal cells and CCK+BCs. IPSCs recorded on CCK+BCs had shorter latency and T50 value of decay time compared to IPSCs on PCs. CCK+BC-CCK+BC pairs displayed frequency-dependent short-term facilitation, and asynchronous release appeared at frequencies higher than 10 Hz suggesting, that CCK+BCs may act as a low pass filter of synaptic inputs.

Our results highlight the differences of synaptic transmission among different PTIs. These interactions could be generally important in the generation and maintaining of physiological oscillations.

P109. Cholinergic and Endocannabinoid Neuromodulatory Effects Overlap on Neurons of the Pedunculopontine Nucleus of Mice

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The pedunculopontine nucleus (PPN) is part of the reticular activating system (RAS) and one of the main sources of the cholinergic fibers in the midbrain. Several neuromodulatory mechanisms were described in the PPN, but overlaps of these mechanisms have not been shown yet.

In our study we observed that PPN neurons respond to carbachol in a heterogenous way: they can be depolarized, hyperpolarized or lack response. The effect of carbachol was similar to our previous observations with type 1 cannabinoid receptor (CB1R) agonists; therefore, we investigated whether different neuromodulatory effects elicit the same or completely different actions on the same neuron. In order to achieve this, after application and washout of carbachol, the CB1R agonist ACEA was used and the changes of the membrane potential and firing rate were detected. A marked but not full overlap was revealed: all neurons depolarized by carbachol were depolarized by ACEA, as well; and all neurons lacking response to carbachol lacked response to ACEA. However, neurons hyperpolarized by carbachol were depolarized, hyperpolarized or not affected by ACEA.

These observations suggest that different neuromodulatory mechanisms have similar but not identical effects on neurons of the PPN, which might be responsible for the differences of the effects of these drugs observed in in vivo experiments.

P110. Synaptic targets of GABAergic fusiform cells in the primary visual cortex of the cat.

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GABAergic neurons represent a diverse neuronal population in the cerebral cortex, each type providing synapses to a particular region of its target cells' surface, e.g. soma, proximal dendrites, distal dendrites, dendritic spines. Of these, one the least known cell type is the so-called fusiform cell that has been implicated in mediating inhibition to distal dendritic branches and calcium spike generation of pyramidal cells with high efficacy. Here we studied the synaptic target distribution of fusiform cells in layers 2-5. All cells were revealed using Golgi-impregnation. Sixteen fusiform cells were selected based on the completeness of impregnation of axonal and dendritic processes, which were reconstructed at x1000 magnification. Each cell was tested for GABA content using postembedding GABA-immunolabelling on semithin somatic sections. Ten cells showed GABA immunopositivity, four cells were negative and for one cell the immunolabelling was inconclusive. To test the nature of synaptic targets, a correlated light- and electron microscopy was carried out on three fusiform cell axons. Altogether 83 synaptic junctions were identified each of which established type 2 (symmetric) synapse. Of these, 73.5% (n=61) were found on small diameter dendritic shafts receiving no, or only few synapses in addition to the one from the fusiform cell. Dendritic spines represented 24.1% (n=20) of synaptic targets examined, whereas 2.4% (n=2) of the synaptic targets could not be identified unequivocally. The lack of somata, axon initial segments, large caliber dendrites amongst the postsynaptic targets and the fact that none of the target dendrites showed features of GABAergic neurons leads to the conclusion that fusiform cells preferentially innervate the small distal dendritic branches of presumed glutamatergic neurons, such as pyramidal and spiny stellate cells. Further studies aim to elucidate whether GABAergic fusiform cells also innervate other GABAergic cells.

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P111. Synapses Established by the Median Raphe Contain Glutamate Receptors in the Mouse Forebrain

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The typically serotonergic median raphe nucleus (MR) takes part in the subcortical modulation of cortical information processing, however our workgroup showed recently, that MR establishes fast and precise, partly glutamatergic excitatory synaptic contacts with a specific subpopulation of GABAergic interneurons in the hippocampus (HC). These synapses contain vesicular glutamate transporter type 3 (vGluT3) and AMPA-type glutamate receptors. Because NMDA receptors (NMDARs) play an important role in excitatory transmission, we tested, whether MR synapses can act via NMDARs in its different target areas. Using double immunohistochemistry against vGluT3 and NMDAR in the HC, we found that at least 90% of these synapses are NMDAR-positive, while they lack the GABAergic marker neuroligin-2. Using anterograde tracing and NMDAR immunohistochemical staining, we showed that at least one-third of the synapses established by the MR in the HC, medial septum (MS) and prefrontal cortex (PFC) contain NMDARs. Finally, using double retrograde tracing, we found that there are cells in the MR that innervate the HC and PFC or MS and PFC simultaneously. These observations suggest that MR can act via fast glutamatergic NMDA receptors not only in the HC, but also in MS and PFC and these areas may be synchronously stimulated by the same MR neurons.

P112. Different availability of potassium currents distinguishes two functionally distinct populations within CCK-expressing perisomatically-targeting interneurons in the CA3 area

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Specific functions of various voltage-dependent ionic channels contribute to the functional divergence of GABAergic cell types by resulting in different excitability properties. We distinguished two populations of CCK-expressing GABAergic cells (CCK-IN) in the CA3 region of the hippocampus based on the presence or the absence of a potassium current that leads to state-dependent firing properties using recordings from anatomically identified GABAergic cells in acute slices. We found that in a large subset of CCK-INs, action potentials are inhibited at the onset when the firing patterns were evoked from hyperpolarized membrane potentials. However, this transient outward rectification (TOR) was absent in the same cells during firing patterns from depolarized membrane potentials. Interestingly, the second subset of morphologically indistinguishable CCK-INs did not show state-dependent inhibition of action potential generation (non-TOR cells). Voltage-clamp recordings revealed that TOR CCK-INs activated substantial A-type potassium currents below their action potential threshold, whereas in non-TOR cells, potassium currents were available only above the threshold potential. Altogether, the results revealed that different availability of inactivating potassium conductances distinguishes two functionally different populations among anatomically homogeneous CCK-INs in the CA3 region and results in state-dependent firing in one of the subpopulations of CCK-INs.

P113. Influence of P2X7 Receptor on Spine Synapse Density in the Dentate Gyrus

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Our main purpose is to explore the potential relationship between the purinergic P2X7 receptor (P2X7R) and major depressive disorder. In previous behaviour tests P2rx7 ^{-/-} mice displayed an antidepressant-like phenotype, as they did not express the typical symptoms of depression.

The hippocampus is highly sensitive to detrimental effects of depression concerning memory and learning processes, therefore loss of hippocampal spine synapses is a revealing feature of the disorder. Our study aimed to compare the amount of spine synapses in the dentate gyrus of mice with functional and invalidated P2X7R. The invalidation of P2X7R was obtained both genetically, using P2rx7 ^{-/-} mice and pharmacologically, by administering the P2X7R selective antagonist Brilliant Blue G (BBG) intraperitoneally (4 mg/kg, for five days). An electron microscopic stereological analysis was performed to determine the density of spine synapses in the molecular layer of the dentate gyrus in P2rx7 ^{+/+}, ^{-/-} and BBG treated mice. This method proved to be precise and reliable for synapse counting.

The results indicate significantly lower spine synapse density when the P2X7R was blocked, both in P2rx7 ^{-/-} mice and BBG treated mice as well. Further experiments are to define whether these baseline data may change after having evoked a depression-like state with the learned helplessness paradigm, thereby confirming the suspected connection between P2X7R and major depressive disorder.

P114. Anatomically diverse populations of CB₁ receptor-expressing interneurons provide similar GABAergic control along the somato-dendritic axis of CA3 pyramidal cells

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We investigated a subpopulation of GABAergic cells in cortical structures that expresses CB₁ cannabinoid receptors (CB₁) on their axon terminals. First, we determined the ratio and the origin of CB₁-expressing inhibitory boutons in the CA3 region of the hippocampus. Using immunocytochemical techniques, we estimated that approximately 40 % of GABAergic boutons in different layers of CA3 also expressed CB₁. To identify the inhibitory cell types expressing CB₁ in this region, we recorded and intracellularly-labeled interneurons in hippocampal slices. CB₁-expressing interneurons showed distinct axonal arborization, and were classified as basket cells, mossy-fiber-associated cells, dendritic-layer-innervating cells or perforant-path-associated cells. In each morphological category, a substantial variability in axonal projection was observed. In contrast to the diverse morphology, the active and passive membrane properties were found to be rather similar. Paired recordings of inhibitory postsynaptic currents made by basket or mossy-fiber-associated cells onto pyramidal cells were large and fast, while synaptic events obtained in dendritic-layer-innervating interneuron-pyramidal cell pairs were smaller and slower, most likely due to dendritic filtering. Our data suggest that CB₁-expressing interneurons with different axonal projections may contribute to a similar proportion of GABAergic inputs along the somato-dendritic axis of CA3 pyramidal cells.

P115. Synapse-Specific Distribution of Neuroligin-2 in the Hippocampus

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Neuroligin 2 (NL2) plays a critical role in the maturation and proper function of GABAergic synapses and they form a trans-synaptic signal transduction complex and mediate a bidirectional signaling between the presynaptic axon and the postsynaptic target. Different populations of GABAergic hippocampal interneurons play different roles in the local network activity; therefore, NL2 may have a different contribution to these synapses. We examined two distinct GABAergic interneuron populations in the CA1 region of the hippocampus: the parvalbumin- (PV-) positive and CB1 endocannabinoid receptor positive basket cells. Using electron microscopy, we reconstructed their synapses and using preembedding immunogold method, we quantified their NL2 content. We found that the density of NL2 was 50% higher in the synapses established by parvalbumin positive, compared to those made by CB1 positive interneurons. On the other hand, the total amount of NL2 content is higher in synapses established by CB1 positive interneurons. The exact function of NL2 is still unknown, but its higher density in the synapses of PV positive cells may be essential for maintaining its fast spiking activity.

P116. Pharmaco-genetic manipulation of odour discrimination in freely moving mice

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Pharmacogenetic techniques enable cell type-specific and reversible manipulation of neuronal networks without physical attachment of any device. This uncompromised freely moving state of the animal compared to fiber- or wire-attached or head restrained states allows the most intact observation of the induced behavioural changes. One of the pharmacogenetic approaches is based on the rescue of the zolpidem sensitivity of zolpidem-insensitive mutant GABA_A receptor $\gamma 2$ subunits. Purely transgenic implementations of this technique showed various successes, mainly due to the low activity of the cell-type specific promoters. Our alternative approach is to use AAV based gene delivery and CMV promoter for strong, but non selective expression of the zolpidem-sensitive GABA_AR $\gamma 2$ subunit and Cre recombinase. To test the effectiveness of the virus mediated zolpidem-sensitivity rescue in olfactory behaviour, we have virus injected the main olfactory bulbs (MOB) of mutant mice. Trained mice were capable of discriminating odour mixes up to 70% similarity, and this ability was not affected by the *per os* treatment with 0.1 g/bwkg zolpidem tartarate. In control mice, this treatment caused severe impairments in movement coordination, demonstrating effective zolpidem concentration in the brain. Our results suggest that high level, but not cell type-selective viral expression of the zolpidem-sensitive GABA_ARs is not effective enough to evoke large and behaviourally significant changes in the MOB.

P117. Strategically positioned inhibitory synapses of axo-axonic cells potentially control spiking of amygdalar principal neurons

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Axon initial segment (AIS) is a key region of neurons, where the generation of action potentials can be controlled most effectively by GABAergic inputs. It has been shown that in the neocortex and hippocampus the AIS of principal cells (PCs) is innervated mainly by axo-axonic cells (AACs), however much less is known of the properties of AACs in the basolateral amygdala.

In this study we recorded from monosynaptically connected pairs of AACs and PCs in amygdalar slices. Perforated patch recordings showed that the majority of AACs had inhibitory effect, since the reversal potential of postsynaptic responses was below the resting membrane potential of the postsynaptic neurons. Using whole-cell recordings, our measurements uncovered that AACs could powerfully inhibit or postpone the spiking of PCs depending on the timing of presynaptic cell firing. Using simultaneous soma and axon bleb recordings we measured that action potentials are generated predominantly between 20-50 μm from the soma along the AIS of PCs. Correlated light and electron microscopic analysis showed that AACs preferentially contacted the AIS with the highest density in the region where the action potentials are generated.

Our data revealed that the synapses of AACs are strategically positioned to reduce the probability of AP generation in the output neurons of the basolateral amygdala. Thus, these GABAergic interneurons are in the position to powerfully influence the memory formation in this cortical area.

P118. Abundance of D-aspartate in regions of different proliferative activity in postembryonic chick brain.

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L-aspartic acid (L-asp) has neurotransmitter properties in the central nervous system of various vertebrates. Our earlier results suggest that L-asp and L-glutamate (L-glu) are co-released in the striatum. Unlike D-glutamate, D-asp is also synthesized in the brain of mammals and birds by the asp-racemase enzyme. Relatively high concentration of D-asp during development implies that D-asp plays a role in the early development of the brain. D-asp likely modulates adult neuroplasticity, probably through regulation of neurogenesis. In the present study, the concentration of L-glu, L- and D-asp were measured in samples from brain regions relevant to passive avoidance learning (intermediate medial mesopallium, medial striatum, arcopallium, as well as the subventricular proliferation zone (SVZ), lateral nidopallium and cerebellum of young and adult domestic chicks. Homologous regions of adult rats were also analysed. Capillary electrophoresis coupled with laser induced fluorescent detection was used to quantify the two enantiomers extracted from tissue homogenates or dialysates collected from implanted microdialysis probes. The effects of age and passive avoidance training were investigated. D- but not L-asp concentration decreased during postembryonic development, suggesting that its developmental action still persists after hatching. There is a species difference between adult rats and chicks in D-Asp (but not L-Glu or L-Asp) concentrations. We were unable to detect learning related changes in any of the measured amino acid enantiomers in chicks. D- but not L-asp showed higher concentration in the SVZ as compared to a telencephalic region with lower proliferative activity. L-glu showed no difference between the two brain regions. Abundance of D-asp in a proliferating brain region supports a specific involvement of D-asp in the neurogenesis of young domestic chicks.

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P119. The Cannabinoid Receptor 1 Antagonist Rimonabant Directly Inhibits Delta Opioid Receptor Specific Ligand Binding and Agonist-stimulated G-protein Activity

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What is known: There is a growing number of evidence showing that the cannabinoid receptor 1 (CB₁) antagonist rimonabant can affect the opioid system. The direct effect of rimonabant on opioid receptors has been studied so far mainly on μ -opioid receptors. However recently the δ -opioid receptor (DOR) receives much more attention as before, due to its potential therapeutic applications in nociception or treatment for psychiatric disorders.

Objectives: To investigate the direct effect of rimonabant on DOR specific ligand binding in radioligand competition binding experiments in membranes of Chinese hamster ovary cells stably transfected with mouse DORs (CHO-mDOR). Furthermore to examine the DOR G-protein efficacy and DOR activator ligand potency in the presence of rimonabant in agonist-stimulated [³⁵S]GTP γ S binding assays in CHO-mDOR and in wild type and CB₁/CB₂ double knock-out mice forebrain membranes.

Results: In CHO-mDOR cell membranes micromolar concentrations of rimonabant inhibited DOR specific binding, G-protein efficacy and also the potency of the DOR activator ligand during receptor stimulation. The two latter results were also observed in wild type and CB₁/CB₂ double knock-out mice forebrain membranes. The decreased efficacy and potency in the presence of rimonabant were proven to be DOR specific since rimonabant did not have any effect on epinephrine-stimulated α_2 -adrenergic receptor G-protein activation in CB₁/CB₂ double knock-out mice forebrain membranes.

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P119a. Possible role of parvalbumin-positive interneurons in epileptiform events

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Epileptiform events represent a pathological high activity network state in the hippocampal brain slice. Compared to physiological activities neuronal firing becomes strongly altered during these events, with the most robust effect on fast spiking basket cells. Since this cell type was shown to be the most active during physiological activities it probably has an important role in regulating network functions. Consequently, it is essential to know, whether its activity can modulate epileptiform events. To address this question, we applied a miniature dissection into str. pyramidale/lucidum in area CA3 and recorded field activity from both sides of the cut. By cutting into the axonal arbor CA3 was divided into two subnetworks of fast spiking basket cells, where inhibitory, but not excitatory connections were dissected. As a result we found a change in the dynamics and shape of epileptiform events, indicating the importance of this cell type in driving epileptiform activity. To further investigate the role of fast spiking basket cells, we optogenetically stimulated parvalbumin-containing neurons (channelrhodopsin was expressed under the control of the parvalbumin promoter). Upon light pulses, these cells fired and epileptiform events could be evoked.

