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ABSTRACTS

Effects of interval training and probiotic supplementation on cognitive function in a transgenic mice model of Alzheimer's disease

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Approximately 23 million people worldwide suffer from Alzheimer's disease (AD). The illness is associated with the loss of acquired knowledge and the ability to learn. Inflammation and neuron loss indicated by Amyloid-beta peptide accumulation underly this disease. So far there is no effective treatment, but it is proven that physical exercise protects against neurodegenerative diseases, while numerous studies support the positive effects of healthy gut flora on these processes.

Experimental design: A total of 32 APP/PS1 transgenic male mice were in the experiment. 8-8 animals in control, exercise, probiotic supplementation and combined treatment groups, respectively. 20 weeks of interval treadmill running and/or probiotic supplementation were used.

Aims: We suggest that the use of interval treadmill running and the probiotic supplementation attenuates the ongoing loss of cognitive function, delays the AD progress and reduces the local inflammation caused by the illness.

Results: Our results are based on morphological measurements, cognitive tests and anti beta-amyloid immunohistochemistry.

The brain/body mass (BM) ratio in the combined group was significantly higher than that of the control group, suggesting minor neuron loss. Heart/BM ratio in exercise group were

significantly increased, musculus quadriceps/BM ratio in combined and training group were higher compared to control group indicating the myogenic effects of exercise.
During cognitive test we have observed continous development in the performance of the animals on combined treatment. During the Moris maze test, on the second day the animals showed significantly better performance, and on the 3rd and 4th day a tendency was shown for improved learning skills. Also in the spontaneous alternation test, a remarkable increase in performance was shown, demonstrating better exploratory activity, meaning a better preservation of cognitive function.
6E10 immunohistochemistry against beta-amyloid plaques revealed, that the number of plaques in hippocampus in the exercise group were significantly lower than in the control and combined group. The area occupied by plaques in hippocampus was significantly lower in all treated groups compared to the control group, suggesting that there is no linear connection between the cognitive function and the plaque density.

Anatomical characterization of the limbic thalamic nuclei

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In the last sixty yeas midline thalamus (MT) has been described as a major thalamic relay station of the ascending reticular activating system (ARAS) towards the neocortex. Previous data have also hypothesized that MT – as a hub station – plays crucial role in setting different levels of arousal in distinct limbic forebrain areas as it receives all kinds of arousal-related modulatory inputs (orexinergic, noradrenergic, serotoninergic) and sends axonal outputs to widespread forebrain targets. Due to the small size, irregular shape and location among functional heterogeneous networks of the midline nuclei, it is impossible to selectively examine the exact role of this thalamic circuitry without a region-specific approach. To solve this problem, we have been searching for a selective tool to label the MT. In this study we tested whether the intracellular Ca-binding protein, calretinin (CR) can be used as a specific molecular marker for the MT nuclei.
First to test whether the MT cells activated in response to an arousing stimulus are CR-positive, foot-shock was delivered as a relatively strong external stimulus. The vast majority of MT cells activated (as measured via cFos expression), co-localized CR (over 98%). Using single retrograde tracing from known targets of midline nuclei such as the medial prefrontal cortex, amygdala, nucleus accumbens, ventral hippocampus, subiculum and the bed nucleus of stria terminalis, we demonstrated that most of the projecting cells were also CR-positive (94-98%). The distribution of the CR-positive thalamic cells projecting to these regions was highly overlapping without any specific topography.
Target-selectivity of the CR-positive thalamic cells was also investigated by using three distinct approaches: double retrograde tracing from target areas; CR-dependent retro-anterograde viral tracing in CR-Cre transgenic animals and single-cell viral labelling. The

results of these approaches together demonstrated that the majority of CR-positive MT cells possesses multiple projections.
Taken together, our results show that CR-positive MT cells provide the major thalamic source to the forebrain limbic system, and via their widespread axonal arbour, it is in a unique position to synchronously modulate brain states in distinct (targeted) brain regions. Indeed, recent physiological experiments from our research group combining optogenetic and viral tools, revealed a crucial role of the MT as a major mediator of arousal levels in the limbic system.

Negative estrogen feedback to female mouse GnRH neurons is mediated via activation of the retrograde endocannabinoid signaling mechanism

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Hypothalamic gonadotropin-releasing hormone (GnRH) neurons play a key role in the control of reproduction. In vivo, 17β-estradiol (E2) controls GnRH release in concentration and estrus cycle dependent manner. In vitro patch-clamp electrophysiological studies on GnRH neurons of ovariectomized, female mice showed that low physiological concentration of E2 decreased spontaneous firing rate which was eliminated by blocking fast synaptic neurotransmission (Chu et al., J. Neurosci., 29:5616, 2009), suggesting pivotal role of the GABAergic excitation in the phenomenon. 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 in 9 from 18 GnRH neurons, which could be abolished by the application of the ERα/β blocker Faslodex. Pretreatment of the brain slices with the cannabinoid receptor type-1 (CB1) inverse agonist AM251 (1 μM) significantly attenuated the effect of E2 on the PSCs. The intracellularly applied endocannabinoid synthesis blocker THL (10 μM) also eliminated action of E2. E2 remained effective in the presence of TTX indicating direct action of E2 in GnRH cells. The ERβ specific agonist DPN (10 pM) significantly decreased the frequency of the mPSCs in GnRH neurons. The effect of E2 was completely blocked by the selective ERβ antagonist PHTPP (1 μM). In contrast, the ERα agonist PPT (10 pM) or the membrane associated G-protein coupled estrogen receptor (GPR30) agonist G1 had no significant effect on the frequency of mPSCs in these neurons. These results indicate that ERβ is required for the observed rapid effect of the E2 on GnRH neurons. AM251 significantly abolished the action of E2 on the mPSCs. Similarly, effect of DPN (10 pM) was eliminated in the presence of AM251. Thus, involvement of the retrograde endocannabinoid mechanism has been revealed in this effect. These results indicate that an interaction exists between estradiol

and endocannabinoid signaling, which represents novel regulatory machinery in the execution of the negative estrogen feedback to GnRH neurons.
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Synchrony of Coupled Cortical Regions and Spread of Low Frequency Oscillation - an ex vivo Model of Cortical Slow Wave Activity

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During slow wave sleep membrane potential of cortical neurons spontaneously fluctuates between active (UP) and inactive (DOWN) states (<1Hz). During UP-states action potentials can be elicited; during DOWN-states neurons are hyperpolarized.
Earlier, using microelectrophysiological methods we identified that the entorhinal (ERh) and perirhinal (PRh) cortices exhibit this spontaneous oscillation in horizontal rat brain slices. Further, we studied the region of initiation; the direction of propagation; the connections required for propagation and the pattern of interlaminar flow with current-source density analysis. Multi- and single-unit activity was extracted from field recordings to correlate field events and single cell firing.
UP-states that we registered ex vivo had similar appearance as those registered in vivo; field potential events were in strict correlation with unit activities. In most cases, the activity initiated in the infragranular layers, then spread upwards.
The frequency of the activity recorded simultaneously in both areas was identical, suggesting the physiological coupling of the two regions. In most cases, PRh cortical UP-states followed ERh cortical UP-states with specific latency, whilst cutting either the supragranular layers or the corpus callosum altered the area of initiation and the propagation of the activity. The synchrony between the two regions remained intact. Cutting either the whole cortex or the cortex along with the corpus callosum caused the disengagement of PRh cortical activity ceasing the synchrony.
Our results indicate that this activity could be the ex vivo model of inherent cortical UP- and DOWN-state activity. In vertical plane, layer V activates the supragranular layers; in horizontal plane ERh cortex may trigger the activity, which then spreads toward the PRh cortex mostly through supragranular and extracortical connections. However, in the absence of connections the initiation area and synchrony between the two regions may be altered.