Still, it remains unknown whether these cells contribute to event generation by hyperpolarizing postsynaptic cells, or by the loss of hyperpolarizing effect, due to strong depression of synaptic release after repetitive activation. Support: Hungarian Scientific Research Fund (OTKA K83251 and OTKA 81357), ERC-2011-ADG-294313 (SERRACO)

P119b. The role of the GAD67 in the gamma oscillations

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The coherence of gamma oscillation between local and distant structures is well correlated with cognitive functions in the visual system. The parvalbumin-containing, fast spiking GABAergic interneurons are important for the genesis of gamma oscillation. Its high GABA level is maintained by GAD67 and GAD65 enzymes. Most of the GABA supply is maintained by GAD67, however it is unclear whether the GAD67 is fundamentally needed for generating gamma oscillations or enough GABA can be produced by the synaptic GAD65. To answer this question we tested the visual steady state responses evoked by 25 Hz flashing LED light as the model of induced gamma band activity in PV+ GAD67 knockout (KO) mice *in vivo*. Control (Balb/c) mice present stable gamma band oscillations, and most likely can precisely track the 25 Hz light during stimulation. In contrast, in GAD67 KO mice this gamma oscillation becomes clearly unstable and fragmented in time and frequency as well. Our results suggest that GAD67 is an essential enzyme in maintaining stability of gamma oscillations of the GABAergic interneurons in the visual cortex and thus GAD67 might be a major target in revealing the pathomechanisms of mental disorders with gamma decrease, like schizophrenia and bipolar disorder.

P119c. Patterns of sharp-wave ripple complexes in the rat hippocampus in vitro

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Hippocampal sharp wave-ripples (SPW-Rs) occur during slow wave sleep and behavioral immobility and are thought to play an important role in memory formation. We investigated the cellular and network properties of SPW-Rs with simultaneous laminar multielectrode and intracellular recordings in a rat hippocampal slice model. Spontaneous SPW-Rs were generated in the dentate gyrus, CA3 and CA1 regions. These events were characterized by a local field potential gradient (LFPg) transient, increased fast oscillatory and multiple unit activities. Two types of SPW-Rs were distinguished in the CA3 region based on their different LFPg and current source density (CSD) pattern. Type 1 (T1) displayed negative LFPg transient in the pyramidal cell layer, and the associated CSD sink was confined to the proximal dendrites. Type 2 (T2) SPW-Rs were characterized by positive LFPg transient in the cell layer, and showed CSD sinks involving both the apical and basal dendrites. In both types, consistent with the somatic CSD source, most pyramidal cells were hyperpolarized, while most interneurons increased firing rate before the LFPg peak. T1 and T2 recurrence frequency was decreased when reducing the activity of parvalbumin-positive perisomatic interneurons. Further suppression of cannabinoid receptor-positive basket cell firing blocked the remaining SPW-R activity. Our results suggest that the CA3 neuronal network can generate multiple patterns of population activities in vitro. Our data indicate the importance of inhibitory cells, particularly the perisomatic cells in the SPW-R generation.

P120. The effect of urocortin I on the hypothalamic ACTH secretagogues

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Urocortin I (UCN I) is a structural analogue of corticotropin-releasing factor (CRF), which, together with arginine-vasopressin (AVP), are the principle adrenocorticotrophic hormone (ACTH) secretagogues in mammals. The aim of the present experiments was to investigate the effect of UCN I on the hypothalamic CRF and AVP and its impact on the hypothalamic-pituitary-adrenal (HPA) axis. First, male Wistar rats were injected intracerebroventricularly (ICV) with 0.5, 1, 2 and 5 µg of UCN I. After 30 minutes, hypothalamic CRF and AVP and plasma ACTH and corticosterone concentrations were determined by immunoassay and chemofluorescent assay. Second, rats were pretreated ICV with selective antagonists of CRFR1, CRFR2 and AVPR3 (0.1 µg antalarmin, 1 µg astressin 2B and 0.1 µg deamino-Pen¹,Tyr²,Arg⁸-vasopressin, respectively) and treated ICV with the most effective dose of UCN I (5 µg). After 30 minutes, plasma corticosterone concentration was determined by chemofluorescent assay. UCN I induced dose-dependent increase of the hypothalamic CRF and AVP concentration, which were associated with dose-dependent increase of the plasma ACTH and corticosterone concentration. The most significant effect of UCN I on the plasma corticosterone concentration was abolished by antalarmin, but was not influenced by astressin 2B or deamino-Pen¹,Tyr²,Arg⁸-vasopressin. The present experiments demonstrate that UCN I modulates the concentration of the hypothalamic ACTH secretagogues and, in parallel, the concentration of the plasma ACTH and corticosterone. Our results suggest that UCN I activates the HPA axis by stimulation of the hypothalamic CRF and/or AVP production and this process is mediated by CRFR1, but not by CRFR2 or AVPR3.

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P121. Age-related central leptin-resistance in different nutritional states

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Middle-aged individuals tend to develop obesity, while old ones show anorexia and cachexia. As these trends also appear in other mammals, complex regulatory changes are assumed in the background. Leptin, a catabolic adiposity signal, suppresses food intake (FI) and increases metabolic rate (MR) leading to weight loss. Both aging and obesity have been shown to cause leptin resistance.

In the present study we aimed to analyze how catabolic leptin effects are influenced by nutritional states during aging.

Heart rate (indicating MR) and core temperature of male Wistar rats of different nutritional states [normally fed, calorie-restricted (CR) and high-fat diet-induced obese (HF)] and age-groups (from 6- to 24-months) were registered during an intracerebroventricular 7-day leptin infusion (1 µg/µl/h) in a biotelemetric system. Body weight (BW) and FI were measured daily.

In HF rats leptin-induced hypermetabolism of the younger middle-aged group (HF6) and hypermetabolism plus anorexia of the aging one (HF18) were suppressed. Calorie-restriction reduced BW and fat mass to a similar extent in all (CR6, CR12 and CR24) age-groups. It strongly enhanced leptin-induced hypermetabolism at all ages, and suppressed anorexigenic actions of leptin with the exception of the oldest group. This latter finding suggests an unexpected increase of responsiveness to anorexigenic leptin actions in old rats. (SROP 4.2.4.A/2-11-1-2012-0001, 34039/KA-OTKA/13-02, 13-25, SROP-4.2.3-12/1/KONV-2012-0028)

P122. Characterization of the thermoregulatory response to pituitary adenylate cyclase-activating polypeptide

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Intrabrain administration of adenylate cyclase-activating polypeptide 1-38 (PACAP) causes hyperthermia, but systematic characterization of the thermoregulatory response to central versus peripheral injection of PACAP has not been conducted.

We measured the thermoregulatory responses of Wistar rats to intracerebroventricularly (ICV) or intravenously (IV) injected PACAP and the circadian changes of deep body temperature (T_b) and locomotor activity in freely moving *Pacap* knockout (KO) and wild-type mice.

ICV injection of PACAP caused a marked (~2°C) rise of T_b in rats, which was brought about by elevated heat production and transiently lower heat loss. IV infusion of the same dose of PACAP also increased T_b, but with a significantly smaller magnitude (~0.3°C). The *Pacap* KO mice were hyperactive throughout the day and maintained a significantly (p<0.05) higher daytime T_b.

We conclude that through CNS-mediated mechanisms injection of PACAP increases deep T_b via activation of autonomic cold defense effectors (elevated heat production and reduced heat loss). Long-term absence of the peptide in KO mice results in hyperactivity, which also leads to higher T_b, but this hyperthermia is absent under resting conditions.

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P123. Changes of lysyl-oxidase expression in myenteric ganglia in different gut segments of streptozotocin-induced untreated and insulin-treated diabetic rats

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We recently demonstrated that the susceptibility of nitrergic myenteric neurons and the mesenteric capillaries supplying them to diabetic damage and to insulin treatment is strictly regional. These indicate the importance of molecular differences in the neuronal microenvironment in different gut segments. Lysyl-oxidase (LOX), a cross-linker of extracellular matrix molecules, has a key role in maintain the matrix integrity which is critical in diabetes. Therefore, the aim of this study was to reveal the quantitative differences in LOX expression in different gut segments of streptozotocin-induced diabetic, insulin-treated diabetic, and control rats.

Ten weeks after the onset of diabetes, segments from duodenum, ileum and colon were processed for post-embedding immunogold electron microscopy. The quantitative changes in LOX expression were evaluated in myenteric ganglia, mesenteric capillaries and intestinal smooth muscle.

In diabetic rats, the number of LOX labelling gold particles increased significantly in the duodenum, decreased in the colon, and remained around the control level in the ileum. The immediate insulin replacement markedly decreased the LOX expression in all investigated gut segments and compartments.

Our data indicate that the region-specific quantitative changes of LOX expression are closely related to the regional damages of the enteric neurons and capillaries in diabetes. Revealing the molecular background of these changes is now in progress in our laboratory.

P124. Molecular Requirements of Ubiquitination Mediated Regulation of Thyroid Hormone Activation via Type 2 Deiodinase

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Thyroid hormones (TH) are crucial regulator of brain development and function. The prohormone thyroxine (T4) lacks the ability to bind to the thyroid hormone nuclear receptors and needs to be activated to T3 by type 2 deiodinase (D2) in order to affect the TH-dependent transcriptome. Thus D2 is major player in the regulation of TH-action in the brain. D2 is a tightly-regulated endoplasmic reticulum (ER) located enzyme that undergoes substrate-induced ubiquitination. D2 is the only ubiquitinated member of the deiodinase-family but the molecular background of this difference and the minimal requirements of D2 ubiquitination are poorly understood. We designed chimeric proteins inserting D2-specific molecular elements (ubiquitinated lysines, D2-specific loop) into type 1 deiodinase (D1) and directed the overexpressed chimeras into the ER of HEK-293 cells followed by assessment of their stability and activity. The chimera that contained both elements could be ubiquitinated in the ER as revealed by Western blot while lack of any of these elements impaired ubiquitination and their activity were not upregulated by the proteasome inhibitor MG132. Using fluorescence resonance energy transfer we demonstrated that the ubiquitinated chimera interacts with the D2-specific ligase WSB-1. These data suggest that the ubiquitinated lysines, D2-specific loop and the ER-localization together are required and sufficient for recognition of a deiodinase as target of the ubiquitin-proteasome system.

P125. Effect of Endocrine Disruptor Compound Zearalenone on Hypothalamic Neuropeptide Gene Expression

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Zearalenone (ZEA) is a nonsteroidal estrogenic mycotoxin produced by several *Fusarium* spp. on cereal grains. ZEA is a natural endocrine disruptor compound (EDC) that induces dramatic changes in uterus and gonad development and advances puberty in immature rats. However, the effects of mycotoxins on the central neuroendocrine regulation remain to be elucidated. The present study investigated the effect of zearalenone and 17-beta estradiol on hypothalamic expression of gonadotropin-releasing hormone (GnRH), kisspeptin and kisspeptin receptor (GPR54). Immature female rats (postnatal, PN 18-27 days) were treated daily *per os* with ZEA (10 mg/kg bw) and body weight, food intake and vaginal opening were followed and the weight of the uterus and ovaries was measured at sacrifice (PN 28). Brain samples were collected for real time PCR analysis of neuropeptide expression and for *in situ* hybridization histochemistry. ZEA treatment increased the uterus weight and decreased body weight gain without significant effect on food intake. ZEA administration resulted in a significant advance in vaginal opening without any reliable changes in GnRH and kisspeptin expression in the hypothalamus. Real time PCR analysis of hypothalamic samples, however, revealed 4-fold increase in kisspeptin receptor (GPR54) in response to ZEA. Using *in situ* hybridization histochemistry we found site specific increase of GPR54 mRNA signal in the arcuate nuclei but not in the preoptic area of rats treated with the mycotoxin. These results point to the hypothalamic kisspeptin/GPR54 mechanism as the target of endocrine disruptor mycotoxin.

P126. Melanocortin system in the regulation of energy homeostasis in SHR rats

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Caloric intake and body weight (BW) of spontaneously hypertensive rats (SHR) developed for the study of essential hypertension are lower than those of age-matched normotensive controls. Their BW does not reach that of controls even on a high-fat diet suggesting a dysregulation of their energy homeostasis. We assumed an enhanced activity of the hypothalamic anorexigenic melanocortins (MC) in the background, that has been shown to contribute to their hypertension.

Food intake (FI) of adult male SHR rats and normotensive Wistar rats (NT) was recorded in an automated FeedScale system upon intracerebroventricular injection of a MC agonist (5 µg alpha-melanocyte-stimulating hormone, α-MSH, spontaneous night-time FI) or infusion of MC antagonist HS024 (1 µg/h, 7 days). Using immunofluorescent labeling we determined the number of α-MSH producing cells and also performed the quantitation of α-MSH specific signal density (SSD) in the arcuate nucleus of the hypothalamus.

The α-MSH-injection reduced FI more efficiently in SHR than in NT rats. HS024 started to increase FI and consequently BW in the NT group already from the 1st, in SHR animals only from the 3rd day. We did not find difference between SHR and NT rats in terms of α-MSH-producing cell counts in the arcuate nucleus, but the SSD of α-MSH immunosignal was significantly higher in SHR rats than in NT animals.

Both our pharmacological and morphological findings suggest higher MC-production and -responsiveness in SHR rats, that may contribute to the explanation of the dysregulation of their BW. (SROP 4.2.4. A/2-11-1-2012-0001, SROP-4.2.3-12/1/KONV-2012-0028)

P127. Investigation of nesfatin-1 action on thermoregulation

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Nesfatin-1 affects energy balance by reducing food intake and elevating body temperature. To achieve the latter, heat loss can be reduced or thermogenesis activated. This may be realized by initiating cutaneous vasoconstriction, provoking shivering, increasing locomotion and/or turning on non-shivering thermogenesis. Sympathetic nervous system is induced in all cases. Heat loss through the skin is regulated primarily by adjusting tail blood flow in rats. Non-shivering thermogenesis takes place in the brown adipose tissue (BAT), where uncoupling protein-1 (UCP1) is able to separate oxidative phosphorylation from ATP production in mitochondria with energy dissipated as heat. To reveal how nesfatin-1 acts on the thermoregulation, we applied 100 pmol of nesfatin-1 or saline icv and monitored the behavior of animals for 2 hours. Just before the experiment and directly after the observation period the skin temperature was measured by using an infrared camera. At the end, the rats were sacrificed, BATs were removed, and UCP1 levels were determined by RT-qPCR. Nesfatin-1 treated rats did not show any sign of shivering and alteration in locomotion, but they went to rest later than controls, suggesting an increased sympathetic activity. Additionally, tail temperature of nesfatin-1 treated rats decreased, while UCP1 mRNA level remained unchanged. The results suggest that nesfatin-1 does not induce fever (no shivering), but it elevates body temperature promoting heat conservation.

P128. Effects of alarin on the thermoregulation of rats

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In the background of obesity, regulatory alterations in energy balance affecting peptide systems may also be assumed. Regulation of energy balance does not only involve maintenance of body weight but also that of metabolic rate and core temperature. The contribution of alarin, a new member of the potentially orexigenic galanin peptide family to the regulation of energy metabolism has been recently suggested. Our aim was to analyze the thermoregulatory effects of alarin in rats.

Adult male Wistar rats received full-length alarin, its truncated form or scrambled alarin in various doses intracerebroventricularly at cool or slightly subthermoneutral ambient temperatures. Oxygen consumption (VO₂), heat loss and core temperature (T_c) of rats were recorded in an indirect calorimeter system.

Upon alarin injection at 25 °C, an increase in VO₂ and continuous tail skin vasoconstriction induced a slow rise in T_c that reached 0.5 °C by 120, and 1.0 °C by 180 minutes. At cooler ambient temperatures similar responses were seen. Neither the truncated nor scrambled alarin elicited any significant thermoregulatory response.

Alarin appears to elicit a slow hypermetabolic, hyperthermic response in rats. Such a thermoregulatory response would characterize a catabolic (anorexia and hypermetabolism) mediator. Further investigations are needed to clarify the complex role of alarin in energy homeostasis. (SROP 4.2.4.A/2-11-1-2012-0001, SROP-4.2.3-12/1/KONV-2012-0028, 34039/KA-OTKA/13-02)

P129. Interleukin-1 β (187-207)-induced hyperthermia is inhibited by interleukin-1 β (193-195) in rats

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Interleukin-1 β (IL-1 β) is a pro-inflammatory cytokine, which plays an important role in the immune response and signal transduction both in the periphery and the central nervous system. Different domains within the IL-1 β protein are responsible for distinct functions. The IL-1 β domain in position 208-240 has pyrogenic properties, while the domain in position 193-195 exerts anti-inflammatory effects. There is little evidence about the effect of the domain in position 187-207 on body temperature. Therefore, the aim of the present study was to investigate the action of IL-1 β (187-207) and its interaction with IL-1 β (193-195) on the body temperature. IL fragments were administered intracerebroventricularly and the body temperature was measured rectally in male Wistar rats. IL-1 β (187-207) induced hyperthermia, while IL-1 β (193-195) did not influence the core temperature considerably. In co-administration, IL-1 β (193-195) completely abolished the IL-1 β (187-207)-induced hyperthermia. The non-steroid anti-inflammatory drug metamizole also reversed completely the action of IL-1 β (187-207). Our results provide evidence that the IL-1 β domain in position 187-207 has hyperthermic effect. This effect is mediated through prostaglandin E2 stimulation and other mechanisms may also be involved in the action of IL-1 β (187-207). It also suggest that IL-1 β domain in position 187-207 and IL-1 β (193-195) fragment may serve as novel target for treatment of disorders accompanied with hyperthermia.

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P130. Reversal of age related learning deficiency by the vertebrate pituitary adenylate cyclase activating polypeptide (PACAP) in *Lymnaea*

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The pond snail (*Lymnaea stagnalis*) has been extensively used as a model system for studying the cellular and molecular mechanisms of associative learning and memory. The main advantage of this system is that animals can learn after a single trial food reward conditioning and the memory can be recalled even 3 weeks later. However, this robust, "flash-bulb" like memory can only be induced in young adults (3-4 months old); aged snails (over 6 months) cannot learn the association after only one training trial.

Recently we have shown that the homolog of the vertebrate PACAP and its receptors exist in *Lymnaea* and PACAP activates the AC enzyme, just like in the vertebrate nervous system. We first tested the hypothesis that PACAP plays an important role in the formation of robust long-term memory after classical food-reward conditioning. Our earlier findings provided the first evidence that PACAP38 is both necessary and instructive for fast and robust memory formation after reward classical conditioning in young animals. Here we tested the role of synthetic PACAP38 in learning in aged animals by looking at its effect on the formation of long-term memory after single trial appetitive conditioning. Our new results show that systemic injection of PACAP boosts memory formation in old animals.

Since PACAP is a highly conserved molecule, our results indicate that it has an important role in learning and memory in general and it may also be used as a memory "rejuvenating" agent during normal biological ageing.

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P131. The Fractalkine/Cx3CR1 System is Implicated in the Development of Metabolic Visceral Adipose Tissue Inflammation in Obesity

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Diet-induced obesity and related inflammation are major risk factors for metabolic, neurological and psychiatric diseases. The chemokine fractalkine (Cx3CL1) and its receptor Cx3CR1 play a pivotal role in recruitment, infiltration and proinflammatory polarization of leukocytes and microglial cells, however, the role of fractalkine signaling in the development of metabolic inflammation is not fully resolved. To address this issue, fractalkine receptor deficient (Cx3CR1 gfp/gfp) mice were exposed to normal or fat-enriched diet (FatED) for 10 weeks and physiological-, metabolic- and immune parameters were compared to control (Cx3CR1 +/gfp) mice. Mice with intact fractalkine signaling develop obesity, mild glucose intolerance, recruit cells expressing gfp into the visceral white adipose tissue (WAT), and display increased expression of pro- and anti-inflammatory cytokines when exposed to FatED. Cx3CR1 (gfp/gfp) mice gain less weight on FatED, do not develop glucose intolerance, recruit less gfp-positive monocytes and express significantly less cytokines in the WAT than control animals on FatED. The effect of FatED on cytokine expression pattern was specific for the WAT, as we did not detect significant elevation in the liver or in the hypothalamus. These results highlight the importance of fractalkine signaling in recruitment and polarization of adipose tissue immune cells and identify fractalkine as a target to fight obesity-induced inflammatory complications.

P132. The mechanisms of induction of amylin in the maternal preoptic area

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Amylin is the only gene, which is selectively expressed in the maternal brain. It was identified in our previous microarray study in the preoptic area, the center of maternal motivation whose lesion abolished maternal behaviors. Examining the time course of amylin induction, a dramatic increase was observed in rats and mice immediately after parturition in the preoptic area. The distribution of amylin neurons was the same as the neurons showing Fos activation by pup exposure: medial preoptic nucleus, parts of the medial preoptic area, and the ventral part of the bed nucleus of the stria terminalis. Ovariectomy had no effect on the activation of amylin neurons suggesting sexual steroid independent mechanisms. However, amylin expression was induced in virgin but maternally behaving (sensitized) virgin females with the same expression pattern as in dams. Almost all amylin neurons expressed Fos in response to pup exposure suggesting their activation in dams. Since our previous studies suggested that maternal motivation may be modulated by posterior thalamic neurons expressing tuberoinfundibular peptide of 39 residues (TIP39), we examined the relationship of amylin and TIP39. Fiber terminals containing TIP39 and the parathyroid hormone 2 receptor (the receptor of TIP39) have the same distribution as amylin neurons in the preoptic area. Furthermore, amylin neurons were closely apposed by TIP39 terminals suggesting their innervation by TIP39 neurons. These results imply that amylin is a novel neuropeptide with maternal functions, and that its maternal induction could be driven by posterior thalamic TIP39-containing neurons, which were shown to relay suckling information from the spinal cord to the hypothalamus.

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P133. 3-D reconstruction of the distribution of melanin-concentrating hormone producing neurons in the mouse hypothalamus.

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Melanin-concentrating hormone (MCH) producing neurons are best known by their contribution in the central regulation of feeding behavior. Perikarya of MCH neurons present predominantly in the lateral hypothalamic area and the zona incerta. Recent studies revealed that the general pattern of the distribution of MCH perikarya is similar in the mouse and the rat, with interesting differences at certain levels.

We identified MCH neurons in the mouse brain in series of coronal slices by immunohistochemical method using polyclonal anti-MCH M8440 (Sigma-Aldrich). Slices were photographed with a digital camera and the data were loaded into 3D Slicer software under Linux. We used the „Treshold”, „Wand” and „Paint” tools of the „Editor” modul to select the regions of interest, and the „Modell” modul to reconstruct their 3 dimensional distrubution. The model was imported into 3DS MAX where the external borders of the hypothalamus where added manually based on The Mouse Brain in Stereotaxic Coordinates (Ed. by Paxinos and Franklin). This work has been supprted by by the Hungarian Scientific Research Fund (OTKA K81419)

P134. Central catabolic effects of leptin during the course of aging

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In the course of aging we observe changes in the regulation of energy balance. Middle-aged people often develop obesity, while old people tend to loose body weight. Changes in the activity of catabolic and anabolic peptides are assumed in the background. Leptin is a catabolic adiposity signal produced in white adipose tissue. It suppresses food intake and enhances energy expenditure acting in the hypothalamus. Both aging and obesity have been associated with leptin resistance.

In the present study we aimed to analyze how central catabolic leptin effects are influenced by aging.

Core temperature and heart rate (indicating metabolic rate) of male Wistar rats of different age-groups (4-months young adult, 6-months younger middle-aged, 12-months older middle-aged, 18-months aging and 24-months aged) were registered during an intracerebroventricular 7-day leptin infusion (1 µg/µl/h) in a biotelemetric system. Body weight and food intake were measured daily.

Leptin resistance of older animals affected hypermetabolic actions, whereas leptin-induced anorexia remained significant in all age-groups. Weight reducing effect of leptin diminished in middle-aged and aging animals, to become significant again in the oldest group.

Accordingly, leptin-induced anorexia and hypermetabolism change in disparate ways with aging. Overall catabolic effects become pronounced again in old rats. (SROP 4.2.4.A/2-11-1-2012-0001, 34039/KA-OTKA/13-02, 13-25, SROP-4.2.3-12/1/KONV-2012-0028)

P135. Impact of ovarian hormone deficiency and subsequent treatments with estrogen receptor agonists on the innate immune system of the hippocampus in middle-aged female rats

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The innate immune system has a major contribution to maintenance of the physiological functions of the hippocampus. The hippocampus is vulnerable to age-related changes ranging from gene expression to network connectivity. The risk of hippocampal deterioration increases with the decline of gonadal hormone supply. To explore the impact of hormonal milieu on the function of the innate immune system in middle-aged female rats, we compared mRNA expression after gonadal hormone withdrawal, with or without subsequent estrogen replacement using estradiol (E2) and isotype-selective estrogen receptor (ER) agonists. Targeted profiling assessed the status of the immune system (macrophage-associated receptors, complement, inhibitory neuronal ligands), local E2 synthesis (P450 aromatase) and estrogen reception (ER). Results established upregulation of macrophage-associated and complement genes, altered neuronal regulation of microglia and downregulation of aromatase in response to ovariectomy. Analogous changes were observed in the hippocampus of postmenopausal women. E2 replacement attenuated Iba1, Cd11b, C3, increased Mrc1, Cd163, reversed Sema3a, but did not restore aromatase expression. While the effects of ER α agonist closely resembled those of E2, ER α agonist also attenuated the expression of several macrophage-associated and complement genes. Our data indicate that the innate immune system of the aging hippocampus is responsive to the gonadal hormone milieu and estrogen treatment.

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P136. Colocalization of cocaine and amphetamine regulated transcript with kisspeptin and neurokinin B in the human infundibular region

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Kisspeptin (KP) and neurokinin B (NKB) neurons in the mediobasal hypothalamus play a pivotal role in the regulation of pulsatile gonadotropin-releasing hormone (GnRH) secretion. Unlike in rodents, KP and NKB neurons in the human rarely express dynorphin- but often exhibit Substance P (SP) immunoreactivity, indicating that the phenotype of these neurons can show considerable species differences. In search for additional neuropeptides in human KP and NKB neurons, we carried out immunohistochemical experiments on hypothalamic sections from five postmenopausal women. In immunofluorescent studies, $9.9 \pm 2.2\%$ of the KP-IR and $1.5 \pm 0.8\%$ of the NKB-IR cell bodies co-contained immunoreactivity for the excitatory neuropeptide cocaine and amphetamine regulated transcript (CART); $24.0 \pm 4.1\%$ of KP+NKB neurons were triple-labeled for KP, NKB and CART. Fiber projections of the CART-IR KP and NKB neurons innervated the hypophysial portal capillaries and also established afferent contacts with other cells of the infundibular region, including KP-IR and GnRH-IR neurons. Quadruple-immunofluorescent studies revealed that a subset of CART-IR KP and NKB fibers also contain SP. Presence of CART in human, but not mouse, KP and NKB neurons represents a new example of species differences in the neuropeptide complement of hypothalamic KP and NKB neurons. Target cells, receptor sites and physiological effects of CART in the efferent communication of human KP and NKB neurons require clarification.

P137. Age-associated alterations in the acute central effects of corticotropin-releasing factor (CRF) on energy balance

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In the background of middle-aged obesity and aging anorexia regulatory alterations are assumed, since they also appear in mammals. We have described such changes of the hypothalamic body weight (BW) reducing catabolic melanocortin (MC) system: a weak response in middle-aged and a strong one in old age-groups. We tested age-associated alterations in the responsiveness to catabolic corticotropin-releasing factor (CRF) downstream to MC.

The effects of intracerebroventricular (ICV) CRF injections on energy balance [food intake (FI), BW, oxygen consumption (VO₂), core temperature (Tc)] were analysed in male and female Wistar rats of different age-groups: young adult, younger/older middle-aged, aging and old (3-, 6/12-, 18- and 24-months, respectively). Anorexigenic responsiveness (0.3 ug) was tested during 120-min re-feeding following 24-h fasting. Thermoregulatory analysis (1 ug) was performed using calorimetry for VO₂ complemented by thermocouples recording Tc and tail skin temperature indicating heat loss.

ICV CRF injection suppressed FI, increased VO₂ and Tc in young rats. In males, all effects declined with aging. Females showed significant responsiveness in all age-groups. Age-associated alterations in the responsiveness of male rats to CRF differ from those of MC. Gender differences may be explained by endocrine factors (anorexigenic estrogens) or alterations of body composition. (SROP-4.2.4.A/2-11-1-2012-0001, SROP-4.2.2.A-11/1/KONV-2012-0024, 34039/KA-OTKA/13-02, 13-25)

P138. Differential Gene Expression in the Male and Female GnRH Neurons

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GnRH neurons play a pivotal role in the regulation of the reproductive system. Release of the GnRH peptide is pulsatile and regulated by estradiol acting on feedback centers in the hypothalamus. In both sexes, gonadal steroids exert feedback inhibition on GnRH secretion. However, at proestrus in females, the rising serum estradiol level activates the positive feedback center/s leading to the preovulatory GnRH surge and ovulation. We hypothesized that functional differences between the male and female GnRH systems are represented in the transcriptome of GnRH neurons. This study was aimed at the exploration of differential expression of genes in male and female GnRH neurons at the time of the negative feedback by using adult male and metestrous female GnRH-GFP transgenic mice. Sampling of GnRH-GFP neurons by laser capture microdissection was followed by RNA isolation, whole transcriptome amplification and microarray analysis. For validation, we applied single-cell cytoplasm harvest from GnRH neurons and quantitative RT-PCR. Analysis of microarray data showed that in male GnRH neurons 546 genes were overrepresented compared to female ($p_{\text{adj}} < 0,002$). Gene annotation analysis revealed significant enrichment of the overrepresented genes in functional clusters of GO terms including „synaptic transmission” and „chromatin modification”. These results suggest a sexually dimorphic regulation of gene expression in GnRH neurons.

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P139. Neuronal connections of the central amygdaloid nucleus with refeeding-activated brain areas

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Inhibition of food intake after consumption of a certain amount of food is a key mechanism responsible for the maintenance of normal body weight. Following a prolonged fast, satiety, which is observed within two hours after reintroducing food, is accompanied by activation of neurons in several brain areas including the central amygdaloid nucleus (CeA). Since activation of CeA neurons can inhibit food intake, we hypothesized that the CeA can contribute to the termination of a meal during refeeding. To reveal satiety-related afferent and efferent connections of the CeA, iontophoretic injections of retrograde (β subunit of Cholera Toxin, CTB) and anterograde (Phaseolus Vulgaris Leucoagglutinin, PHAL) tracers were placed into the CeA of male rats. Ten days later, the animals were fasted for two days and then refed for 2h. Double-labeling immunohistochemistry for c-Fos, a marker of neuronal activation, and for CTB or PHAL was performed to determine the neuronal connections between the CeA and other refeeding-activated brain regions. Numerous CTB-cFos double-labeled neurons were detected in the paraventricular thalamic nucleus, subthalamic nucleus (STh), parasubthalamic nucleus (PSTh), central medial (CM) and posterior intralaminar thalamic (PIL) nuclei, parabrachial nucleus (PBN) and periaqueductal gray (PAG). Triple immunofluorescent labeling revealed that several refeeding-activated proopiomelanocortin producing neurons in the arcuate nucleus also project to the CeA. Few CTB-cFos double-labeled neurons were detected in the prelimbic, agranular insular, dysgranular insular, piriform, primary and secondary somatosensory cortex, bed nucleus of the stria terminalis (BST), ventromedial hypothalamic nucleus, perifornical nucleus, lateral hypothalamus (LH), zona incerta and in the nucleus tractus solitarius (NTS). Mapping of the efferents of the CeA in the refeeding-activated areas revealed high density of PHAL-labeled axons in the BST, LH, posterior part of the hypothalamic paraventricular nucleus (PVN), STh, PSTh, PAG, NTS, CM and in the PBN. Low density of the fibers was found in the hypothalamic paraventricular nucleus. These results display potential pathways promoting the termination of food intake and demonstrate that the CeA establishes reciprocal connections with hypothalamic, thalamic and brainstem nuclei. Further experiments are required to determine the functional consequences of these connections.

P140. Association of afferent and efferent connections of the parabrachial nucleus (PBN) with refeeding-activated brain areas

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Following fasting, satiety is observed within two hours after reintroducing food and accompanied by neuronal activation in distinct areas in the brain including the parabrachial nucleus (PBN). While the PBN is a well known feeding regulatory center, its connections with other refeeding-activated neuronal groups is unknown.

To elucidate the connections of the PBN refeeding-activated neuronal groups in the brain, the anterograde tracer, Phaseolus vulgaris leucoagglutinin (PHA-L) and the retrograde tracer, cholera toxin B (CTB), were administered into PBN of rats under stereotaxic guidance. One week after the surgery, the rats were fasted for two days, allowed to refed for two hours and then perfused transcardially with 4% PFA. Double-labeling immunocytochemistry was performed to detect c-Fos as a marker of neuronal activation, and either PHA-L or CTB. Refeeding-activated neuronal groups were mapped by comparing the number of c-Fos-immunoreactive (IR) neurons in brain areas of refed and fasted rats.