GnRH input to hypothalmic centers controlling GnRH secretion

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Besides forming the final common pathway regulating reproduction, gonadotropin-releasing hormone (GnRH) neurons also innervate hypothalamic and extrahypothalamic loci involved in the regulation of neuroendocrine functions and/or associated behavior. Among those, the rostral periventricular area (RP3V) and the arcuate nucleus (Arc) have particular significance due to their critical role played in the control of GnRH and prolactin secretion. These areas contain neurons producing kisspeptin (KP) and dopamine that highly co-localize in neurons of the (RP3V), but appear in completely distinct subpopulations of the Arc.

The aim of these studies was to reveal whether GnRH neurons communicate with KP and dopaminergic neurons in the RP3V and Arc. Confocal and electron microscopic studies were carried out on immunohistochemically double-labelled brain sections to trace juxtapositions of GnRH-immunoreactive (IR) varicosities on KP- or dopamine-secreting, tyrosine hydroxylase (TH)-IR neurons in mouse and human brains. In addition, interaction of GnRH neurons with KP neurons was studied by in vitro electrophysiology.

At light microscopic level, GnRH processes were seen to course through and form varicosities in the RP3V and Arc. Confocal microscopic analysis and 3D reconstruction of the labelled cellular profiles revealed GnRH-IR axon varicosities juxtaposed onto KP-IR and TH-IR neurons in both hypothalamic areas. Such contacts were also observed in humans. The identified GnRH-IR axon varicosities were in apposition to approximately 25% of the KP-IR neurons in the RP3V and 50% of them in the Arc. At the ultrastructural level, asymmetric synaptic contacts were observed between GnRH axon terminals and both KP- and TH-IR neurons, not only in the RP3V, but also in the Arc.

To study the effect of GnRH on KP neurons, in preliminary experiments, whole cell and cell-attached patch-clamp recordings were carried out. KP neurons in brain slices of the RP3V responded to GnRH (10 μM) with a significant depolarization (n=9; +7.45±2.157 mV; $p<0.05$) and with an increased firing rate compared to the pre-treatment control rate (n=3; +172.7±8.37 % of the control recording; p=0.013). In contrast to the RP3V KP neurons, those in the Arc did not respond to GnRH. In turn, administration of the GnRH antagonist Antide (100 nM) resulted in a slight membrane depolarization (n=6; +15.2±5.98 mV) in these cells.

These findings indicate that the GnRH neurons innervate KP and dopamine neurons in the RP3V and Arc and GnRH depolarize KP neurons and increase their firing frequency in the RP3V, but not in the Arc. Using this communication, GnRH neurons may exert an estrus cycle-dependent feed forward and/or feedback effect on functionally distinct subpopulations of KP and dopaminergic neurons.

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Cortical electrical stimulation evoked high frequency oscillations in the human hippocampus

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Introduction

Cortical electrical stimulation (CES) and electrically evoked potentials (EP) are widely used investigational methods to study neural connections and networks both in rodent and human brain.

High frequency oscillations (HFO, ripples and fast ripples) participate in memory consolidation and epileptogenic processes predominantly in and around the hippocampal formation (HcF). There are sparse and not focused publications about HFOs activated by CES. We examined the effect of CES under general anesthesia in the HcF of 8 temporal lobe epilepsy (TLE) patients.

Methods

We used laminar multielectrodes to record local field potential, and spectral activation elicited by CES (0.1 ms, 5-15 mA; 0.5 Hz). Hippocampal regions were reconstructed based on histological assessment of the removed HcFs, and subregions were determined: cornu ammonis (CA), dentate gyrus (DG), subiculum (Sub). Ripples were detected manually. Evoked spectral power and spontaneous multiple unit activity were calculated (MUA).

Results

Significant evHFO activity was evoked in all HcF regions (CA, DG, Sub). The peak times after the stimulations are 12 ± 0.9 ms in CA, 27 ± 1.7 ms in DG. In Sub we observed two different latencies, 15 ± 1.7 ms and 40 ± 1.36 ms. The duration time of evHFO is 22 ± 1.9 ms derived from all cases. The mean maximal power frequency values are the following: 95 ± 10.5 Hz in CA, 108 ± 10.8 Hz in DG and 169 ± 21.8 Hz in Sub. Higher frequency components were detected by empirical method decomposition: 66-229 Hz in CA, 46 - 650 Hz in DG, 70 - 371 Hz and 371 - 504 Hz in Sub. In different regions, the influencing and the modified frequencies are 11 - 33.4 Hz and 14.6 - 322.7 Hz in CA, 11-57.3 Hz and 47-478.9 Hz in DG, 11-35.9 and 46.3 - 507.7 Hz in Sub. Significant overlap were between the HFO amplitude and MUA amplitude depth profile in 32% of the stimulations.

Conclusion

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

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Bidirectional analog modulation of back-propagating action potentials enables dendritic hybrid signaling

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Backpropagating action potentials (bAPs) and the associated dendritic calcium signals play fundamental roles in neuronal information processing by providing digital feedback to the input zone of the neurons. We investigated whether and how a dynamically varying analogous parameter, namely the subthreshold somatic membrane potential, can control this dendritic digital feedback signal in rat dentate gyrus granule cells. Dual somato-dendritic recordings revealed multiple location-dependent modulatory effects of the membrane potential on the shape of bAPs. In the proximal dendrites, somatic hyperpolarization (from -64.6 ± 0.6 mV to -77.2 ± 0.6 mV) accelerated the repolarization phase of bAPs while the peak potential is less affected. In contrast, in the distal dendritic region hyperpolarized bAPs reached lower peaks. These changes of the bAPs are paralleled by somatic hyperpolarization-induced distal reduction and proximal enhancement of bAP-evoked dendritic calcium signals. We next explored the underlying mechanisms that link the bAP related dendritic calcium signals to the analog state of the cells. Combination of dynamic clamp and calcium imaging experiments showed that the faster repolarization alone (i.e. without changes in the preceding membrane potential) was able to enhance calcium signals in the proximal dendritic region. Computational simulations and voltage clamp experiments suggested that faster bAP itself can promote larger calcium influx if fast-inactivating, high voltage activated calcium channels are present. Consistent with this hypothesis, blockade of R-type calcium channels eliminated the increase of bAP-evoked proximal calcium signals. Using simulations, we demonstrate that the distally observed loss in the bAP peak results in less calcium influx because the calcium channel activation is submaximal during the small distal bAPs.

We also show that a more physiological dendritic hyperpolarization, the GIRK channel activation through mGlu2 receptors, was able to induce bi-directional changes in bAP-evoked calcium signals similar to the somatically introduced hyperpolarization. Importantly, the above dendritic bAP and calcium mechanisms also enable reporting of the phase of somatic voltage fluctuations in the theta frequency. Altogether, the results show that the analog information about the somatic membrane potential is translated to gradual and location-dependent changes in the bAP shape and in the associated calcium signals, representing the substrate and the potential readout mechanisms of analogous modulation of an essentially digital signal in the dendrites. Thus, bAPs are hybrid signals that retain graded information and relay it to the dendrites in a location-dependent manner.

Discrete Sampling of Extracellular Potential leading to Rise of Current Source Monopoles

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Performance evaluation of chronically implanted, cylindrically-shaped polymer-based neural implants

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One aim of the NeuroSeeker project funded by the European Commission is to develop cylindrically-shaped depth probes with an increased number of recording sites. These probes could be used for example for a more accurate localization of the seizure focus in patients with focal epilepsy or to explore human cortical microcircuits by recording the activity of many single neurons across several cortical layers and simultaneously deep inside the brain. Prior to the application of these probes in human patients, thorough validation experiments in animal models are required. In this study, in vivo validation was performed by chronically implanting shorter probe modules for several months into the brain tissue of rats. The cylindrically-shaped implants (length:1.5 mm, diameter: 800 μm) consist of a polyimide foil with 32 integrated platinum contact sites (diameter: 35 μm). The contact sites are arranged in four rows where in each row the contacts are positioned equidistantly around the circumference of the probe cylinder. Individual rows are 200 μm apart from each other. To evaluate the electrophysiological performance of the implanted devices, we recorded local field potentials, as well as multi- and single-unit activity (MUA, SUA) either from the cortex or from the thalamus of the animals on a daily basis. Animals were kept still during the recordings by the intramuscular administration of a small dose of Ketamine/Xylazine. Spike sorting was used to separate the activity of single units from MUA. We also developed a method to estimate the amount of the useful signals (population activity) recorded with the individual contact sites. For the analysis of the long-term performance we monitored the temporal change in the number of isolated single units, in the amount of the useful signals, and in the impedance and noise of the electrodes. Our results showed that the amount of useful signals and the number of separable single neurons can increase during the first week after implantation, but these values started to decrease after the second week, probably due to the effect of chronic tissue response. However, in some cases MUA and SUA of moderate quality could even be recorded after 3 months. Based on our results we can conclude that the investigated cylindrical probe could be a useful tool for future clinical applications.

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The effects of early environmental enrichment in a rat model of Parkinson's disease

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

Wistar rats were used in our experiment. Animals were divided into standard and enriched groups according to their environmental conditions. Animals of the standard group were placed under regular conditions. For environmental enrichment, during the first five postnatal weeks we placed pups in larger cages supplemented with toys, objects, running tunnels and rotating rods of different shape, size and material. Half of the toys were changed daily. Three months later rats were treated with unilateral injections of 2 µl 6-OHDA (5 µg/µl) into the left substantia nigra, control animals received 2 µl physiological saline. Behavioral experiments were done preinjury, and 1, 10 days after the operation. After the behavioral testing tyrosine-hydoxylase immunohistolochemistry was performed to label dopaminergic cells of the substantia nigra.

Behavioral examinations have shown hypokinesis due to the operation. Our morphometric studies revealed a significant cell loss in the substantia nigra in the 6-OHDA-treated animals of the standard group compared to the saline-treated animals of the same group. In contrast, in case of the enriched animals there was no significant difference between the saline- and the 6-OHDA-treated group.

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

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Target cell type-dependent differences in [Ca^{2+}] in hippocampal glutamatergic terminals

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Probability of the neurotransmitter release (Pr) is a key determinant of modulating the strength of synapses. Density and distribution of voltage-gated Ca^{2+} channels in the presynaptic active zone (AZ) are known to influence the Pr of docked vesicles. Here we combine two-photon Ca^{2+} imaging, triple immunofluorescent labelling and three-dimensional electron microscopy (3D EM) reconstruction to measure functional Ca^{2+} channel densities at high- and low-Pr excitatory synapses established by hippocampal CA3 pyramidal cells with local interneurons (INs). Evoked EPSCs onto fast spiking (FS) INs display short-term depression whereas onto metabotropic glutamate receptor 1α (mGluR1α) positive INs mostly facilitation. Ca^{2+} concentration in axon terminals evoked by a single action potential, measured from the peak [Ca^{2+}] transient amplitudes, is significantly larger in boutons with high-Pr (PV contacting) than in boutons with low-Pr (mGluR1α contacting). 3D EM reconstructions show that the AZ area normalized to the bouton volume was significantly larger in mGluR1α than in PV targeting boutons. Therefore the calculated total amount of fluxed Ca^{2+} per unit AZ area is ~2 times larger in PV than in mGluR1α targeting boutons, predicting a larger Ca^{2+} channel density in the AZ. These results indicate that high Pr is supported by a higher Ca^{2+} influx at unit AZ area, suggesting a target cell type-specific difference in the density or modulation of presynaptic Ca2+ channels.

Receptor affinity and G-protein activation of nociceptin-opioid hybrid peptides in rat brain membranes

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Although the three opioid receptors (mu, delta and kappa) as well as nociceptin receptor have an important role in pain mechanism and modulation, they are very distinct. While the conventional opioid receptors mediate analgesia, the nociceptin receptor causes increased sensation of pain. These G protein-coupled receptors (GPCRs) constitute dimeric/oligomeric complexes in the cell membrane, thus one may design such ligands that target these homo- or heterodimers simultaneously. The aims of this research were to synthesize and characterize three bivalent ligands, H-YGGTGGGRYYRIK-NH2, H-YGGFRYYRIK-NH2, Ac-RYYRIKGGGYGGFL-COOH. These bivalent ligands consist of YGGF sequence, a frequent motif in the N-terminus of endogenous opioid ligands, and Ac-RYYRIK-NH2, which was isolated from the peptide library as an antagonist that inhibits the biological activity of nociceptin, an endogenous ligand for nociceptin receptor. The activity of these compounds was investigated in two different assays, competition receptor binding and G-protein activation assays for each opioid receptor (mu, delta, kappa) and the nociceptin receptor. The bivalent ligands were compared to DAMGO, Ile5,6-deltorphin II, U69593, nociceptin, selective to mu, delta, kappa, nociceptin receptors, respectively.

[35S]GTPγS assay, which measures the agonist-mediated G-protein activation, has demonstrated that YGGTGGGRYYRIK-NH2 and H-YGGFRYYRIK-NH2 both stimulated the regulatory G-proteins with high potency. In binding experiments, the affinity of YGGTGGGRYYRIK-NH2 and H-YGGFRYYRIK-NH2 was the highest for the kappa receptor and lower for the mu, delta and nociceptin receptors. Ac-RYYRIKGGGYGGFL-COOH showed less activity in both receptor binding and G-protein activation assays.

Selective activation of thalamic and extrathalamic inhibition - effects on cortical activity and behavior

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Inhibition has a crucial role in the proper function and regulation of neuronal networks. In the thalamus, inhibition is provided by thalamic and extrathalamic (ET) sources. Thalamic inhibition originates in the nucleus reticularis thalami (nRT), whereas ET inhibition consists of fibers originating in the zona incerta, anterior pretectal area (APT), pontine reticular formation (PRF) and ventral pallidum.

Thalamic and ET inhibition have distinct anatomical and physiological properties. Previous results demonstrated that selective optogenetic stimulation of the nRT with single laser pulses evoked sleep spindles in the first order ventral posteromedial nucleus (VPM). Selective stimulation of ET fibers (PRF) with a train of pulses in the higher order intralaminar nucleus, however, evoked slow cortical oscillation and behavioral arrest. Whether the evoked effects are specific to the type of inhibitory afferent or the thalamic nucleus involved is presently unclear. To answer these questions we used selective labeling of the afferents by conditional AAV-channelrhodopsin-2 constructs injected into vesicular inhibitory amino acid (VIAAT)-CRE mice. We activated GABA-ergic terminals from the nRT and ET sources with single pulses and also with trains of stimuli (5Hz, 9Hz, 30Hz) and recorded the behavior and the evoked LFP activity from parietal and frontal cortices in freely moving animals.

Stimulation of the nRT terminals with single pulses in the midline nucleus resulted in cortical field response in the frontal but not in the parietal recordings but no spindles could be evoked. Trains of stimuli, however, evoked large amplitude rhythmic cortical LFP activity but only during the awake states. The oscillation was stimulus locked and it followed the frequency of stimulation. Thirty Hz stimulation elicited a unique behavioral response. During the sleeping period the animal was wakened, but when the mouse was already awake it started to show an exploratory behavior. Stimulation of nRT terminals with trains was less effective in the VPM. An oscillation appeared on the parietal cortex during the stimulation but it was smaller and no behavioral response was apparent.

Stimulation of the APT terminals in the intralaminar nuclei had no effect on the cortical LFP or the behavior and no spindles could be evoked by stimulating PRF terminals in these nuclei either.

Accordingly to our data, thalamic inhibition seems to play bigger role in recruiting oscillation in the midline thalamus than ET inhibition, probably due to a more precise and

synchronous inhibitory control which enables thalamic cells to fire in a specifically defined time window. In addition, nRT stimulation has greater effect in the midline thalamus than in the VPM, since higher order thalamic nuclei receive a layer V. input from the cortex which could be crucial for strengthening an emerging oscillation.