Large numbers of refeeding activated neurons that project to the PBN (CTB containing) were found in the hypothalamic paraventricular nucleus (PVN), paraventricular nucleus (PSTh) and nucleus tractus solitarius (NTS). Moderate numbers of double-labeled neurons were observed in the bed nucleus of stria terminalis (BSTN), central nucleus of amygdala (CeA), lateral hypothalamic area (LH) and periaqueductal central gray (PAG). Scattered c-Fos and CTB-containing neurons were detected in the agranular insular cortex, anterior hypothalamic area (AH), nucleus arcuatus, dorsomedial hypothalamic nucleus (DMN), zona incerta (ZI), tuberomammillary nucleus, and area postrema (AP). A high density of PHAL immunoreactive fibers were observed to closely associate with refeeding-activated neurons in BSTN and CeA. Less intense PHA-L-IR fiber networks were observed in association with c-Fos-containing neurons the AH, PVN, arcuate nucleus, DNM, LH, ZI, PSTh, PAG and AP, and a low density was found in the agranular insular, ectorhinal and perirhinal cortex and NTS.

These data suggest that the PBN is highly interconnected with a large number of refeeding-activated neuronal groups in the brain that may have important role in the regulation of feeding. These groups include the bed nucleus of stria terminalis that may mediate the anorexigenic effect of stress, the hypothalamic paraventricular nucleus, a key regulator of energy homeostasis, and the central part of amygdala that may integrate satiety-related inputs with information arriving from cortical and limbic systems.

P141. Mitochondrial metabolic lateralization in the rat hypothalamus.

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

P142. Effects of estrogen, thyroid hormones and Bisphenol A on the regulation of estrogen- and thyroid hormone receptor expression in the developing cerebellum.

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Estrogen (E2) and thyroid hormones (THs) regulate cell migration, differentiation, proliferation and synaptogenesis during cerebellar development. These events involve the binding of hormone ligands to their cognate receptors to activate relevant genes for the adequate orchestration of developmental processes. Recent reports implicate a complex mechanism through which E2 and THs influence the expression levels of each other's receptors (ERs and TRs). In addition, an growing body of evidence suggests that endocrine disruptors (EDs) influence hormone-regulated physiological events. Here we examined the effects of the presence or absence of E2, THs and three EDs (bisphenol A, arsenic and zearalenone) on the expression levels of ER and TR receptor mRNAs and proteins in cerebellar granule cell cultures. Receptor expression levels were determined by quantitative PCR and Western blot techniques. Results were compared to non-treated controls and to samples obtained from age-matched *in situ* cerebella. Additionally, we determined the effects that glial cells might have on the regulation of ER-TR expression levels. Results show that: (i) ER and TR expression levels depend on the individual, as well as combined presence of E2 and THs; (ii) glial cells are important mediators in the hormonal regulation of neuronal ER-TR expression, and (iii) EDs have characteristic, nevertheless distinct effects on ER-TR expression levels. These observations suggest that both E2 and THs, in adequate amounts, are required for the precise orchestration of cerebellar development and that interactions between endogenous and exogenous hormon-like signals may influence cerebellar development in a manner more complex than previously thought.

P142a. Low Concentration of 17 β -Estradiol Decreases Frequency of Postsynaptic Currents in GnRH Neurons of Metestrous Mice via Endocannabinoid Signaling

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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 (50 $\pm$ 14.5% of the control) in 9 from 18 GnRH neurons. 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 (93 $\pm$ 8.2%). These data suggest that endocannabinoids are involved in the mediation of the effect of E2 on suppression of fast neurotransmission onto GnRH neurons. 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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P143. Localization of glutamine-synthase positive cells in the developing subventricular zone

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Az oldalkamra (VL) szubventrikuláris zónája (SVZ) azon néhány terület közé tartozik, ahol felnőttkorban is neurogenesis zajlik. Az utóbbi évtizedben egyre több tanulmány foglalkozott ennek a területnek a sejttöszetével, az itt végbemenő neurogenesis lépéseivel és a migrációs utak elhelyezkedésével. Ennek ellenére a SVZ fejlődéséről még mindig korlátozott információ áll rendelkezésre.

Jelen tanulmány az oldalkamra mentén elhelyezkedő glutamin-szintetáz immunpozitív (GS-ip) sejtpopulációk megjelenésével és migrációjával foglalkozik patkányban a perinatális időszakból (E14-P20) a felnőttkorig (P60). E 18-tól kezdődően találtunk GS-ip sejteket a VL mediális falának középső részén, valamint a foramen Monroi körül, amelyek a kamrával szomszédos rétegben helyezkedtek el. Újszülöttkorban a mediális és laterális fal felső részén is voltak kamrakontakt GS-ip sejtek, amelyek a GFAP-val immunnegatívnak bizonyultak.

Későbbi életkorokban (P6-P20) a GS-ip sejtpopuláció fokozatosan a ventrikuláris zóna (VZ) alá szorul és leginkább a laterális fal felső részének SVZ-ban található meg. A GS-ip sejtek közvetlenül a radiális glia GFAP-ip nyúlványai mentén voltak megfigyelhetők, amelyek valószínűleg kijelölik a sejtek migrációs irányát. A kamrarendszer mentén kétféle GFAP-ip nyúlványrendszer volt található,

a) egy általános, a kamrával párhuzamosan, és közvetlenül a VZ alatt elhelyezkedő „tangencionális” nyúlványrendszer és

b) egy a kamrafalra merőleges, és csak a laterális SVZ-ben mintegy 100 µm mélységben megtalálható „radiális” nyúlványrendszer.

Az, hogy felnőttkorban a laterális SVZ-ban a rostrális migrációs útvonalnak megfelelően a „radiális” nyúlványok mentén hasonló sejthalakokat látunk, mint a fejlődés során, azt valószínűsíti, hogy ekkor is keletkeznek és migrálnak GS-ip sejtek.

P144. Development of GABA-erg neurons in the mouse superficial dorsal horn

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Neurons of the dorsal spinal cord develop from six types of progenitor cells. The six progenitor cell population give rise to eight types of postmitotic neurons: six early (E10-12.5) and two late (E11-E13) horn cell population. The late born, so called dIL_A and dIL_B neurons migrate into the superficial dorsal horn and will gain the major function of receiving and processing nociceptiv sensory inputs. The dIL_A and dIL_B cells differentiate to GABA-erg and glutamaterg neurons, respectively.

In the present study we aimed to identify and investigate the distribution of GABA-erg neurons based on their transcription factor expression pattern during embryonic development. The cervical spinal cord of E11,5-P3,5 wild type, GAD65-GFP and GAD67-GFP transgenic mice embryos and newborns were removed. The spinal cords were sectioned and immunostained for NeuN (general neuronal marker) and Pax2 (a marker for GABA-erg neuron).

The NeuN immunohistochemistry revealed, that the neurons of the spinal dorsal horn born before E14, than they migrate to their final destination. Pax2 immunoreactivity first appears early stage of neurogenesis (before E14) and labelled also postmitotic neurons. GAD67 and GAD65 expression appeared at E11 and E13, respectively. Pax2 showed substantial colocalisation with GAD65 and GAD67, indicating that most of the GABA-erg neurons in the superficial dorsal horn can be identified by Pax2 transcriptional factor.

Our results indicate that GABA-erg neuron of the superficial spinal dorsal horn born between E11-E13. They migrate their final destination between E14-E18. Their markers and distribution do not show major changes after this.

P145. Organization of extracellular matrix in the brainstem of chicken embryo

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The young chicken, that just hatched, is soon able to walk, see, chirp or feed itself. These behavioural actions require an early but considerable development of CNS areas responsible for basic motor actions, sensory functions and homeostasis. Previous studies revealed, that macromolecules of the extracellular matrix (ECM) around neurons highly correspond to their activity, and vice-versa. The aim of this study was to investigate the expression of hyaluronan (HA) in the ECM assembly around neurons, during embryonic development, within the vestibular nuclei of chicken embryos. We gained embryos by aborting incubation at certain developmental stages. After paraffin embedding and sectioning, histochemistry was performed to visualize the HA. Parallel, PCR analysis was made against hyaluronan synthases' mRNA (HAS2 & HAS3). Both in all stages examined. HA is present in all vestibular nuclei from the earliest stages of development. In the lateral vestibular nucleus its presence increases from E11, but perineuronal net (PNN), a condensation of the ECM around neurons, first forms moderately at E13, then its intensity gradually increases, in the neuropil the staining intensity rises till E13. In the medial vestibular nucleus neuropil labelling enforces till E15, and moderate PNN is identifiable at hatching stage. In the superior vestibular nucleus HA presence grows in the neuropil till E15 stage, and moderate PNN is seen in E20. In the descending vestibular nucleus it accelerates early, till E11, and PNN is visible from E15 on, coating not all neurons. PCR also showed the presence of HAS2 and HAS3 mRNA in the examined stages.

These findings suggest the HA's importance in neuronal circuit establishment, as well the strong ECM at vestibular areas may prove the early development of those, responsible for posture and gaze, motor and sensory modulation. These might be the character of precocial birds.

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P146. Apoptotic effects of the 'designer drug' methylenedioxypropylamphetamine (MDPV) on the neonatal mouse brain

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The designer drug of cathinone family, methylenedioxypropylamphetamine (MDPV), has been on the market for some time as one cheap and frequently used psychoactive drug of abuse. However, its mechanism of action, particularly its potential detrimental effect on the developing brain, is largely unknown, despite the fact that pregnant females may occur among the users. The objective of our study was to identify the brain areas sensitive for a possible apoptotic effect of the widely abused MDPV on the developing brain. To this end, we used a mouse model which can be compared with the developing human fetus of third trimester, considering the developmental stage of the brain. Litters of 7-day-old C57BL/6 mice were treated either with i.p. injection of 10 mg/kg b.wt. MDPV or vehicle (saline), and sacrificed after 24 h. The dose was chosen according to our behavioral observations on adult mice. The brains were then processed for anti-caspase 3 (Casp) immunohistochemistry and the apoptotic cells were identified and counted in different brain regions. We found prominent increase in the number of apoptotic cells in several areas of the brain: piriform and sensorimotor cortices, retrosplenial area, cingulate gyrus, hippocampus CA1 and in the caudate-putamen. The apoptotic cell types included pyramidal and non-pyramidal (stellate) neurons in cortical regions, as well as medium spiny multipolar neurons of striatum. The neurons of the nucleus accumbens appeared to be especially sensitive for MDPV at this stage of the development: Casp-immunoreactive cells marked out the core and shell regions of the accumbens. However, when we repeated the experiment with adult mice, we did not observe the same effect on the brain; the MDPV did not seem to cause apoptotic changes in the mature nervous system. The results are in agreement with the assumption that cathinones (in particular MDPV) may adversely affect neural integrity in the developing CNS. Grant sponsor: OTKA PD-105251.

P147. Migratory pathways and morphological maturation of neurons populating the superficial spinal dorsal horn neurons

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Neurons in the superficial spinal dorsal horn arise from late-born progenitor cells and assemble into circuits underlying nociceptive information processing. During their development they may follow unique migratory pathways, since they have to migrate through or around the earlier born neurons populating the deep dorsal horn. To reveal how they migrate we labelled neurons in the cervical spinal cords of mice embryos at the time of their birth at E12.5 with GFP by in utero electroporation. One day to one month after electroporation the cervical spinal cord was removed and processed for further morphological studies. Labelled neurons were revealed and their distribution was mapped. Time-lapse microscopy was also used on electroporated spinal cord explants investigating the migration dynamics of these cells. We have revealed two GFP labelled cell groups; neurons in the first group migrated into the superficial dorsal horn, whereas neurons in the second group remained close to the central canal and found their final destinations in lamina X. Lamina I and II cells were migrating radially while lamina X neurons were migrating tangentially close to the ventricular zone. Our results indicate that neurons of the superficial dorsal horn and the lamina X become postmitotic at the same time but because of some unknown reasons they segregate from each other immediately after their birth and show different developmental courses.

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P147a. Morphological Consequences Of Intravitreal PACAP Injection In The Developing Rat Retina

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The pleiotropic pituitary adenylate cyclase-activating polypeptide (PACAP), a member of the secretin/glucagon/VIP family, exerts versatile effects on neuronal development. Abundant expression of its receptor (PAC1-R), predominantly the Hop1 isoform has been demonstrated during the second week of postnatal retinal development. Immunostainings showed PAC1-R expression in ganglion, amacrine, horizontal and rod bipolar neurons and in glial Muller cell processes but its function is not well understood. Therefore, we aimed to investigate the effects of PACAP on retinal morphogenesis from postnatal day 5 (P5) through P10.

Three groups of Wistar pups were treated intravitreally with 100 pmol PACAP, applying one, two or three injections, followed by dissections at P7, P9 and P10, respectively. To determine the relative proportions of retinal layers and changes in cell numbers, morphometric measurements were performed on semithin sections. To test apoptosis, activated caspase 9 and 3 levels were assessed by western blots and TUNEL assays, while anti-PKC and anti-HPC1 immunolabeling detected changes in bipolar- and amacrine cell population, respectively.

We found that PACAP injection caused a 45% increase in GCL cell numbers, induced apoptosis, presumably, in the bipolar cells of the distal INL and acted as a negative regulator for differentiating amacrine cells. Our data indicate that PACAP may have multiple, occasionally opposing roles in the maturation of the mammalian retina.

P148. Myelination is impaired in the human hippocampal formation in Down syndrome

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Down syndrome (DS) caused by trisomy 21 is accompanied by mental retardation and learning disability. Previously, we have reported lower density of myelinated axons in the hippocampal formation in DS, especially in the dentate hilus, when myelination was detected with light microscopic immunohistochemistry. However, question remained whether this result is due to impaired formation of the myelin sheath, or to lower numbers of axons or to both. Therefore, ultrathin sections containing the alveus, the strata oriens and lacunosum-moleculare of CA1, the hilus and the molecular layer of the dentate gyrus as well as the corpus callosum of 3 DS patients and 4 controls of various postnatal ages were examined using an electron microscope. The numbers and the diameters of the myelinated axons were quantified. No difference was found when the numbers and the diameters of the myelinated axons of the corpus callosum, the str. oriens of the CA1 and the alveus was compared in controls and in DS patients. The difference was significant in the str. lacunosum-moleculare and in the outer 1/3 of the dentate molecular layer. The most striking difference between the controls and DS patients was observed in the hilus, right below the granular layer as well as 100-200 micrometers deeper. We conclude that the lower numbers of the myelinated axons and the impaired myelination of the perforant pathway and the septal GABAergic afferents can contribute to learning disability characteristic to DS.

P149. Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP) Prevents Monosodium Glutamate (MSG) Induced Functional Disturbances in the Mouse Retina

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MSG provokes a chronic activation of postsynaptic neurons leading to excitotoxic effects. We studied the short term functional consequences of MSG treatment in the mouse retina. We investigated whether administration of PACAP, a neuroprotective peptide, could rescue retinal ganglion cells (RGCs) from MSG-induced effects. Spontaneous and light-evoked spikes of RGCs from mice were recorded using a multielectrode array. Green light was used to generate full field stimuli. RGCs were also analyzed using the fluorescent Ca²⁺ dye fluo-4. Retinas were treated with MSG (10 mM) or a mixture of MSG+PACAP (10 µM; 15 min). MSG remarkably elevated the free intracellular calcium concentration and increased the spontaneous spiking 4-5 minutes after drug application. During this time, spike correlations between RGC pairs were reduced. After 10-15 minutes of MSG application, the spontaneous activity of most RGCs was dramatically reduced or eliminated. Pretreatment with PACAP prevented the MSG effects as indicated by little or no change in the spontaneous spiking patterns. Moreover, the intracellular Ca²⁺ concentration was noticeably decreased by PACAP. In addition, MSG blocked the light-evoked responses of all recorded cells. The light-evoked responses of approximately 40% of RGCs were retained following pretreatment with PACAP. We found that MSG had short term effects on the spontaneous and light-evoked spiking of mouse RGCs. PACAP also rescued RGCs from the short term MSG-induced insults.

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P150. Grafted neuroectodermal stem cells rescue damaged rat retinal ganglion cells otherwise destined to die

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Human glaucoma is associated with gradual deterioration of vision and progressive retinal ganglion cell (RGC) loss. Here we investigated whether grafted neuroectodermal stem cells are able to prevent RGC loss.