Near-infrared spectroscopy based functional brain networks in auditory streaming

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The phenomenon of perception stochastically switching back and forth between possible interpretations of an unchanging stimulus is termed bi-/multi-stable perception (Schwartz et al., 2012). The auditory streaming paradigm (van Noorden, 1975) consists of sequences of sounds of the form ABA-ABA-..., where 'A' and 'B' denote two different sounds and '-' stands for a silent interval with the same duration as A and B. Possible perceptions of these stimuli can be grouped into three categories: integrated (ABA-ABA-), segregated (A-A- and B---B---), and combined (e.g., AB--, BA--, A---, etc.). With sufficiently long sequences, listeners are spontaneously switching between these possible perceptual organizations. Previous studies showed (Farkas et al., in prep; Pressnitzer & Hupé, 2006) that listeners can voluntarily bias their perception to switch more or less (voluntarily biased instructions) than they would do when their task is only to report their current perception as it happens (neutral instruction). Here, we used Near-Infrared Spectroscopy (NIRS) to identify the functional brain networks during the perception while listening to sequences of the auditory streaming paradigm. Our primary aims were to identify the brain networks activated while experiencing the different perceptual alternatives and to compare these networks between voluntarily biased and neutral-instruction conditions. The combined percept yielded the most centralized network with many interhemispheric connections. Further, listeners are characterized by more hierarchical networks when they voluntarily switch as often as they can than when they switch as seldom as they can with the neutral-instruction condition falling in between.

PACAP heterozygous mice in the three hit theory model of depression

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The three hit theory is an accepted concept: genetic predisposition, epigenetic factors and stress together cause depression. Mice lacking pituitary adenylate cyclase-activating

polypeptide (PACAP) show depression-like behavior. The role of extra-hypothalamic corticotropin releasing factor (CRF) in the bed nucleus of the stria terminalis (BNST) is not well understood. The CRF peptide family member urocortin1 (Ucn1) is neglected in stress research, however it activates CRF2 receptors with higher affinity than CRF. Ucn1 is expressed primarily in the central projecting Edinger-Westphal nucleus (cpEW).
Our first aim was to set up and validate a mouse model for studying depression at behavioral level. Second, we also planned to examine functional-morphological changes in the BNST-CRF and cpEW-Ucn1 neurons.
Offspring of PACAP heterozygous mice (genetic factor) were subjected to severe maternal deprivation (epigenetic factor) vs. controls. Half of male adult offspring was exposed to chronic variable mild stress (environmental stress) for two weeks, while other half remained unstressed.
Knockout mice subjected to maternal deprivation and stress showed low survival rate suggesting severe maladaptation. In forced swim test, our heterozygous mice showed increased immobility time upon stress with maternal deprivation history, which was supported by increased adrenal weights in these mice. The highest CRF cell count and specific signal density accompanied by increased FosB expression in the oval nucleus of BNST was found in mice with three risk factors. In contrast, cpEW-Ucn1 neurons in heterozygous mice show blunted activity upon stress.
Our adrenal weight data prove that the stress model was effective. Increased BNST-CRF and decreased cpEW-Ucn1 neuronal activity may explain the observed depression-like phenotype in PACAP heterozygous mice carrying all three risk factors. We conclude that PACAP heterozygous mice in our model could be reliable tools to study major depression.
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Eye-movement correlates of natural reading of horizontally and vertically presented texts

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Neural underpinnings of reading have been studied by both fMRI and PET, however recently the value of electroencephalogram (EEG) is being recognized as the need to acquire information about the time course of neural information processing during natural reading has increased. Yet there is still very little known about the neural mechanisms of natural reading, especially about the effects of changing text orientation, since the majority of studies investigating reading used Serial Visual Presentation (SVP), not allowing for saccades to occur. The aim of the present study was to unveil the relations between different eye movement parameters when reading horizontally or vertically and also to

determine predictors of reading speed in both conditions, in order to provide the basis for the analysis of neural mechanisms of reading in ecologically valid free-viewing situations in the future.
Horizontal-vertical natural reading and related eye movements were investigated with an eye-tracker while simultaneously measuring EEG. Eye-movement events (fixations, saccades) were determined using an adaptive algorithm. To date, only the behavioral and eye-movement related data were analyzed. Results confirmed that reading speed is significantly slower for vertical texts. Additionally, in the vertical condition significantly smaller saccade amplitudes, slower saccades, more regressive saccades, and longer fixation durations were registered. Significant positive correlation was found between saccade amplitude and saccade peak-velocity, and a negative one between saccade duration and glissade amplitude in both conditions. Conducting regression analysis revealed that in both conditions the most robust predictor for reading speed is the saccade amplitude (positive relationship) followed by fixation duration (negative relationship), although these relationships were weaker in vertical condition. Further analysis employing hierarchical regression revealed that in both conditions saccade duration along with frequency of regressive saccades were also significant predictors. While saccade duration is negatively related to reading speed in both conditions, the frequency of regressive saccades was found to be negatively related to it in the horizontal condition and positively in the vertical condition.
Taken together these results are in accordance with previous findings, and show that smaller visual span might indeed be an important limiting factor for reading speed yet they also reveal that it is not the only one, as oculomotor factors should also be considered as potentially affecting reading speed. The results could also contribute to more reliable analysis of EEG data by helping to reduce eye-movement related artifacts, and therefore to gain new information about the effects of text orientation to different neural mechanisms during natural reading.

Resting state functional network determines properties of visual mismatch negativity

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In this EEG study we aimed to investigate the relationship between the resting state functional networks and visual mismatch negativity (vMMN). VMMN is an event-related response (ERP) measured during passive (task irrelevant) stimulus presentation elicited by deviant stimuli violating the sequence of standard stimuli. VMMN has a promising clinical usage, however, it is characterized by a great inter-subject variability, which makes the interpretation ambiguous. Resting state functional networks were proved to be an indicator

of numerous clinical and psychological factors. Identification of the resting state networks determining the amplitude and latency of vMNN may contribute to the clinical application of vMMN. The EEG data of 22 subjects were recorded by 61 channels. The functional networks were calculated from a 5 minutes eyes open resting state section before the vMMN experiment. Strength of functional connectivity between all pairs of electrodes was investigated based on the measurement of phase synchronization (phase lag index-PLI) in delta, theta, alpha and beta frequency bands. Windmill patterns were presented in a passive oddball sequence, and vMMN was measured by subtracting the averaged response to the frequent standards from the averaged response to the rare deviant in the 100-350 ms range post stimulus. The correlation between the early (100-200ms) and late (200-350ms) components (latency, amplitude) of the vMMN and the PLI values were determined. Our results mainly restricted to the theta band. The latency of the early and late components was negatively correlated with the right centro-occipito-parietal network. The amplitude of the late component was positively correlated with the left fronto-temporal network. Previous findings localized the primary source of vMMN to the right prestriate cortex. Our results suggest that the increased resting state functional coupling of the prestriate cortex indicate a more rapid processing. The significance of the fronto-temporal network support the notion, that attentional and memory processes are involved in the generation of vMMN.

Induced Pluripotent Stem cell-based in vitro neurotoxicology models

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There is a great need for disease models for neurodegenerative diseases (e.g. Alzheimer's, Parkinson's, Huntington's), as well as for in vitro platforms for neurotoxicology screens (the food and cosmetics industries, for example, represent high demand for routine toxicity studies). Most developmental neurotoxicity studies are carried out in rodents, despite of the high cost and the low translational value of the results due to the species differences. Accordingly, a new initiative seeks to convert the traditional animal-based developmental toxicity tests to in vitro assays using human cells to detect chemical hazards. However, the highly complex structure of the human brain makes in vitro modelling difficult. There is only a limited number of human neuronal cell lines, and human tissue suitable for developmental neurotoxicity studies comes from aborted fetuses and biopsies. Human pluripotent stem cells are highly capable to fill this niche. These cells may originate from blastocyst stage embryos (human embryonic stem cells, hESCs) or they can be derived from somatic cells reprogrammed with specific transcription factors (human induced pluripotent stem cells, hiPSCs). One of the main advantages of hiPSCs is that the cells can originate from patients with different neurological disorders, but they also can be derived from different genetic origin of healthy individuals, providing a wide range of allele distributions. Additionally, a large number of diverse cells can be established from hiPSCs using specific differentiation protocols. We have established hiPSC-based in vitro toxicology assays that

can be used to test toxicity at different stages of cell differentiation. The basic procedure is that cells are exposed to different toxic treatments and then cell viability is measured with an MTT assay (a simple absorbance measurement which gives a quantitative readout of the metabolic activity of the cells). The viability assay can be performed immediately after the toxic treatment of hiPSCs, or following cell differentiation. The latter design can yield information about the possible effects of the treatment on the neural development. Alternatively, we can test toxins on mature differentiated neurons, neural progenitor cells, or neural rosettes, and we can infer the expected effects on the adult brain. In this study, we measured the effect of H_2O_2 and ethanol on the viability of neural progenitor cells using an MTT assay. We investigated the concentration-response and time-course of the treatments. The results demonstrated that iPSC-based in vitro neuronal models can be used in various academic and pharmaceutical applications to test neurotoxicity. Acknowledgment: This work was supported by grants from EU FP7 aprojects (STEMMAD, PIAPP-GA-2012-324451; EpiHealthNet, PITN-GA-2012-317146; D-BOARD, FP7-HEALTH-2012-INNOVATION-1-305815) and H2020 projects (EU-ToxRisk H2020-PHC-2015-681002) as well as Research Center of Excellence 9878/2015/FEKUT project.

Origin of olfactory inputs to brainstem dorsal raphé nucleus neurons

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The neuromodulator serotonin (5-hydroxy-triptamine, 5-HT) originating from neurons in the brainstem dorsal raphé nuclei (DRN) is involved in many brain functions including the regulation of sensory perception and mood and is the major target in several psychiatric disorders. DRN neurons show slow state dependent fluctuations in their firing rate, but also respond to sensory events including olfactory, auditory and somatosensory stimuli with transient (< 1 sec) modulation of their firing. Optogenetic stimulation of DRN 5-HT neurons rapidly inhibits the spontaneous firing of olfactory cortical neurons, but entirely spares sensory-driven firing. Using a combination of retrograde tracing methods and optogenetics we revealed two olfactory information receiving brain regions which heavily project to the DRN, namely the lateral hypothalamus (LH) and the orbitofrontal cortex (OFC). Using local photostimulation of ChR2 expressing LH and OFC axons in the DRN we show that these target both 5-HT and GABAergic neurons. Interestingly, LH axons give rise to widespread AMPA/KA receptor mediated excitation and GABAA receptor mediated inhibition of DRN neurons, whereas OFC axons seem to exert AMPA/KA receptor mediated excitation in a small subset of DRN neurons. These results identify the origin of olfactory input to the DRN and argue that the olfactory system can regulate its own activity via LH and presumably OFC derived transient firing rate changes in DRN neurons. Olfactory information processing may thus be placed under the control of hypothalamic and higher order cortical areas.

Serotoninergic neurons of dorsal raphe nucleus in the three hit theory of depression

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Introduction: Depression is one of the most common psychiatric diseases, which causes increasingly severe health care social and economic problems. The exact pathogenesis of depression is still unknown, which might be explained in part by the lack of equivocally accepted animal model in basic research. Based on the three hit theory of depression, simultaneous occurrence of genetic, epigenetic and environmental factors cause the disease. A partial or total lack of pituitary adenilate cyclase-activating polypeptide (PACAP) causes depression-like behaviour in mice. Therefore, these animals can be applied as a suitable model for genetic predisposition of depression. Maternal deprivation is widely applied tool to examine epigenetic effects of early life stress. The chronic variable mild stress (CVMS) is a model of every-day stress. The hypothalamus-pituitary-suprarenal gland axis and higher order centers, such as serotoninergic neurons of dorsal raphe nucleus (DR) play crucial role in stress adaptation. FosB is a chronic neuronal activation marker expressed upon stress.

Hypothesis: We put forward that mice exposed to all three hits will show the symptoms of depression in behavioural tests. We anticipated that alterations in serotonin and FosB expression in DR will also prove the validity of our model.

Methods: We exposed PACAP KO, heterozygous and wild type mice to short (15 min) and long term (180 min) maternal separation in the first 14 postnatal days vs. groups which did not suffer maternal deprivation. Half of animals of each group was exposed to CVMS between postnatal days 106 and 120 including a final forced swim test (FST). One day latermice were transcardially perfused. Indirect immunofluorescence for serotonin and FosB was performed in the DR.

Results: In FST, maternally separated mice showed increased depression like behaviour compared with controls. Histological examination revealed that mice with maternal deprivation history upon CVMS showed a significant FosB and a tendentious serotonin cell count decrease in all three genotypes.

Conclusion: Our behavioural and histological test supports that in genetically predisposed animals, maternal deprivation blunts the function of DR-serotnin systems. The altered stress response proves the validity of our murine depresson model based on the three hit theory.

SU-8 implants in the rat central nervous system: a biocompatibility study

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AnimalTracker: an ImageJ-based programming tool to create custom-made behavioral analyzes

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Most behavioral investigations are carried out on video recordings of the observed animals and changes in different activities are analyzed manually or automatically by certain software. While commercially available programs provide wide range of evaluation methods, they offer limited possibilities to customize image processing and data analysis. In this study, we present a universal tracking application programming interface (API), which provides an easily applicable toolkit to create custom-made tracking and analyzing programs in Java. The Animal Tracker API is based on the widely used ImageJ image processing program and offers the possibility to implement additional ImageJ plugins and extensions within the custom-made analysis. The Animal Tracker is an opensource project and is available from the animaltracker.elte.hu webpage. The Animal Tracker API consists of two main packages. The Tracking package processes the input video and locates the observed animal, based on five consecutive steps (filtering, binarization, post processing, blob detection, blob comparison). Our API provides a certain number and type of algorithms for the tracking steps but if needed, the existing toolkit can be modified and extended without breaking the image processing chain. The Analyzing package offers the use of standard measurement parameters (e.g. distance, time, speed) as output values but custom-made special parameters, such as angle preference in the Morris Water Maze, can be also implemented. Output values are handled by the ImageJ Results Table, with a possibility to group values according to custom-made aspects. The additional Zone Designer package provides extended possibilities to freely define individual or overlapping zones within the observation area using geometric primitive objects. In order to prove the general utility of our API, we provide ready-to-use plugins to analyze Morris Water Maze and radial maze tests with mice or rats. Based on raw data obtained from each frame, output values include distance travelled and time spent within the pre-defined zone, immobility time, momentary speed and orientation of movement in a table format, suitable for further data processing e.g. by Excel. We hope that our API will be suitable for the scientific community to create custom-made programs for behavioral analyses.

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Connection between a nested oscillation system and the interictal-epileptic discharges explored by cortico-cortical evoked potentials

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Introduction

Averaged cortico-cortical evoked potentials (CCEP) as a response to direct brain surface stimulation are suitable for mapping brain effective connectivity. We analyzed the sweep to sweep variance of the CCEP territory and amplitude, and compared the result to the occurrence of interictal-epileptic discharges (IEDs).

Methods

CCEPs were elicited (2 s, 10 mA, 0.5 Hz, 80-450 stimuli) and analyzed in sleeping and awake grid and strip implanted epilepsy patients (n=5) undergoing presurgical evaluation. The time course of the number of the activated electrode contacts (peak amplitude exceeding 3 or 6 SD relative to the -450--50 ms baseline) was determined in the times of 10-50 ms (N1) and 50-500 ms (N2) for every stimulation epochs. Fast Fourier transform, wavelet and empirical mode decomposition were calculated (Neuroscan, MATLAB). These methods were repeated on stimulation free data. IEDs were detected manually between stimuli (500-1500 ms after each stimulus epoch). The positions of subdural electrode arrays were reconstructed based on MRI imaging.

Results

The number of the activated grid points showed significant variance in time (in highest varying case: 2-50/52 contacts). The time-frequency analysis resulted in 0.02-0.04 Hz modulation both in sleep and awake states. The similar modulation was found in the stimulation-free activity with lower amplitude. IEDs were increased at the troughs of the 0.02-0.04 Hz oscillations (n=1). In one case three other frequency bands were observed in theta (4-10 Hz), slow delta (0.25-0.16 Hz) and ultraslow (<0.003 Hz) ranges. The amplitudes of the faster rhythms were coupled to the phase of the slower rhythms.