Glaucomatous condition leading to RGC death was induced by thermocoagulation of the episcleral and perilimbal veins on the left eye of SD rats. Treated rats received neuroectodermal stem cells (500.000 cells/2-3 µm/eye) intravitreally 3 and 6 weeks after injury and were allowed to survive for further 6 weeks. Control animals received no graft and survived for 3, 6, 9 and 12 weeks. The intraocular pressure was monitored throughout the study. The numbers of retinal ganglion cells and optic nerve axons were determined in both the operated and intact eyes. The stem cell derivatives were mapped and their phenotype was immunohistochemically characterized.

The number of ganglion cell showed a gradual decrease in controls (3 weeks: 80.4 %; 6 weeks: 75.2 %; 9 weeks: 63.81 % and 12 weeks: 61.88 %) accompanied by a slow normalisation of IOP values. Early transplantation prevented the RGC loss by approximately 50 % while late grafting rescued only 25% of RGCs. These findings were confirmed by the numbers of remaining myelinated axons in the optic nerve. The transplanted cells developed to neurons and astrocytes and produced IL10 and BDNF in the first 10 days after grafting.

These findings suggest that grafted neuroectodermal stem cells induce RGC survival by a paracrine mechanism.

P151. The effects of nNOS inhibitor, N^w-propyl-L-arginine (L-NPA), pre-treatment on early latent period of epileptogenesis in a kainic acid mouse model of epilepsy.

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Using a highly selective neuronal NO synthase (nNOS) inhibitor, N^w-propyl-L-arginine (L-NPA), we investigated the effects of nNOS in the development of seizures and neurobiological changes, during status epilepticus (SE) and the early latent period, using a kainic acid (KA)-induced seizure model in C57BL/6J mice. Three complementary approaches were used to characterise the acute effects of pretreatment with L-NPA. Behavioural indicators of seizure severity were scored using a modified Racine scale, EEG was recorded using an implantable telemetry device, and expression of c-Fos (a marker for neuronal activity) 2 hours following seizure were used to get a measure of seizure severity. Activity-dependent synapse formation was quantified during the early latent period using synaptophysin and markers of microglia and astrocytes, respectively. In comparison to vehicle pre-treated controls, L-NPA reduced the severity and duration of convulsive motor seizures. L-NPA also reduced the number of spikes/hour during first week post-KA administration; neuronal expression of c-Fos in dentate granule cells at 2h post-KA and synaptophysin immunoreactivity in the outer molecular layer of the dentate gyrus. These results suggest that nNOS plays a contributory role in seizure severity during SE and is important for neurobiological changes, associated with the development of chronic epilepsy, in the early latent period of epileptogenesis.

P152. Evoked high frequency oscillations in the human hippocampal formation

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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).

Evoked HFO (evHFO) were studied in the HcF under general anesthesia in ten temporal lobe epilepsy (TLE) patients. We used laminar multielectrodes to record local field potential, and spectral activation elicited by CES (0.1ms; 5-15mA; 0.5Hz). 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).

Significant HFO activity was evoked in 7 cases (70%). Subregion distribution of evHFO is the following (significant HFO (ripple) / case number): CA1: 1(0)/3, CA2: 0(0)/1, DG 6(1)/8, distal Sub 5(3)/7, proximal Sub and proSub 2(1)/2. Sub had lower stimulus amplitude threshold (5mA) than DG (10mA). Stimulation closer to the temporal pole evoked HFOs with higher probability. We found no significant correlation of evoked HFO and seizure outcome.

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.

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P153. *Mycobacterium avium* chronic infection induces alterations in the hippocampal cytokine

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In the last decades, one of the most remarkable findings in neurosciences was the discovery of a bidirectional interaction between the immune and central nervous systems. A disruption in this interplay has been suggested as a potential cause of depression, ie, the cytokine theory proposes that increased levels of pro-inflammatory cytokines are in some circumstances responsible for depression.

Increased production of pro-inflammatory cytokines occurs naturally as part of the immune response to infection. In animal models, acute stimulation of the immune system, by administration of LPS or pro-inflammatory cytokines, leads to a sickness behavior with a pattern of acute and transitory behavioral alterations similar to the ones observed in depressed individuals. However, while the sickness behavior alterations disappear rapidly, depression is a chronic process.

By now, only few reports have address the effect that chronic infections may have on mood disorders, despite their high prevalence and longstanding altered cytokine profile. In our work we used the model of peripheral chronic infection with *Mycobacterium avium*, known to induce persistent alterations in the peripheral cytokine profile. Our results show that it also causes significant changes in the hippocampal cytokine profile. Currently, further studies are being performed to unravel how this altered cytokine milieu affects hippocampal neurogenesis and neuronal plasticity, and depressive-like behavior phenotype.

P154. Effect of exogenous PACAP on ischaemia-reperfusion induced kidney injury

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Pituitary adenylate-cyclase activating polypeptide (PACAP) is a neuropeptide, its neuroprotective and cytoprotective effects have been shown in several studies. We have revealed that exogenous PACAP decreases the damage after kidney ischaemia-reperfusion in male rats.

The aim of the present study was to investigate the effect of exogenous PACAP during ischaemia-reperfusion in the kidney of female rats.

Wistar rats were subjected to one-sided renal artery clamping for 60 minutes followed by 24-hour, 48-hour or 14-day reperfusion. PACAP (100 µg) was administered intravenously before arterial clamping in half of the rats in each group. Histological evaluation of the sections was performed with Adobe Photoshop and Scion Image programs. In the focus of our investigation was the tubular damage, that plays a very important role in the function of the kidney.

The tubular damage was significantly less severe in the PACAP-treated groups after 48-hour and 14-day reperfusion compared to the control groups. In case of PACAP-treated rats the duration of the reperfusion had no influence on the severity of the damage, but in the control rats the damage was severe after a longer reperfusion.

Based on our results it can be concluded, that exogenous PACAP is protective against ischaemia-reperfusion induced kidney injury in female rats.

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P155. Changes in Brain Histone Acetylation might underlie anxious phenotype

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Epigenetic changes are likely to underlie psychiatric diseases, including depression and anxiety disorders. However, this aspect of affective disorders is poorly studied. Here, two inbred mouse strains having extremes in anxiety related behaviour (AX and nAX, respectively) were analyzed for differences in levels of various brain histone acetylations by using Western-blot method. Histone acetylation is regulated by two opposing class of enzymes, HAT and HDAC, which are widely expressed in key brain areas of anxiety, including the hippocampus and prefrontal cortex.

We correlated the effects of acute and chronic HDAC inhibitor treatments on histone acetylation level with behavioural data. Chronic treatment with the anxiolytic Buspirone caused a similar pattern of Ac-histone changes than HDACi treatments. Interestingly, the effects of HDACis and buspirone on behaviour were similar. These results suggest that modification of brain Ac-histone levels might underlie the chronic dysfunction of the neuronal fear circuitry.

P156. Antipsychotic Drug-Candidate Egis-11150: Pro-Cognitive Efficacy in NMDA-Antagonist Preclinical Models and Facilitation of Hippocampal Neuronal Plasticity.

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This study investigated the effects of Egis-11150 (PMID: 22824189) in NMDA antagonist-induced preclinical models, on hippocampal long-term potentiation (LTP) and on the levels of dopamine (DA) and norepinephrine (NE) in brain micro-dialysates. In an attention-set shift paradigm, the cognitive performance of male C57Bl6 mice receiving phencyclidine (PCP, 3 mg/kg) was impaired when compared with vehicle treated controls. Egis-11150 (0.03-0.1 mg/kg) abolished the PCP-induced deficit whilst having no effect in vehicle treated animals. In a reversal-learning version of the rewarded T-maze, sub-chronic treatment with PCP diminished the efficiency of task reversal. Acute treatment with 0.01 or 0.03 mg/kg Egis-11150 normalised the PCP-induced deficit – no effect in vehicle-treated animals. In mouse hippocampal slices, submaximal theta-burst LTP in the CA1 area was enhanced by Egis-11150 (30-300 nM). Diminution of maximal theta-burst LTP, produced by prior acute stress *in vivo*, was normalized by the drug. Microdialysis was carried out in conscious male SPRD rats. In the dorsal hippocampus, administration of Egis-11150 (0.2 mg/kg) produced significant increases of DA and NE as well as their respective metabolites. The magnitude of the changes were similar to those produced by 1 mg/kg risperidone. Collectively, these data demonstrate the therapeutic efficacy of EGIS-11150 in acute and chronic NMDA antagonist models of cognitive deficits associated with schizophrenia.

P157. Evaluation of bilaterality in temporal lobe epilepsy

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Temporal lobe epilepsy (TLE) is the most common form of therapy resistant focal epilepsy. Temporal lobectomy provide 70-80% seizure freedom in cases where unilateral seizure onset was observed. Factors contributing 20-30% bad outcome are not known. We hypothesize that bilaterality measured in a more detailed way may explain the outcome.

TLE patients undergoing intracranial video EEG monitoring before unilateral temporal lobectomy were included. Laterality index (LI) was determined for MRI, neuropsychological deficit pattern, interictal and ictal EEG and ictal semiology (IS). LI for IS was determined using lateralizing signs scored in a systematic way. Seizure outcome was evaluated by Engel's score. We calculated linear correlation for MRI and IS LIs.

We were able to reliably score the IS and use it as a LI. Four TLE patients was selected. Average follow up time after surgery was 7.5 months. Three patients had Engel 1A, one patient had Engel 3 outcome. MRI LI correlated better than IS LI with outcome, but none of it was statistically significant.

We were able to build a multimodal laterality index system for TLE. Based on our small sample number we could not show significant correlation with outcome. We plan to involve more patients, and more variables, like high frequency oscillations to get better statistical power.

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P158. Ultrastructural Changes in the Hippocampus of G72 Gene-expressing 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 mice that express the human 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 in these animals, so we investigated whether there are any morphological changes behind those changes seen in physiological measurements. Using both pre- and post-embedding methods, we examined these excitatory synapses of stratum moleculare with electron microscope. We found, that synapses are at least 33% larger in transgenic (TG, n=5) mice compared to wild type (n=5) littermates, and contain around 2 times more NMDA receptors, while the thickness of the postsynaptic density was unchanged. The reason of these observed changes could be a G72-protein induced mitochondrial alteration, or a change in the concentration of the NMDA receptor co-agonist D-serine.

Because N-acetyl-cysteine (NAC)-treatment ameliorated behavioural alterations in G72-TG animals after, we tested whether this treatment would rescue morphological changes, as well. Our preliminary data suggest that several weeks of treatment may indeed reverse observed changes in these synapses.

P159. Neurotoxic effect of oligomerised β -amyloids (o β A): suitability as a pharmacological screening assay

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Synaptotoxic and neurotoxic effects of o β A are thought to be involved in the aetiopathology of Alzheimer's disease. The molecular species of o β A underlying these pathologies as well as the respective mechanism(s) of action remain to be defined. This study aimed to devise an in vitro pharmacological screen for blockers of the neurotoxic effects of o β A. Neuron-enriched primary cultures of embryonic rat brain cortex were examined at various days after plating (DIV 15-25). Exogenous glutamate and NMDA (10-100 μ M) caused marked cell death over 24h, which could be prevented by NMDA receptor antagonists. Inhibition of glutamate uptake by DHK (300 μ M) or TF-TBOA (15 nM) increased medium glutamate concentration significantly to 5-15 μ M within 4 h and produced neurotoxicity by 24h. Exposure of the cells to o β A preparations resulted in the attachment of beta-amyloid immunoreactive material to the somatodendritic compartment of neurons, no labelling of glial cells was apparent. The o β A contained aggregates (>75K) that behaved as amyloid derived diffusible ligands. However, neurotoxic effects over 24h at up to 5 μ M nominal concentration of beta amyloid monomer were inconsistent. When apparent, o β A neurotoxicity was significantly reduced by NMDA antagonists. Collectively, these data indicate that glutamate uptake systems are important for neuronal viability in neuron-enriched cultures and that o β A derived from synthetic beta-amyloid is not a reliable tool for pharmacological assays.

P160. Investigation of sex differences in major depression using the active escape paradigm in rats

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Major depressive disorder is a devastating disease with a high lifetime prevalence worldwide. Stressful life events, especially in western societies contribute to an increasing tendency. Human studies report greater susceptibility of females to stress related disorders, including depression. The underlying mechanism of this obvious gender difference are still largely unknown. Researching of the mechanism is essential to have a good animal model. We hypothesize that female rats are more sensitive to stress; therefore they are prone to develop depressive symptoms more frequently compared to males. CD(SD) intact male and ovariectomized female rats were used and their sensitivity to stress and the development of depressive-like behavior was tested in an active escape paradigm. In the whole range of footshock intensity studied females showed better escape performance than males, indicating that females have reduced tolerance to footshock. Our results show that the learned helplessness-active escape paradigm routinely used in our laboratory reliably reproduces gender differences, providing an excellent basis for future studies on the mechanism of sex differences in major depressive disorder.

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P161. Neuroprotective effect of the total flavanones from *Iris tenuifolia* against oxidative stress-induced cell death through the activation of phosphorylation of mitogen-activated protein kinase

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Increasingly flavonoids have been observed to exert their cellular effects via the modulation of signaling pathways, such as the MAPK pathway. Much attention has turned to the role of ERKs in depression. The flavanones possess neuroprotective effect against H₂O₂-mediated neurotoxicity, neurons were pre-treated with the flavanones (0.032, 0.32, 1.6, 3.2, 6.4 mM) for 24 h prior to the addition of hydrogen peroxide (25 μM). The greatest degree of protection evident at 1.6 mM. We found that total flavanones induced the activation of both MAPK/ERK and AkT (a component of the PI3K pathway). The activation of both the MAPK and PI3K pathways indicate it's involving in protection action of total flavanones. The pattern of protection induced by total flavanones directly mirrored their potential to modulate the AkT and ERK1/2 kinase which followed a bell-shaped activation curve with time-course in response to the flavanones. Furthermore, the ability of total flavanones to inhibit hydrogen peroxide-induced cell death was significantly blocked by U0126 and LY294002 inhibitors that confirmed involvement of both MAPK and PI3K pathways in the protection effect of total flavanones from *Iris tenuifolia*. Our results propose its mechanism of protection that the up-regulation of both ERK1/2 and Akt kinase signaling pathway.

P162. Cortico-cortical evoked potentials in the seizure onset zone

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Cortico-cortical evoked potentials (CCEP) provide a simple method to test short and long range connections in the human brain. Using CCEP physiological and pathological responses can be evoked in epilepsy patients.

We aimed to analyze the connectivity pattern of the seizure onset zone (SOZ) in focal epilepsy patients using CCEPs. 25 patients implanted with subdural intracranial electrodes (IcE) to explore the seizure focus was involved in the study. CCEPs were evoked by single sock electrical stimuli applied to the adjacent contacts of IcE. CCEPs were detected by semi-automatic algorithms. All electrodes were assigned to the respective underlying Brodman's area. Average number of CCEPs (AN), connectivity, CCEP amplitude, and Brodman connectivity (BC) was analyzed. SOZ was determined using clinical criteria.

The CCEP amplitude decreased with the electrodes distance until 4cm, but further increasing the distance did not contribute to further decrease in amplitude. The AN was significantly higher stimulating the SOZ and recording from the not SOZ within 2cm distance compared to SOZ only.

Our results suggest that the number of short range connections between SOZ and the neighboring notSOZ is higher than outside or inside the SOZ. Connectivity pattern between functional networks are indicative for SOZ also. We think that CCEP connectivity may be used as an additional biomarker for epileptogenicity.

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P163. Uridine Modulates Neuronal Activity and Inhibits Spike-Wave Discharges of Absence Epileptic Rats

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In the present study, simultaneous extracellular single unit recording and microiontophoretic injection of the pyrimidine nucleoside uridine (Urd) was used to provide evidence for the presence of Urd-sensitive neurons in the thalamus and the cerebral cortex of Long Evans rats. Twenty-two neurons in the thalamus (24% of recorded neurons) and 17 neurons in the cortex (55%) responded to the direct iontophoresis of Urd. The majority of Urd-sensitive neurons in the thalamus and cortex increased their firing rate in response to Urd. In contrary, adenosine (Ado) and uridine 5'-triphosphate (UTP) decreased the firing rate of all responding neurons in the thalamus, and the majority of responding neurons in the cortex. Functional relevance of Urd-sensitive neurons was investigated in spontaneously epileptic freely moving Long Evans and Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats. Intraperitoneal injection of 500 mg/kg Urd decreased epileptic activity (210-270 min after injection) in both rat strains. Intraperitoneal administration of 1000 mg/kg Urd decreased the number of spike-wave discharges (SWDs) between 150-270 min and 90-270 min in Long Evans and WAG/Rij rats, respectively. The higher dose significantly decreased the number of SWDs even 24 hours after Urd injection. The present results suggest that Urd-sensitive neurons in the thalamus and the cerebral cortex may play a role in the antiepileptic action of Urd possibly via modulation of thalamocortical neuronal circuits.