Conclusions

The size of the CCEP area showed 0.02-0.04 Hz infraslow oscillation (n=12) which correlated with the appearance of IEDs (n=1). This result questions the usage of averaging method measuring brain connectivity. Further investigation is needed to find the connection between the members of nested oscillations and the occurrence of IEDs.

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

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Background

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

Method

We studied single (n=10) and paired-pulse (n=6) electrical cortical stimulation on drug-resistant epilepsy patients implanted with subdural grid and strip electrodes, and laminar multielectrodes (24 contact, 200μm). We applied a brief single (10mA, 0.2ms, 0.5Hz) and pair (ISI: 6.6, 10, 20, 30, 40, 50, 100, 200, 500, 1000ms, on the best single response electrode-pair) current pulses on adjacent contacts of the grid and strip electrodes. Various phases of cortico-cortical evoked potentials (CCEP) were analysed using custom scripts in Matlab.

Result

We found P1, N1, P2, N2 components with average latencies of 7, 25, 80, 250 ms respectively. The larger was the distance between stimulating and recording electrodes on the same gyrus, the longer latency components were evoked only. The laminar profile showed surface sink and layer IV source for N1, surface source and layer IV sink for P2, and cortical wide source for N2 in the middle layers. Amplitude difference of P1-N1, N1-P2, P2-N2 was correlated with ISI. Values measured at 2000 ms ISI were used as baseline. We found the characteristic of the N1-P2 curve similar in 5 out of the 6 patients evaluated with an excitation at ISI 7 and 10ms, inhibition at ISI 20-50ms and a long interval excitation at ISI 200-500ms.

Conclusion

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

Heterogeneous neuronal firing patterns during human neocortical population activity in vitro

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Epilepsy is a disorder associated with neuronal hyperactivity. Surgical tissue removal is considered as an alternative when pharmacological treatment is ineffective. Human brain tissue obtained this way generates spontaneous population activity (SPA), which has previously been attributed to epileptic processes.

Here, epileptic neocortical tissue was compared to non-epileptic neocortical tissue obtained during brain tumor surgery, which also generates SPA in vitro. This study investigates the differences between the epileptic and the non-epileptic tissue and the involvement of different neuron types in the population activity.

For this purpose, the local field potential gradient was recorded from neocortical brain tissue slices using a laminar multielectrode. Both SPA and single neuron activity were detected and analyzed using crosscorrelations. Interestingly, while the neurons showed very heterogeneous firing patterns across all groups, the extent to which neurons were involved in the population activity significantly differed between epileptic tissue and non-epileptic tissue.

As the SPA could be observed in both epileptic and non-epileptic tissue, it cannot be directly related to epileptic processes. Investigating these types of neuronal activities in epileptic and non-epileptic human tissue helps to elucidate the subtle border between physiological and pathological neuronal population activity.

Ras and Rab interactor 1 (RIN1) controls dendritic arborisation and filopodial motility in a protein kinase D-dependent manner

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Actin cytoskeleton plays a pivotal role in fine-tuning cellular shape and morphological plasticity in neurons. Ras and Rab interactor 1 (RIN1) is predominantly expressed in the nervous system, especially in forebrain structures, with a known role in synaptic plasticity. Recently, RIN1 was proved to be a direct target of protein kinase D (PKD), possessing serine 292 (S292) as a PKD-specific phosphorylation site. S292 phosphorylation results in 14-3-3 binding and the sequestration of active RIN1 from the cytoplasm. PKD has been shown to regulate dendritic arborisation and actin dynamics in dendritic spines in an activity-dependent manner. Therefore, we aim to investigate the importance of RIN1 as a downstream target of PKD in relation to dendritic and/or spine morphology and motility.

To reveal the importance of PKD-mediated S292 phosphorylation, RIN1 mutants possessing S292A or S292E point mutations – hindering or mimicking constant phosphorylation, respectively – were created. RIN1 has the ability to signal through two downstream pathways, Abl-mediated cytoskeletal remodelling and Rab5-mediated endocytosis. Accordingly, E574A and RIN1QM point mutants, which selectively impair the interactions of RIN1 with Rab5 and Abl kinase, respectively, have been also created.
Neuronal cultures were prepared from RIN1ko embryonal hippocampi and cultured neurons were transfected with fluorescently labelled wild type or mutant RIN1 constructs. Dendritic arborisation was analysed by the Sholl analysis Fiji plugin on confocal microscopic images recorded from transfected pyramidal neurons. According to our results, RIN1 overexpression increased the extent of the dendritic tree, which was completely blocked by mutating the S292 site of RIN1. These results indicate that PKD-mediated phosphorylation of serine 292 is required for the RIN1-dependent regulation of dendritic arborisation.
Live cell imaging recordings were also made from transfected neurons. Filopodial and dendritic spine motility in the transfected neurons was investigated by an Image J plugin developed recently in our laboratory (Dendritic Filopodia Motility Analyser). Using this tool, we show that overexpression of RIN1 as well as its phospho-mimetic mutant form (S292E) significantly increased motility. This effect was completely blocked if the non-phosphorylatable S292A RIN1 mutant was expressed.
To reveal the importance of Rab5 versus Abl kinase mediated RIN1 pathways, analyses on the RIN1 double point mutants are currently in progress.
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Investigation of the role of GABAergic inhibition in epileptic human neocortex

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Is the portable EEG technology ready to bring Brain Machine Interfaces to the everyday user?

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Intro

From recreational activities to improving the quality of the life of people with permanently limiting injuries of the motor system Brain Machine Interfaces (BMI) are becoming more and more important. Recent developments of accessible portable home EEG devices (eg. Emotiv Epoc) bring the BMIs closer to mainstream applications. The vast majority of BMI

techniques were developed using sophisticated laboratory level EEG systems and their compatibility with these new more accessible tools have not yet been investigated extensively.

Method
Using randomized blocks of visual cues (left/right index finger tapping, left/right and both hand movement) we recorded samples from four healthy and one paraplegic subject. Spectral analyses, phase amplitude coupling (PAC) and a modified version of cross-frequency coupling estimator were assessed as local parameters. Other subjects were investigated with invasive electroencephalography grid arrays and high-density clinical EEG devices to record the neural activity during the finger tapping protocol.

Results
Motor related evoked potentials were detected during finger tapping with all recording devices. We found the usage of the Emotiv EEG device is limited by the non-uniform SNR on the electrodes and the differences between individual headsets. We found that the placement and the number of electrodes impacted the performance of our analyses. Nonetheless, we still could extract useful information regarding to motor planning and execution activity from the recorded neural patterns.

Conclusion
The advent of these light weight home EEG devices brings the Brain Machine Interface technology to the everyday user. However, our results suggest that higher signal quality or at least industry standardized SNR comparable over each electrode could deliver dramatic improvement. We plan to further investigate the possibilities of modifying these techniques to better adapt them on home EEG devices.

COMBINED TWO-PHOTON IMAGING, ELECTROPHYSIOLOGICAL AND ANATOMICAL INVESTIGATION OF THE HUMAN NEOCORTEX IN VITRO

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Spontaneous synchronous population activity (SPA) can be detected by electrophysiological methods in cortical slices of epileptic patients and maintained in physiological medium in vitro. In order to gain additional spatial information about the network mechanisms involved in the SPA generation, we combined electrophysiological studies with two-photon imaging. Neocortical slices prepared from postoperative tissue of epileptic and tumor patients were maintained in a dual perfusion chamber in physiological incubation medium.

SPA was recorded with a 24 channel extracellular linear microelectrode covering all neocortical layers. After identifying the electrophysiologically active regions of the slice, bolus loading of neuronal and glial markers was applied on the tissue. SPA related Ca^{2+} transients were detected in a large population of neighboring neurons with two-photon microscopy, simultaneously with extracellular SPA and intracellular whole cell patch clamp recordings. The intracellularly recorded cells were filled for subsequent anatomy. The cells were reconstructed in three dimensions and examined with light- and transmission electron microscopy. Combining high spatial resolution two-photon Ca^{2+} imaging techniques and high temporal resolution extra- and intracellular electrophysiology with cellular anatomy may permit a deeper understanding of the structural and functional properties of the human neocortex.

Does the Budapest Reference Connectome Server Shed Light on the Development of the Connections of the Human Brain?

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Similar Ca^{2+} channel densities in glutamatergic synapses with different release probabilities

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It has been demonstrated that synaptic connections of the hippocampus are heterogeneous in terms of efficacy and short-term plasticity. Pyramidal cell to fast spiking (FS) parvalbumin-containing (PV) interneuron (IN) synapses show high initial release probability (Pr) and short-term depression, whereas pyramidal cell to metabotropic glutamate receptor 1α (mGluR1α) expressing IN connections have low initial Pr and display short-term facilitation. A modelling study suggested that target cell type-specific differences in the density of presynaptic Ca2+ channels could underlie the distinct short-term plasticity of these synapses. Here we applied SDS-digested freeze-fracture replica technique to directly test the density of Cav2.1 and Cav2.2 subunit proteins in the active zone of boutons that contact Kv3.1b (expressed in PV cells) and mGluR1α expressing INs. We found no significant difference in the Cav2.1 and Cav2.2 subunit densities in the active zone of boutons contacting Kv3.1b or mGluR1α immunolabelled profiles. Furthermore, we found that the Cav2.1 and Cav2.2 synaptic gold particle number showed strong positive correlation with the area of active zones in both bouton populations. These results indicate that the target cell-dependent difference in the Pr is not the result of differing Ca^{2+} channel density in these boutons, suggesting a target cell-specific modulation of Ca^{2+} channel function.

ERP signatures accompanying auditory figure-ground segregation

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In everyday situations, we are constantly confronted with multitudes of sounds originating from separate, active sound sources. The auditory system's task is to parse the mixture of sounds that reach the ears into meaningful objects or streams. This is helped by figure-ground segregation which requires the grouping of elements over time and frequency in order to separate a sound from other background stimuli. When some tonal elements repeat within a stimulus with randomly varying tonal elements, listeners can detect the repeating pattern as a figure over a randomly changing background. Electroencephalogram was recorded while participants were asked to perform a figure-detection task. Detection performance improved with higher figure salience (i.e., with higher number of repeating tonal components) as well as with longer repetition periods. Figure detection elicited the object related negativity (ORN) and P400 event-related potential (ERP) components, the amplitude of which increased together with figure salience and duration. However, only the P400 amplitude was correlated (positively) with detection performance. The results provide further evidence that ORN and P400 reflect processes involved in detecting the emergence of a new auditory object in the presence of other auditory objects and that while ORN represents the likelihood of the presence of two or more concurrent sound objects, P400 is related to the perceptual decision about the presence of multiple auditory objects.

Sleep spindle modulation by varying brain and core body temperature

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Body core temperature varies across states of consciousness as well as different stages of sleep, and correlates with occurrence of different brain rhythms. Here we investigated the properties of sleep spindles in mice under urethane anesthesia maintained at different body temperatures. We recorded multiunit spindles from the ventrobasal nucleus of the

thalamus with multisite silicon probes while varying the temperature between 34 and 39 °C.
The frequency of spindles showed a strong positive correlation with body temperature, whereas no clear relationship was found between spindle prevalence or duration and temperature. At the lowest and highest applied temperature levels (34 and 39 °C), few or no spontaneous spindling was observed.
To establish whether this correlation is mediated by some indirect regulator, or is a direct effect of local brain temperature, Zoltan Fekete's group developed a thermosensitive silicon probe, able to simultaneously measure both the local temperature and the multiunit activity. We found that brain temperature correlates with body temperature to a certain degree, albeit with a variable delay. Our preliminary data suggest that heat modulation of sleep oscillations needs to be considered as an experimental factor as well as a possible diagnostic and therapeutic tool applied in sleep disorders.

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Extra-hypothalamic corticotropin releasing factor systems in the three hit theory model of depression in mice

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According to the three hit theory of depression the genetic predisposition, epigenetic and environmental factors may lead to manifested major depression. Mice lacking pituitary adenylate cyclase-activating polypeptide (PACAP) show depression-like behavior. The function of corticotropin releasing factor (CRF) in the hypothalamus- pituitary -adrenal gland axis is well known, however the role of extra-hypothalamic CRF in the amygdala and bed nucleus of the stria terminalis (BNST) is largely elusive.
Our aims were to set up an applicable mouse model for depression and to study the functions of CRF neurons in amygdala and BNST.
Litters from PACAP heterozygous mice (genetic factor) were separated from their dams daily either for 15 or 180 mins versus maternally non-deprived animals (epigenetic factor). The half of adult offspring was subjected to chronic variable mild stress (environmental factor). Finally, animals were perfused, adrenal glands and body weight were determined, and double immuno-fluorescence (CRF-FosB) labelling was carried out on the sections of the forebrain.
Our results revealed that adrenal gland weights significantly increased in groups which have suffered all three hits. The highest CRF cell counts and specific signal density in the ventral and oval nuclei of BNST was found in mice carrying all the risk factors. In line with this, the number of FosB containing CRF neurons was also increased in this group.

On the other hand, CRF in the amygdala showed the highest density in animals who experienced stress without maternal separation history. The number of FosB positive neurons had increased upon maternal deprivation.
In summary, increased adrenal gland weights suggest that the stress response took place. The co-incidence of all risk factors altered the functions of BNST-CRF neurons, which may refer to maladaptative changes. The three hit theory of depression in mice seems to be an applicable model for further investigation of mood disorders.

Establishment of an in vitro drug screening assay for Alzheimer's disease based on induced pluripotent stem cells

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Despite intensive efforts, there is no cure for the most common form of dementia, Alzheimer's disease (AD) which progressively destroys the brain. Moreover, the early diagnosis of the disease is still not possible, therefore our knowledge is very limited on the early phase of the pathology. It would be important to develop in vitro cellular systems which are able to mimic the progression of the disease and suitable for drug testing. Reprogramming adult human somatic cells to induced pluripotent stem cells (iPSCs) is a novel approach to produce patient-specific stem cell lines for disease modelling. This new preclinical model system is especially useful when the disease affects cells which are inaccessible and animal models prove to be not predictive enough as in the case of various disorders of the central nervous system (CNS). Our aim is to develop human iPSC-based in vitro cellular model systems for better understanding of the pathomechanisms and drug development. To reach this goal we genetically reprogrammed fibroblasts and mononuclear blood cells from genetically and clinically well-characterized patients with early and late onset AD and healthy controls into iPSCs. The iPSC lines were characterised by alkaline phosphatase activity, immunocytochemistry for pluripotency markers (Oct4, Nanog, E-cadherin, Sox2) and were karyotyped. The pluripotency was proved by in vitro differentiation towards the three germ layers (ecto-, meso- and endoderm). In the modelling experiments iPSCs were induced to become neural precursor cells (NPCs) by dual SMAD inhibition. The NPC cultures were characterised and compared by marker gene expression (Pax6, Nestin and Sox1). Furthermore, altered secreted amyloid-beta 40/42 ratio were detected in neurons of early onset AD patients compared to healthy controls. As a next step we will characterise and compare the sporadic AD patient iPSCs-derived neuronal cultures, as well. In conclusion, these findings demonstrate the suitability of iPSC-derived neuronal cultures to examine the pathophysiology of neurological and psychiatric disorders such as Alzheimer's disease that may be also used in drug development.
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Dynamics of finger tapping-related functional brain networks

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Although limited information on the neural mechanisms underlying planning and execution of motor actions is already available, network properties of these complex processes are far to be well elucidated. The aim of this study was to investigate the dynamics of finger tapping-related functional brain networks in the spectro-temporal domain.