P164. Gap Junction Blockade and PACAP Administration Can Prevent Vascular Changes in Retinopathy of Prematurity

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Retinal neovascular diseases remain a leading cause of vision loss worldwide. Electrical transmission via gap junctions has been shown to be an important mode of retinal communication. Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors occur throughout the nervous system, including the retina. The in vivo protective effects of PACAP have been shown in retinal degeneration induced by monosodium-glutamate and ischemia. However, the role of gap junctions and the connection between gap junctions and PACAP in retinal proliferative disease such as retinopathy of prematurity (ROP) have not examined yet. We aimed to study the PACAP administration and global gap junction blockade in retinal ischemia and subsequent retinal neovascularization. Well described method of oxygen induced retinopathy (OIR) in rodents was used to provoke retinal ischemia and neovascularization. Intravitreal injection with gap junction blocker (MFA) or PACAP results in a noticeable decrease in vaso-oblivation and neovascularization compared to the untreated control retina in rodents. Connexins appear to be important messengers in the neovascular cascade though their exact role has not been clarified yet. Connexins and potential retinoprotective PACAP may have important role in the vascular changes of OIR.

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P165. Global optogenetic activation of inhibitory interneurons effectively suppresses epileptiform activity

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Optogenetic techniques provide powerful tools for bi-directional control of neuronal activity and for investigating alterations occurring in excitability disorders such as epilepsy. One strategy for seizure-suppression is to activate inhibitory interneurons in the cortex or hippocampus by targeting them with Channelrhodopsin-2. There are several subclasses of interneurons in these areas with different functional connectivity with principal neurons, e.g. targeting their perisomatic or dendritic compartments. Therefore, one could optogenetically activate specific population of interneurons, or alternatively a mixed population for their concerted action with presumably stronger inhibitory effect on principal cells. We chose to explore such conceptually novel strategy involving simultaneous activation of mixed populations of interneurons by optogenetics, in mouse acute hippocampal slices. Here we demonstrate that this optogenetic approach is a powerful strategy to suppress epileptiform activity. We also show that this effect is due to a massive GABA release and resulting inhibition of principal cells, rather than to mere perturbation of network synchrony. Our results suggest that an optogenetic strategy engaging broader interneuron populations is a more effective alternative to counteract hyper-synchronous network oscillations in epilepsy.

P166. Role of nitric oxide in trigeminovascular activation of mice

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Nitric oxide (NO) induces vasodilatation and sensitization in the trigeminovascular system (TRGVS) related to migraine. The NO-donor nitroglycerol (NTG) causes migraine-like headache in migraineurs, but only mild transient headache in healthy people. It is commonly used for chemical activation of the TRGVS in rodents, but its mechanism and specificity are not clear.

We compared the effects of 3 NO donors on TRGV activation in mice: one alcoholic (Nitrolingual aerosol) and 2 aqueous solution (Nitropohl and isosorbide mononitrate: ISM).

Light aversive behaviour was investigated in a custom-made light-dark box, meningeal microcirculation by laser Doppler imaging, and the early neuronal activation marker c-Fos by immunohistochemistry after i.p. injection of the compounds and respective vehicles in male CD1 mice.

Only Nitrolingual in a vehicle containing ethanol and peppermint oil induced significant light aversive behaviour, meningeal vasodilatation and c-Fos activation in the trigeminal nucleus caudalis (TNC) and ganglia (TRG), but its solvent evoked similar responses. The aqueous NTG solution Nitropohl resulted in similar c-Fos increase in the TRG and TNC, but not meningeal vasodilatation and light aversive behaviour. ISMN did not induce TGV activation.

Vascular and neuronal responses in the TRGVS do not change parallelly, NO is only involved in neuronal sensitization. The NO theory in chemical TRGV activation should be re-evaluated.

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P167. Imaging neuroinflammation in the ischaemic brain with high speed two photon microscopy *in vivo*

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Inflammation plays an important role in the pathogenesis of cerebral ischaemia. However, it is still unclear how brain injury is influenced by altered activity of complex glial and neuronal networks. Microglia are key inflammatory cells in the brain and their contribution to blood brain barrier damage and neuronal injury is controversial. To investigate microglial inflammatory responses *in vivo*, we induced cerebral ischaemia by middle cerebral artery occlusion (MCAo) in mice combined with high speed 3d two photon microscopy and examined neuronal and glial interactions in real time. Brain injury induced rapid activation of microglia in the core of the infarct and in penumbral regions. Using Oregon Green 488 BAPTA-1 acetoxymethyl-ester (OGB-1 AM) and genetically encoded calcium indicators (GCaMP6f) we monitored intracellular calcium concentration changes and neuronal network activity. Stimulation-evoked neuronal activity was impaired after induction of cerebral ischaemia, which was associated with altered microglial responses and recruitment of microglia in the injured hemisphere. We also assess focal activation of microglial cells to blood brain barrier injury after cerebral ischaemia. Collectively, our investigations link early glial inflammatory responses to changes in neuronal network activity with the aim of identifying the cellular mechanisms by which neuroinflammation contributes to brain injury.

P168. Effects of Arctigenin In Seizure Induced *ex vivo* Brain Slice Preparations

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The plant lignan arctigenin is a phytoestrogen, which has important protective role in plants. Its antiviral and antioxidant properties were demonstrated in mammals as well; moreover it reduces the level of inflammatory cytokines and stress-induced signal transduction molecules. It is proved that it possesses also neuroprotective property. Beside the estrogen-like effect, arctigenin can modulate neuronal excitability by inhibiting non-NMDA receptors.

Previous experiments performed in the “*Ex vivo* Electrophysiology Research Group” of Eotvos University demonstrated the inhibitory effect of arctigenin on normal evoked responses in brain slices. It was found that arctigenin significantly reduced the amplitude of evoked responses in a dose-dependent manner.

The goal of the present experiment was to study the eventual inhibitory effect of arctigenin on synaptic transmission and spontaneous activity in an *in vitro* seizure model. Brain slices were prepared from rat somatosensory cortex. Two types of convulsive substances (4-aminopyridine-containing and magnesium-free artificial cerebrospinal fluid) were applied, which resulted in increased neuronal excitability. The effect of arctigenin on the early and late components of electrically evoked field responses and spontaneous discharges were analysed. Although arctigenin effectively repressed general excitability, its inhibitory effect was weaker in convulsant-treated slices than in normal slices.

P169. Microspikes on intracortical microelectrode recordings – a tuberous sclerosis case report

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Tuberous sclerosis (TS) is a rare genetic disorder, featured by multiple dysplastic cortical tubers. The prevalence of epilepsy is high in TS, but only a subset of tubers is epileptically transformed (ETT). Recent publication showed micro-spikes recorded by micro-electrodes (MIE) that were not apparent on macroelectrodes (MAE).

One TS patient was implanted with subdural MAEs to localize the ETTs for resective surgery: a grid over the left temporo-parietal, and bilateral strips over the temporo-medio-basal areas. Multicontact laminar MIE was inserted nearby the supposed ETT underneath the grid. Interictal epileptiform discharges (IED) were selected visually and IED triggered averages and inter IED time histograms were calculated.

We found frequent micro-IEDs (MID) on the MIE that was not reflected on the overlaying MAE. Large amplitude macro-IEDs (MAD) were recorded in the temporo-medial strips bilaterally, that showed no correlation in time with the MIDs. The seizure onset zone (SOZ) colocalized only with the MADs. Surgical intervention was not possible due to the bilateral SOZ.

According to our single case experience, MIE recording may be misleading, and indicates that MIE may have high electrical sensitivity and low epileptic specificity. However the presence of the MIDs in the not ETT may be an indicator of a precursor state of epileptogenicity. Further investigations of TS and MIE, MAE recordings are needed.

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P170. Expression of receptors of transforming growth factor beta proteins following focal ischemia in the rat brain

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Transforming growth factor- β s (TGF- β 1-3) regulate cellular proliferation, differentiation, and survival. TGF- β binds to type I (TGF- β RI) and type II (TGF- β RII) transmembrane kinase receptors, and an accessory type III receptor (TGF- β RIII). TGF- β may utilize a second type I receptor, ALK1. TGF- β is neuroprotective as injection of TGF- β decreased, while its antagonist increased the infarct size following middle cerebral artery occlusion (MCAO). Recently, we reported the expression pattern of TGF- β 1-3 after MCAO.

The present study describes the induction of TGF- β RI, RII, RIII and ALK1 at 3h, 24h, 72h and 1 month after transient MCAO using in situ hybridization. At 24h after the occlusion, TGF- β RI and RIII were slightly induced in the striatum around the lesion while TGF- β RII and ALK1 did not show any changes. At 72h following MCAO, TGF- β RI and RII appeared in the penumbra and also in the ischemic core. TGF- β RIII was not induced within the lesion while ALK1 showed elevated expression around the vessels within the infarct area at this time point. All four TGF- β receptors were induced within the lesion 1 month after the occlusion. Most TGF- β RI and RII expressing cells are neurons as the signal co-localizes with neuronal markers.

These data suggest that TGF- β receptors are induced after MCAO in a timely and spatially regulated fashion, which is also coordinated with the induction of TGF- β s at earlier time points.

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P171. Multi-phase translational research with epilepsy patients

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National Institute of Clinical Neuroscience runs translational epilepsy research since decades. The core of this research has been multi-scale electrophysiological approaches, studying micro- and macro-scales. In the latest years additional modalities has joined to the lineup.

The observations include 4 phases: 1) preoperative evaluation, 2) intraoperative electrophysiology, 3) in vitro electrophysiology, and 4) in vitro two photon microscopy.

In phase one, the patients undergo standard preoperative evaluation to locate the epileptic focus, including video-EEG monitoring and various imaging methods. During this phase spontaneous seizures, and interictal activity is recorded with intracranial macro- (MAE), and intracortical microelectrodes (MIE).

In phase two the electrocorticography is repeated during the respective surgery of the patients. The recording areas include the hippocampus and the cerebral cortex, where spontaneous, low -, and high frequency stimulation induced activity is recorded enabling us to record interictal and ictal like events right before phase three.

In phases three and four, researchers may investigate the tissue in a more controllable setting, studying neuronal dynamics involving the pre-, and postsynaptic levels.

During our collaborative study, we hope to be able to pose elementary questions about epileptogenicity from ion channel level to the intact brain.

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P172. *De-novo* Expression of Parvalbumin in Ependymal Cells in Response to Brain Injury Promotes Ependymal Niche Remodeling and Wound Closure

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The calcium-binding protein parvalbumin (PV) hallmarks subpopulations of interneurons in the murine brain. We serendipitously observed the *de-novo* expression of PV in ependymal cells of the lateral ventricle wall following *in-vivo* lesioning and the preparation of organotypic hippocampal slice cultures (OHSCs). In OHSCs, *de-novo* PV-expression begins shortly after the onset of culturing, and the number of ependymal cells implicated in this process increases with time. PV-immunopositive ependymal cells aggregate and form compact cell clusters characterized by lumen-formation and beating cilia. Numerous single cells acquire a reactive phenotype and undergo marked alterations in shape. Exposure of OHSCs to NF-KB inhibitors and to antioxidants reduces PV-expression in ependymal cells, thereby implicating this process in injury-induced inflammation. Indeed, *in-vivo* stab injury enhances PV-expression in ependymal cells adjacent to the lesion. Furthermore, fusiform PV-immunopositive cells around the ventricular zone and the lesioned site participate in scar formation. Such reactions are not observed in PV-knock-out mice, which manifest an impaired wound-healing response to *in-vivo* injury, and profound cell-morphological alterations in OHSCs. Whole-transcriptome analysis of ependymal-cell clusters in OHSCs revealed down-regulation of genes involved in cytoskeletal rearrangement, cell motility and cell adhesion in PV-knock-out as compared to wild-type mice, whereas those implicated in the response to hypoxia and oxidative stress were up-regulated. Our data indicate that the injury-triggered up-regulation of PV-expression is mediated by inflammatory cytokines, and promotes the motility and reorganization of ependymal cells, thereby contributing to tissue remodeling and repair after injury.

P173. Imaging central and systemic inflammation after cerebral ischaemia in vivo, using SPECT/CT in mice

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Imaging techniques to assess brain injury in a non-invasive manner are routinely used in patients and in experimental studies. However, it is technically challenging to visualize inflammation, blood brain barrier (BBB) breakdown and brain injury simultaneously *in vivo*, which is further limited by image resolution and the small size of a rodent brain. Since systemic inflammation is often associated with the development of neurological disorders such as stroke, novel tools to assess the contribution of inflammation to BBB breakdown and brain injury *in vivo* would be needed. To address these questions, we used single-photon emission computed tomography (NanoSPECT/CT Plus) utilizing specific radioligands to investigate central and systemic inflammation after experimental stroke in mice. BBB breakdown was visualized by using penetration of ^{99m}Tc-DTPA into the brain parenchyma, and colocalised with alterations on MRI (nanoScan PET/MRI) images after stroke. Inflammatory changes were simultaneously monitored by imaging ¹²⁵I-BSA in the same mice, which enabled visualization of systemic inflammation induced by bacterial lipopolysaccharide prior to experimental stroke and also peripheral inflammatory changes induced by cerebral ischaemia. Our imaging protocols might be useful tools to assess inflammation and brain injury in small rodents *in vivo*, and to develop techniques for evaluating systemic inflammation and BBB permeability changes in patients presenting with neurological disorders.

P174. Changes of the pyramidal cells in the primary motor cortex of schizophrenia patients

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One of the most consistent observations in the morphology of the schizophrenic brain is the reduction of the volume of several areas, e.g. prefrontal and temporal cortices. In the clinical practice, the most patients suffer from psychomotor impairment. Therefore, cytoarchitectural changes of the motor cortex can be present in schizophrenic brains as well. In the present study, post mortem human brain samples of the primary motor cortex (Brodmann's area 4) have been investigated with histological and immunohistochemical (NeuN, PV and SMI32) stainings. We analysed the cell size and the density of the pyramidal cells of the layer III and V. Significant reduction of the pyramidal cell size in layer III and V was found. The density and size of the giant pyramidal cells (Betz cells) has been reduced as well. In layer III a significant loss in density was measured. These data suggest that motor disturbances may be related to the cytoarchitectural change of the motor cortex in schizophrenia disorder. Further investigations are needed to clarify whether these observations are features of the disease or the effect of the antipsychotic medication.

P175. Alleviated mucosal and neuronal damage at the acute phase of recurrent inflammation in a rat model of Crohn's disease

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Crohn's disease (CD) is a chronic relapsing inflammatory bowel disease with marked intestinal motility disturbances, indicating that myenteric neurons are targets of inflammatory damages. Our aim therefore, was to establish a rat model of chronic colitis suitable to investigate the myenteric neurons and their microenvironment in repetitive recurrent inflammations (RRI) provoked by repeated treatments with 2,4,6-trinitrobenzenesulfonic acid (TNBS). Tissue samples were taken from the inflamed and adjacent proximal and distal segments of the colon in different timepoints of acute intestinal inflammations. The quantitative features of myenteric neurons were investigated on wholemounts after HuC/HuD immunohistochemistry. The expression of heme oxygenase-1 (HO-1) was evaluated by qRT-PCR. Severe mucosal inflammation, widespread loss of the neurons, and marked but transient HO-1 upregulation were demonstrated after the single dose of TNBS. After repeated doses the extension of ulcers decreased markedly, neuronal loss was smaller scale and was limited to the inflamed area. HO-1 mRNA upregulation was approximately 50% greater here than after the first treatment and the high level of overexpression was sustained throughout the timepoints examined. We suggest that experimentally provoked RRI through preconditioning have a protective effect on the mucosal and neuronal damage related to the acute intestinal inflammation. The persistent upregulation of HO-1 may correlate with this effect.

P176. A murine passive transfer-trauma model for Complex Regional Pain Syndrome (CRPS) clinical signs

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CRPS is a chronic persistent pain condition with redness, warmth and swelling of the affected area occurring after a minor limb injury. Since its mechanism is unclear and there is still no cure, an appropriate animal model for experimental investigations is needed to analyse the pathophysiological processes. Important roles of immune responses and complex neuro-immune interactions are suggested to be involved in its mechanisms.

Passive transfer-trauma model of CRPS was set up in female C57Bl/6 mice with a plantar skin-muscle incision followed by repeated treatment with purified serum-immunoglobulin G (IgG) of CRPS patients and healthy volunteers. Mechanical touch sensitivity, paw volume, motor coordination and paw temperature were measured during 8 days. Sensory nerve-derived neuropeptides and inflammatory cytokines were determined from the paw homogenates.

CRPS-IgG injection significantly increased incision-induced paw oedema and touch sensitivity compared to the healthy-IgG-treated group, but did not influence motor coordination and paw temperature. CRPS-IgG elevated the concentration of the sensory neuropeptide substance P (SP) in the paw, but inflammatory cytokines remained unchanged.

This is the first passive-transfer trauma model for CRPS, where serum IgG from patients induced clinical features resembling the human disease. Autoantibodies may contribute to the pathophysiology of CRPS, and removing these proteins could be a new possibility of CRPS treatment.

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P177. Tracking the journey of Shadoo from translation to the nucleus

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The prion protein (PrP) – known for its central role in transmissible spongiform encephalopathies – has been reported to possess two nuclear localization signals and localize in the nuclei of certain cells in various forms. There are growing numbers of evidences about the appearances of various intracellular forms of the prion protein, which might play a role in prion diseases. In this respect, it is intriguing that another prion protein superfamily member – the Shadoo (Sho) – is also found in the nucleocytoplasm of some neurons. The accumulation of Sho is observed in the nucleus of several neural and non-neural cell lines as visualized by using an YFP-Sho construct. This nuclear localization that is mediated by the (25-61) fragment of mouse Sho (encompassing an (RXXX)₈) motif is not expected from a membrane protein. Here we try to trace the journey of various forms of Shadoo from translation to the nucleus.

P178. Changes in the Distribution of Citrate Synthase in Hippocampi of Patients with Temporal Lobe Epilepsy

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Dysfunction of mitochondria is well known in several neurological disorders and in different types of epilepsy. There is a limited amount of evidence about changes of the density of mitochondrial enzymes in human epileptic samples, and the available data are controversial. Therefore, we examined surgically removed hippocampal samples from 18 patients with drug resistant temporal lobe epilepsy (TLE). Post mortem control samples were obtained from 4 subjects without any sign of neurological disorders. Immunohistochemistry was used to assess the density of citrate synthase, which is a widely accepted method for detecting intact and functional mitochondria. Light, electron and fluorescent microscopic analyses were carried out. In control tissue differences in the homogeneity of cellular staining was found only among hippocampal subfields. In contrast, epileptic tissue exhibited inhomogeneous staining patterns within specific subregions, like CA3 and dentate gyrus. Elevated intensity of citrate synthase-immunostaining was observed in the CA2 area of each epileptic hippocampus. In previous studies this region was found to be a spontaneous spike generator in TLE, providing a possible explanation for the presence of the high rate of mitochondrial metabolism.

P179. The epigenetic background of Brattleboro rats' schizophrenia-like behavior

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We aim to clarify if epigenetic changes in vasopressin-deficient (AVP-) Brattleboro rats contribute to their schizophrenia-like (SZ) behavior. Other authors presented SZ-like symptoms in this strain and suggested that SZ might have epigenetic component.

Behavioral (attention deficit by prepulse inhibition (PPI)) and epigenetic (global histone3 (H3) acetylation (ac) by Western-blot and H3 lysine9 (K9) ac by immunocytochemistry) differences were compared between wild type and AVP- animals.

The reduced PPI of AVP- rats confirmed SZ-like behavior in our local colony. The pan-H3ac immunoreactivity was lower in the homogenized brains' prefrontal region in AVP- animals. Thus, we examined the region specific distribution (prefrontal cortex, lateral septum and nucleus accumbens) of a specific acetylation site, the H3K9ac, as one of the most studied epigenetic mark. We found lower immunopositive cell number in the dorsal peduncular prefrontal cortex and the ventral lateral septum of AVP- animals.

We confirmed that the AVP- Brattleboro rat is a good preclinical model of SZ. In this strain the SZ-like behavioral alteration is accompanied by changes in H3 acetylation level in the prefrontal brain region, especially at dorsal peduncular prefrontal cortex and the ventral lateral septum. These reduced acetylation may contribute to disturbances of many neurotransmitter systems (for example GABA, acetylcholine) which can explain the SZ-like symptoms.

P180. Distinct set of receptors mediates evoked fEPSPs and spontaneous spiking: selective effect of amyloid-beta(1-42)

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Neuronal hyperexcitability culminating into unprovoked seizure is a phenomenon associated with early Alzheimer's disease (AD). Abeta1-42 is regarded as a key factor of AD, is shown to induce neuronal hyperexcitation. The effect of Abeta1-42 on LTP is well documented; however data about Abeta1-42 impact on firing connected to synaptic plasticity is less known. To this end we investigated the effects of Abeta1-42 on two electrophysiological hallmarks of excitation, evoked fEPSPs and spontaneous spiking, using wild-type mouse hippocampal slices. Despite fEPSPs remained unchanged within the 2.5 h time frame of the recording, spiking activity greatly increased over time in untreated slices. Moreover, the change of spiking rate was correlated with the initial spiking activity; therefore recordings were divided into two groups based on the Z-value (deviance from the mean). There was a massive enhancement of spiking rate in the "lower" initial spiking activity group ($Z < -2.5$) than the "higher" initial spiking activity group ($Z > -2.5$). Using MK801, NMDA, CNQX and low-MgSO₄ (0.25 mM) ACSF to isolate NMDA receptor (NMDAR) dependent fEPSPs, we have found that the fEPSPs are AMPAR-dependent, but the spiking activity requires mainly NMDAR-activation. Abeta1-42 oligomers induced a significant enhancement of spiking activity in the "higher" initial spiking activity group without altering evoked fEPSPs. These results suggest that Abeta1-42 induced neuronal overactivity is dependent mainly on NMDAR-s.

P181. Investigating the Retinoprotective Effects of PACAP Fragments, Secretin and Glucagon in Ischemic Retinopathy

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Pituitary adenylate cyclase activating polypeptide (PACAP) has neuroprotective effects in different neuronal and retinal injuries. Retinal ischemia can be effectively modelled by permanent bilateral common carotid artery occlusion (BCCAO), which causes chronic hypoperfusion-induced degeneration in the entire rat retina. The retinoprotective effect of PACAP38 and VIP is well-established in ischemic retinopathy. However, little is known about the effects of related peptides and PACAP fragments in ischemic retinopathy. The aim of the present study was to investigate the potential retinoprotective effects of different PACAP fragments (PACAP 4-13, 4-22, 6-10, 6-15, 11-15, 20-31) and related peptides (secretin, glucagon) in BCCAO-induced ischemic retinopathy.

Wistar rats (3-4 months old) were used in the experiment. After performing BCCAO, the right eyes of the animals were treated with PACAP fragments or related peptides intravitreal (100 pM), while the left eyes were injected with saline serving as control eyes. Sham-operated (without BCCAO) rats received the same treatment. Routine histology was performed 2 weeks after the surgery, cells were counted and the thickness of retinal layers were compared. Our results did not reveal retinoprotective effect of the PACAP fragments or related peptides. These results suggest that PACAP 1-38 has the greatest effectiveness in the animal model of ischemic retinopathy.

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P181a. Proteomic investigation of the amygdala in the Clomipramine model of depression

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Clomipramine is a tricyclic antidepressant, which increases the amount of norepinephrine and serotonin in the synapse. It is known, that neonates treated with clomipramine, show depressive-like behavioral and neurological alterations in adulthood. In depression research, there is evidence of the dysfunctionality of the monoaminergic and 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, hence taking antidepressants in childhood is forbidden in some countries. The main object of my study was to explore the protein expression differences in the amygdala - important brain region in emotion processing -, between the clomipramine treated and control group of female Wistar rats. We used the 2D-DIGE method to separate the proteins, and Mass spectrometry (MS) to identify them. In the group treated with clomipramine we found significant expression changes in 23 spots compared to the healthy group. The altered proteins have functions like regulating apoptosis, transcription, protein degradation, and Ca²⁺ signaling. Changing the brain monoaminergic transmission in early brain development stage induces altered protein networks in adulthood giving a firm molecular basis for clinical observation that neonatal psychoactive drug treatment makes high incidence of psychiatric diseases in adulthood.

P181b. Patterns of synchronous population activity in the neocortex of patients with epilepsy or tumour, in vitro

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Similar to interictal spikes recorded on the scalp EEG of epileptic patients, spontaneous population activity (SPA) has been shown to be spontaneously generated in epileptic human tissue in vitro. Tissue slices were prepared from neocortical tissue of patients with epilepsy and as a control, of patients with tumour but without epilepsy. Local field potential gradient (LFPg) was recorded with a 24 channel laminar microelectrode. SPA consists of high frequency oscillations and elevated cell firing superimposed on a local field potential transient. SPA could be detected in 48% of epileptic and 41% of tumour slices. The most frequently, SPA was generated in the supragranular layers (68%-epileptic-, 56%-tumour tissue). Cell clustering analysis showed that 58% and 47% of the neurons elevated their firing rate in epileptic and tumour slices, respectively. In general, supragranular cells showed the maximal firing before, whereas infragranular cells discharged after the LFPg peak. We showed that both excitatory and inhibitory neurons participate in the generation of SPA. Our results suggest that spontaneous SPA is generated in both epileptic and non-epileptic neocortical tissue slices. Supragranular layers have a leading role in the initiation of the SPA, in which both excitatory principal cells and inhibitory interneurons participate. The cellular and network properties of SPAs were similar, which indicates that in vitro occurring SPA cannot be directly related to epileptic processes.

P182. Examination of pituitary adenylate cyclase activating polypeptide-like immunoreactivity in human dental pulp samples

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a multifunctional neuropeptide with neurotrophic, cytoprotective, anti-inflammatory and anti-apoptotic effects. PACAP-immunoreactive fibers were found in the odontoblastic and subodontoblastic layers of the dental pulp, but there is no data about its role in pathological changes. The aim of our study was to examine PACAP38-like immunoreactivity (LI) in various pathological conditions of the pulp. The samples were collected from 60 patients (28 female, 32 male). The samples were divided into groups according to the diagnosis of the pulp (healthy, acute irreversible pulpitis, chronic irreversible pulpitis, gangraena) upon clinical examination and radiographic findings. The samples were also separated on the basis of their locations (front, premolar and molar teeth). PACAP38-LI was measured with radioimmunoassay. Our data revealed significant difference between chronic and acute irreversible pulpitis in the case of premolars. The difference between premolars and molars was also significant in chronic pulpitis. Our results demonstrated that PACAP38-LI increases in chronic inflammation suggesting that it may play an important role in the inflammatory reactions of the pulp.

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P183. A rapid and efficient approach for cell type- and compartment-specific quantitative molecular imaging using STORM super-resolution microscopy

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The brain is a vastly complex structure composed of a diverse population of hundreds of highly compartmentalized cell types. Obtaining quantitative molecular information from subcellular compartments in a cell-type specific manner is extremely challenging. Here, we present a rapid and versatile method based on STORM super-resolution microscopy to surmount this obstacle, with an unprecedented level of efficiency. We show that this method yields robust and reliable data under various experimental conditions. To illustrate the potencial in this approach, we have identified novel principles concerning the molecular organization of cannabinoid-controlled GABAergic synapses in the mouse hippocampal CA1 region. The CB₁ receptor is the most abundant GPCR in the brain, and endocannabinoids are amongst the most important presynaptic regulators of neurotransmission. However, the governing principles which determine the precise spatial localization and density of CB₁ receptors within an axon terminal, and thus their contribution to the control of synaptic transmission, have not yet been identified. We found that the distribution of CB₁ receptors is uniform over the membrane of the axon terminal, as well as between perisomatic and dendritic cell types. However, unexpected quantitative differences in CB₁ abundance support interneuron type-specific efficiency of cannabinoid signaling.

P184. Nanostructured Si Surfaces for Neural Application

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Surface quality of implantable biosensors for recording electric signals in the central nervous system are of great importance with respect to long-term use in chronic animal experiment. In terms of functionality, silicon-based microelectrode arrays are widely used, however, their interaction with the living tissue is still not addressed extensively. Since cells in their native microenvironment interact with three-dimensional features, surface nanostructure of the implanted surfaces plays a key role in biocompatibility. Using nanostructured contact surfaces might be a solution for postponing the formation of glial scar around the implanted microelectrode. Furthermore, nanostructured surfaces provide larger specific surface for the electrical contacts, therefore lower impedance and better signal-to-noise ratio can be achieved.

In our work, nanostructured black-silicon surfaces have been fabricated in poly-silicon thin films by cryogenic dry etching in order to realize seed layer with nanoscale roughness for subsequently metallized electrode recording sites. Surface morphology of the samples has been characterized by electron microscopy and atomic force microscopy. The proposed nanostructuring method can also be integrated in the process flow of an implantable neural microelectrode. The fabricated electrodes were tested by cyclic voltammetry and electrochemical impedance spectroscopy. Samples are prepared to investigate viability and adhesion of neural and glial cells in vitro.

P185. Two-dimensional, high-resolution current-source density analysis of neuronal action potentials

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Action potentials (APs) are the basic computational elements of neuronal networks. The generation and propagation of APs are well described, however two-dimensional extracellular recordings with high-resolution could reveal valuable new findings. The Neuroseeker project aims to develop high-density silicon probes with thousands of small ($\sim 5\ \mu\text{m}$ diameter), closely spaced ($\sim 1\ \mu\text{m}$) contacts. We used the first design of the probes (26 recording contacts) in acute and chronic in vivo experiments to record the unit activity of cortical, hippocampal and thalamic neurons and to investigate the spatiotemporal distribution of transmembrane currents during their APs on an approximately $70 \times 40\ \mu\text{m}$ grid. Rats were anesthetized with ketamine/xylazine, and local field potentials, multiple- and single-unit activity were recorded from multiple regions of the brain. Spike sorting was performed on the recorded data to isolate single neurons. Based on the sorted data, average AP waveforms were calculated from the raw recordings and the two-dimensional current-source density (CSD) was estimated with a Matlab-based software implementing the inverse CSD method. After that, we analyzed the 2D propagation of the APs and the distribution of their transmembrane currents in different cell types, in consecutive spikes of spike bursts and in different brain states. Based on our results we can conclude, that the 2D, high-resolution CSD can be a useful tool in the future.

P186. Feasibility of dynamic stereovision tests in amblyopia screening

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Even though amblyopia is the leading cause of permanent vision loss under the age of 40 and has a high prevalence in the population (4-6%), screening for the disease is not properly implemented. In this work we present results of a feasibility study of dynamic and static stereovision tests (DST & SST) to detect amblyopia. In order to answer these questions we have investigated 403 children in various kindergartens of Trieste, Italy. We have carried out monocular visual acuity (VA) examination, Lang stereotest, DST and SST, Brückner test, cover test and corneal light reflex examination. Out of the 403 examined children, 56(13.9%) had an uncorrected VA difference of more than 0.2 between the two eyes, and 20(5% of total) of them failed the DST as well. Altogether 146(36.2%) failed DST, and 60(14.9%) failed both DST and SST. There were no measurable monocular artifacts in the DST. Positive cases were advised to see an Ophthalmologist. Based on our findings, it is very likely that 5% of children aged 4-6 have amblyopia in Trieste, which is compatible with European average. We have confirmed that the test is easy, efficient and quick to carry out even on a young population and anybody can do it after a short training session. With its present settings, DST on its own does not provide a substitute for complex vision screening tests. However, with minor adjustments we think that its sensitivity can be optimized and will serve as an essential part of vision screening for amblyopia.

P187. withdrawn

P188. FcRn transgenics as a novel method for generating superior CB₁ cannabinoid receptor antibodies

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CB₁ cannabinoid receptors play essential roles in mediating the diverse cellular effects of the endocannabinoid system in the central nervous system. Emerging evidence indicates that CB₁ receptors contribute differentially to physiological or pathophysiological mechanisms in a cell type-specific manner. However, due to low expression levels, it was tremendously difficult to investigate the precise cellular and subcellular distribution of CB₁ receptors in a quantitative manner on profiles other than GABAergic axon terminals. To overcome this limitation, we used transgenic rabbits and mice overexpressing the neonatal Fc receptor (FcRn), as they have augmented immune response because of the role of FcRn in antigen presentation. Accordingly, injection of a CB₁ C-terminus antigen resulted in higher antibody titers and a more diverse B-cell repertoire activation in FcRn transgenic rabbits compared to control animals. Moreover, all 3 FcRn antisera, but only 1 control antiserum were able to visualize CB₁ receptors on mouse hippocampal sections. Notably, a polyclonal antibody derived from one of the transgenic animals was also used to visualize CB₁ receptors on glutamatergic axon terminals both via immunogold electron microscopy and STORM super-resolution microscopy. In the latter case, super-resolution imaging of axon terminals of CA3 pyramidal cells revealed that at least 28% of the recurrent collaterals contain CB₁ receptors. Finally, FcRn-overexpressing mice were also used successfully to generate specific and sensitive monoclonal antibodies against CB₁ receptors providing a new opportunity for multiplex immunostainings. To sum up, FcRn overexpression leads to superior CB₁ antibody production allowing effective labeling of CB₁ receptors even on glutamatergic terminals.

P189. An implantation method of Pt/Ir microwires for chronic neural recording from the orbitofrontal cortex of freely moving mice.

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We present a method for implantation of microwires electrode for chronic neural recording from orbitofrontal cortex (OFCx) in freely moving mice. The uniform microwires (Pt/Ir, 20 µm OD formvar insul.) are inserted to the two parallel tubes (185 µm OD) and protrude for a 2.5 mm forming the brush shape. The tubes are glued to the narrow wall of an Omnetics connector and the wires from opposite site to the electrode site are soldered to the connector's pins. The electrodes are inserted into the two parallel oriented guiding tubes (360 µm OD) that are perpendicularly fixed in a small platform. On the platform the guiding tubes are oriented upward and only a short part (< 1 mm) is oriented downward toward the brain surface. During the surgery the platform with the installed brush electrodes is placed onto the skull and the guiding tubes are positioned in to the trepanation holes above the intact dura mater. The platform is fixed by acrylic cement on the skull and the electrodes are advanced by conventional manipulator through the dura in to the targeted brain area under physiological control. The method of implantation was successfully implemented on seven BALB/c mice. We recorded local field potentials (LFP) and multi-unit activity (MUA) of the OFCx during different behavioral tests. In two cases we were able to record LFP and MUA signals for more than one year post implant. Morphological analysis confirmed that the wires were implanted in to the OFCx.

P190. 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 oscillations and related 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 show volumetric random-access scanning calcium imaging of spontaneous and visual stimulation-evoked, orientation-tuned activity in hundreds of neurons of the mouse visual cortex *in vivo*.

We improved our 3D, acousto-optical, two-photon, random-access scanning microscope (Katona et al. 2012, Nature Methods) to monitor neuronal activity with genetically-encoded calcium indicators in a near-cubic-millimeter scan range (up to $700 \times 700 \times 1,400 \mu\text{m}^3$), with a high scanning speed (up to 500 points/kHz in 3D trajectory scanning mode), with high ($<500 \text{ nm}$) resolution in the center core, and less than $1.9 \times 1.9 \times 7.9 \mu\text{m}^3$ resolution throughout the whole scanning volume.

P191. Optrode for multimodal deep-brain optical stimulation

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Optical stimulation is an emerging method for controlling biological systems. Light is used to influence specific functions of a cell, for example neurons can be fired or silenced. The tissue is sensitive to specific wavelengths and can be sensitised to others. The effect of the stimulation can be verified by measuring local electric potentials. The realization of a microelectrode system where optical stimulation and electrical recording are both available simultaneously would enable high precision, repeatable *in vivo* measurements.

In this work the concept of a deep-brain microprobe with integrated waveguide structures is presented. The supporting silicon of the probe is utilised in the infrared regime. For visible light, a polymer rib waveguide is fabricated from SU-8. To obtain optimal optical performance, the waveguides were simulated using a commercial FEM and custom ray optics software. Preliminary optic measurements show good agreement with simulated results.

The system can be used to explore optogenetics (visible light regime) and infrared neural stimulation (INS).

P192. A Novel Methodology for Identifying Low-Threshold Afferent Driven Inhibitory Neurons in the Spinal Dorsal Horn

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Due to its anatomical position, spinal lamina I, a key element in relaying primary afferent input to supraspinal areas, is accessible for electrophysiological studies in the intact spinal cord. Recently, we identified a subpopulation of anterolateral-tract projection neurons (ALT-PNs) receiving low-threshold primary afferent driven inhibitory inhibition that may function as a postsynaptic gate controlling the nociceptive information flow in the spinal cord. Our hypothesis is that the inhibition is mediated by dedicated inhibitory interneurons reliably excited by low-threshold primary afferents and possessing axon collaterals in lamina I. Identification of this cell population with random whole-cell patch-clamp, and post-hoc morphological identification would give very low yield. Thus, we propose a novel combination of calcium- and IR-LED- imaging methods in a transgenic mouse model that expresses a genetically encoded calcium sensor (GCaMP3) in the GABAergic inhibitory neurons. Our strategy is to 1) locate inhibitory neurons that respond to stimulation of low threshold primary afferents with calcium imaging, 2) record and fill them via visually guided whole-cell patch-clamp and finally 3) perform 3D reconstruction and morphological identification of the cells. We believe that this complex approach will allow faster identification of the putative inhibitory element serving as the disynaptic inhibitory link between low-threshold primary afferents and ALT-PNs.

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P193. Robust silicon-based microelectrode arrays and a microdrive system for in vivo electrophysiology

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Modern silicon-based microtechnology, fuelled by the electronic industry allows precise batch fabrication of microelectromechanical systems (MEMS) with reproducible features and small dimensions, including versatile and cost-effective ways of making microelectrode arrays (MEAs) for implantation into the nervous system. We constructed several types of robust silicon-based probes, with anatomically designed site arrangements in order to record neural activity from the cortex, the hippocampus and several brainstem nuclei in anesthetized or freely moving rats. A stable microdrive was also developed, allowing precise electrode targeting into the tissue. Each probe was attached to a rigid printed circuit board, which was fastened to the microdrive. A flexible cable provided not only electronic connection between the probe and the amplifier system, but sufficient freedom for the movements of the probe as well. The accurate positioning of recording sites on the probe along with the fine-tunable probe location postoperatively allowed us to record parallel neural activity (~ 200 single units) e.g. from three brainstem areas (deep mesencephalic reticular-, pedunculopontine tegmental- and pontine reticular nucleus) concurrently.

P194. Probing identified synapses at the nanoscale: developing software tools for visualization and analysis of correlated confocal- and STORM super-resolution microscopy

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Understanding the molecular mechanisms underlying physiological and pathophysiological processes of the nervous system requires adequate methods to determine the nanoscale targeting of proteins within neurons. The power of high-resolution light- and electron microscopic techniques is often limited by the fact that brain circuits are comprised of several highly compartmentalized cell types. STochastic Optical Reconstruction Microscopy (STORM) is a flexible and efficient way to obtain precise molecule localization data. Combining STORM with confocal microscopy to visualize identified cells opens the ability to analyze super-resolution data in a cell type- and compartment-specific manner. However, while advanced tools exist for the handling of either confocal or STORM image data, to date, the correlated visualization and analysis of both is not available. Moreover, the localization-based data from STORM allows for a wide variety of novel analysis approaches compared to conventional, voxel intensity-based microscopy data, which are not included in available software packages.

Therefore, we aimed to implement the above features in a custom-made program designed for the needs of molecular neuroscience. We developed an easy-to-handle graphical user interface in MATLAB, which we employed to determine the nanoscale localization of molecular components of the endocannabinoid system within axon terminals of identified neurons.

P195. Imaging mass spectrometry of the brain of pacap deficient and wild-type mice

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Imaging mass spectrometry (IMS) is a rapidly developing technique that uses spatially resolved proteomics/peptidomics and metabolomics techniques to simultaneously trace the distributions of biomolecules directly from tissue samples. IMS is suitable for label-free discovery of multiple classes of biomolecules directly on the surface of a tissue section, and can be combined with other routine imaging and proteomic methods.

The most widespread IMS scanning technique is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS). This conventional IMS is a powerful technique that combines the chemical and spatial analysis of dehydrated biological surfaces. High sensitivity and mass accuracy, broad mass range detection, good mass resolving power, high speed, MS/MS capabilities and good spatial resolution make it excellent tool for tissue based imaging purposes. MALDI IMS has been used for discovery of neurodegenerative diseases related lipids, peptides and proteins.

PACAP is one of the neuropeptides implicated in protection against neurodegenerative processes. Its protective effects have already been described in animal models and in vitro experiments in models of Parkinson's disease, Huntington chorea, Alzheimer's disease and motor neuron degeneration. Our aim was to investigate the differences of the local distribution of expressed proteins in the brains of PACAP deficient and wild type mice. Brains were removed from mice, sectioned with cryostat and then the cryosections were coated with MALDI matrix. The samples were analyzed with an Autoflex speed MALDI TOF MS. Our imaging mass spectrometry results show significant differences of the distribution of various proteins in different brain areas, including the hippocampus, mesencephalon and corpus callosum. For example, marked differences could be observed in myelin basic protein, which could explain the differences observed between myelination processes in the two mouse groups. The identified proteins could give an insight into the general protective effect of PACAP and the increased vulnerability to various harmful effects in the nervous system of mice deficient in PACAP.

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P196. Quantitative in vivo imaging in brain science

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In vivo imaging in preclinical research enables measurement and monitoring of biochemical progresses, receptor binding and related anatomical and biochemical changes in the brain. Beyond the in vivo and ex vivo microscopy technologies and immunohistology imaging offers quantitative solutions.

Our aim is to show representative examples of the usage of the PET and SPECT, MRI and CT technologies for brain research.

Blood perfusion in the brain can be measured by high resolution SPECT ^{99m}Tc-albumin microspheres to detect circulation-related disorders in early stage. Our blood-brain-barrier studies give accurate and quantitative answers for the possible damage (with ^{99m}Tc-DTPA) and tissue oxidative stress with ^{99m}Tc-HMPAO. Using SPECT/MRI, potassium and calcium ion trafficking is exactly measured using ionic entrapment of ²⁰¹Tl (for K+) and ²⁰³Pb (for Ca++) concentrations. PET/MRI can measure the local glucose phosphorylation kinetic constants (¹⁸F-FDG) or CNS receptor binding kinetics. The presented studies will include measurements of dopamin D2 receptor dissociation kinetics using ¹¹C-raclopride. Our methods also contain the application of ⁶⁵Zn for zinc and iron related investigations in brain/spinal chord. The presentation will include a quantitative method to follow-up stages of Alzheimer's Disease measuring distribution of a ¹⁸F-labeled amyloid-binding tracer in different brain areas. We are going to present the results and the mathematical basic of quantitative imaging.

P197. *In vivo* Multiphoton Microscopy of Gliovascular Reactions: a Preliminary Study

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Multiphoton microscopy is a method of investigation of living specimens. Astroglia and their vascular connections have been hardly investigated. Present study demonstrates our first steps in this field. The study were performed a Femtonics Femto2D-Inverted multiphoton microscope in the Dept. of Biophysics of Semmelweis University (INMIND 278850). Lesions were performed by focusing the laser of the microscope following craniotomy and removal the envelopes (in a deep ketamine-xylazine anesthesia). Beside 'wild' mice transgenic animals were also used. A strain had green fluorescein-protein labeled fractalkins to visualize microglia, another strain had yellow fluorescent protein labeling endothelial cells. To demonstrate the *post-lesion* leakyness of vessels, fluorescent dyes – sulforhodamine B and fluoresceine isothiocyanate-conjugated dextran (40 kD) were injected *via* the tail vein. Astrocytes were labeled with sulforhodamine 101 smeared on the brain surface. Both astroglial and microglial cells were clearly identified and some gliovascular connections were visible. Laser-combustion of vessels was followed immediately by an intense leakage from the vessel. Within the investigated period (5 -10 min) no astrocytic reaction was observed. Microglial cell reacted with swellings on its processes when the perikaryon. Material then were procesed for conventional fluorescent microscopy. For the transgenic animals thanks to Á. Dénes and F. Erdélyi (Inst. of Experimental Medicine).

P198. Body Doubles in the stage of PCR cloning: A general use of Type IIS Restriction Enzymes for cloning

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The procedure described here allows the cloning of PCR fragments containing a recognition site of the restriction endonuclease (Type IIP) used for cloning in the sequence of the insert. Here, a Type IIS endonuclease - a Body Double of the Type IIP enzyme - is used to generate the same protruding palindrome. Thus, the insert can be cloned to the Type IIP site of the vector without digesting the PCR product with the same Type IIP enzyme. The recognition site of a Type IIS restriction enzyme that cleaves the DNA outside of its recognition site is incorporated in the PCR primer in such a way that the cutting positions contain the desired overhang sequence. Digestion of the PCR product by the Body Double generates the required overhang. Hitherto the use of Type IIS restriction enzymes in cloning reactions has been rather a rare curiosity in special applications: the approach presented here makes Type IIS enzymes as useful as Type IIP enzymes for general cloning purposes. We routinely use the procedure to bypass this frequently arising problem.

P199. Electrophysiological Correlates of Letter Spacing in Natural Reading

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The neural mechanisms of natural reading are still obscure since previous EEG research on reading has been focussed on experiments with fixed eyes. To avoid the prominent EEG artefacts originating from eye and muscle activities, eye-movements were not allowed in these ERP paradigms. However, recent advancement of data acquisition and processing techniques could provide an opportunity for investigation of reading in natural, free viewing conditions.

Here we demonstrate the applicability of concurrent eye-tracking (ET) and EEG data for investigation of natural reading. Our methodological approach mainly consists of simultaneously recoding of eye-tracking (ET) and EEG, adaptive analysis of ET data, artefact elimination using independent component analysis of EEG recordings with incorporated ET information, single-trial regression as well as wavelet transformation based spectral dynamics analysis of fixation-related potentials (FRPs). The obtained results reveal significant effects of letter spacing on FRP parameters during natural reading while controlling for confounding ET variables such as saccade amplitude and fixation duration.

Our results may contribute to deeper understanding of information sampling during natural reading and dyslexia.

P200. A Novel EEG Processing Framework for Analyses of Background and Event-Related Brain Activities Related to Senso-Motoric Processes

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Organization of voluntary movements and their electrophysiological correlates are still poorly understood. Preparation and execution of motor actions is mostly investigated by BOLD fMRI methods. However, despite of its high spatial resolution, this brain imaging modality allows only indirect measurement of neural activity with a low temporal sampling frequency. Accordingly, there is a need for complementary non-invasive approaches addressing these shortcomings.

We introduce a novel methodological apparatus optimized for analyses of brain dynamics related to complex mechanisms of senso-motoric processes. Our approach relies on EEG data recorded with high spatio-temporal resolution and modularly developed software tools implemented in Matlab environment. The developed toolbox allows the assessment of background and event-related brain activities and mainly consists of EEG processing methods for semi-automatic pre-processing, scalp current density transformation, feature extraction such as time-frequency decomposition for spectral dynamics and inter-trial coherency analyses, cluster-based statistical evaluation, 2D and 3D visualization. We demonstrate the usability of our approach by presenting analysis results for EEG data obtained during visually cued index finger tapping and median nerve stimulation experiments.

The presented framework may enhance the investigation of voluntary movements.

P200a. Analyzing dendritic spine movement in cultures: a fast and effective way to identify regulatory pathways influencing spine motility and maintenance

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Dendritic spines are small dendritic protrusions whose number and morphology change actively during the development and maturation of neuronal networks. During synapse formation, filopodial-like structures search for future presynaptic partners. Once the synapse is formed, morphological changes of spines influence synaptic strength. Abnormal spine development and morphology hinder normal neuronal functions and often accompany mental disorders.

Dendritic spines can be visualized in vivo, even in intact and freely moving animals with 2Pi microscopy, however, the time resolution (between minutes to hours) and molecular manipulations within the living brain are quite limited. In order to screen and identify regulatory pathways and molecules influencing dendritic spine dynamics, we developed fast live cell imaging of cultured hippocampal neurons. Neurons were transfected with fluorescently labelled constructs, providing the visualization of individual spines as well as the expression of different mutant proteins. Fluorescent images were recorded in every 2s, allowing the identification of fast filopodial and spine movements. Instead of the time-consuming manual detection of spine outlines, we created an ImageJ plugin to effectively and more reliably follow motility changes within the transfected spines. Using this method, we have already identified several regulatory molecules affecting filopodial and spine motility in cultured neurons.

P200b. Determination of cortical and synaptic layers and synaptic dynamics based on extracellular multi-electrode potential measurements

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We have shown, that it is possible to determine cellular and synaptic layers in the hippocampus and in the neocortex, based on extracellular multi-electrode potential measurement. High density silicon probes provided the basis for high-frequency power map, which clearly marked the pyramidal layer and the dentate-hilar area in the hippocampus. We have calculated the gamma-band coherence between all channels, and clustered them based on the resulted coherence values. The specific laminar distribution of the efferent hippocampal pathways enables identification of the anatomical layers in the dendritic area based on the coherence. This method reliably determined the different anatomical layers not only in the hippocampus, but identified three separate layers, corresponding to the superficial (II/III), middle (IV) and deep (V/VI) layers in the neocortex as well. Finally the temporal dynamics of the LFP and CSD in hippocampal layers were examined, showing the spatio-temporal distribution of synaptic events leading to action potentials in pyramidal and interneurons during theta oscillation and sharp-wave ripple periods.