Twenty healthy young adults performed a finger tapping task with their right index finger. The pace of tapping was determined by a visual cue. Brain activity of subjects was recorded by a 128 channel EEG system with a 2048 Hz sampling rate. Preprocessing of the recordings consisted of a multi-stage semi-automatic elimination of artefacts based on independent component analysis and application of scalp current density transformation in order to attenuate the potential effects of volume conduction. Time-frequency decomposition of EEG activity was performed on data epochs triggered to the finger tapping time and finger tapping-related functional connectivity was estimated for all channel pairs by the Weighted Phase Lag Index (WPLI) in the [-3.5 1.5] s time interval and [0.5 30] Hz frequency range. Network properties were assessed by comparing the obtained WPLI connectivity measures in the [-150 150] ms time interval against the [-3.5 3] s baseline period and by applying FDR correction for multiple comparisons. A complex pattern of significant functional connections was found across the frequency ranges during different phases of preparation and execution of finger tappings. In the preparatory phase this pattern was dominated by broad-band significant fronto-parietal connections contralateral to the finger tapping side. During execution of tappings significant connections appeared between left sensory-motor and supplementary motor areas in the upper theta frequency band (6-8 Hz). Finally, after the execution of tappings broad-band significant connections were found in frontal and prefrontal brain regions.

Our results provide detailed information on the organization of functional neural networks before, during and after finger tappings and accordingly our work may contribute to deeper understating of the human functional brain connectome in general.

Protein kinase D exerts neuroprotective functions during oxidative stress via NFkappaB-independent pathways

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Protein kinase D (PKD), a serine/threonine kinase, fulfills many roles in cellular physiology, including the modulation of cellular stress-induced signaling pathways. All 3 isoforms of

PKD are highly expressed in the mammalian brain. In neurons, PKD regulates dendritic growth and transport processes, and a role in neuronal survival has also been suggested. According to studies on non-neuronal cells, reactive oxygen species released from the mitochondria during oxidative stress activate a PKD-dependent protective signaling cascade. Our knowledge on the relation between oxidative stress and PKD-mediated changes in neurons, however, is still limited.
We examined the effects of oxidative stress on murine cortical neuronal cultures. Oxidative stress induced by H_2O_2 treatment caused transient activation of PKD, as evidenced by western blot analysis or immunocytochemistry experiments involving a PKD reporter or a FRET biosensor. Application of a PKD-specific inhibitor increased cell death evoked by 24-hour H_2O_2 treatment, indicating a neuroprotective role for PKD.
The NFkappaB signaling pathway has been shown to promote non-neuronal cell survival in response to oxidative stress in a PKD-mediated manner. To examine the existence of a similar neuroprotective PKD-NFkappaB pathway in cortical neurons, immunocytochemistry and western blot analyses have been performed. Phospho-NFkappaB and NFkappaB antibodies have been used to detect nuclear translocation in response to oxidative stress. Although anti-NFkappaB staining showed a general increase in signal intensity - which was attenuated in the presence of the PKD inhibitor -, no obvious nuclear translocation was observed. Cytoplasmic and nuclear protein fractions were isolated and probed with antibodies recognizing phosphorylated and unphosphorylated IKKa, NFkappaB p65 or IkappaBa, members of the canonical NFkappaB signaling cascade. H_2O_2 treatment induced the phosphorylation of the NFkappaB pathway in a PKD-dependent manner. However, nuclear accumulation of NFkappaB p65 protein was not observed. Thus, our results indicate that although PKD activates the NFkappaB cascade upon oxidative stress, its neuroprotective effect is mediated independently from NFkappaB nuclear translocation.

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Input pattern requirements for local cooperative LTP in hippocampal CA1 pyramidal cell dendrites

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Simultaneously recorded multimodal signals in the hippocampal CA1 region, in vitro

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The firing mode of thalamocortical neurons correlates with rapid state changes

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In the absence of sensory input, the mammalian brain exhibits a wide array of structured, brain state dependent spontaneous activity. Different stages of the sleep-wake cycle are characterized by different rythmic activities generated within the thalamocortical (TC) network. In addition to these slow changes transient brain state changes can occur during the awake state. Specifically, in cortical networks periods of active wakefulness are associated with depolarized membrane potential and asynchronous firing among neurons and fast oscillations in the local field potential, whereas periods of quiet wakefulness are asociated with hyperpolarized membrane potential, synchronous firing and slow oscillations. The activity of TC neurons during these transient brain state transitions has not yet been characterized. To this end we performed multi-unit and local field potential recordings in the visual cortex with simultaneous extracellular or intracellular recordings of dorsal lateral geniculate nucleus (dLGN) neurons of awake, head restrained mice while monitoring their pupil size. The firing rate of some dLGN neurons showed clear correlations with the pupil size on a sub second time scale indicating that TC neurons also exhibit brain state dependent activity changes on a rapid timescale. Intracellular recordings indicated that high-threshold (HT) bursting is present in some TC neurons during states characterized by intermediate pupil sizes while LTCP mediated bursts are present during minimal pupil sizes and tonic firing during maximal pupil dilatations. These data indicate that the activity of TC neurons can change during brain state transitions on a rapid timescale. We provide the first direct evidence of HT-burst in TC neurons of awake mice.

The role of feedback dependent movement parameters in action related auditory ERP attenuation

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Goal-directed behavior depends on the utilization of information about future events. Such information can be represented by various cognitive subsystems, which may or may not directly interact. The present study investigated the information flow between the motor and the auditory systems. Event-related potentials (ERPs) elicited by action-sound coincidences are smaller than those elicited by sounds in the absence of action. However a valid comparison between the two conditions is only possible if motor and auditory effects

can be separated in the ERPs evoked by action-effect couplings. The auditory contribution to the coincidence waveform is usually estimated by subtracting an ERP related to actions not coinciding with sounds. This logic relies on the assumption that actions and action-related ERPs are identical in the two conditions. For paradigms with self-induced sounds, this assumption is not self-evident: the results of our experiment suggest that predictions about distal sensory consequences can influence physical parameters of actions. Participants pinched a force sensitive resistor (FSR), which triggered the presentation of a sound when the force reached a threshold (Motor-Auditory condition) or had no auditory effect (Motor condition). In the Auditory condition, a replay of a previous sound sequence was presented. Pinch-force profiles were markedly different between conditions: the applied force was stronger, and pressure was maintained for longer in the Motor-, than in the Motor-Auditory condition. Moreover systematic pinch-force changes within blocks differ in the two conditions: In the motor-auditory condition the FSR-amplitudes decreased as a function of time indicating that participants applied less and less force during the course of each block. In the motor condition the opposite tendency could be observed: within each block, the peak amplitude seemed to increase. These results suggest that participants used the self-initiated sounds as feedback to optimize the to-be executed motor patterns. It seems, that the logic behind calculating the auditory component in the action-tone ERP is flawed, as the presence of auditory feedback appeared to have considerable effect on the action parameters. Our results indicate that force related differences in cortical potentials as well as differences in the temporal progression of the action-force profiles could bias the estimation of the ERP attenuation for self-induced sounds, however we assume that a genuine auditory ERP attenuation effect still persists, independent of the movement related factors.

Single bursts in single presynaptic granule cells rearrange the efficacy of the feedforward inhibitory circuit in the CA3 network

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Hippocampal mossy fibers (MF), the axons of DG granule cells (GCs) provide a major synaptic input to the CA3 network. GCs are mostly quiescent but after the several second-long inactive periods they either fire single action potentials or short, high-frequency bursts. Mossy fibers innervate more GABAergic cells than pyramidal cells, and their synaptic output shows diverse postsynaptic cell-type specific short-term dynamics. We asked how single spikes and short, high-frequency burst firing affects the output of single MFs using paired recordings of anatomically identified postsynaptic CA3 neurons, and single presynaptic MFs, either direct recordings from giant MF boutons or from CA3 GCs. Intriguingly, single bursts in single presynaptic MFs (15AP at 150Hz) increased the unitary amplitudes by 281±8% for a relatively long period (0.8-8 sec after the burst) in a subset of postsynaptic GABAergic cells including ivy, fast-spiking basket, axo-axonic and regular-spiking basket cells. Importantly, all these cells types are crucial elements of the feed-forward inhibitory circuits between the DG and CA3 networks. The single burst-induced

amplification developed only after several hundreds of milliseconds, the unitary amplitudes exceeded the maximal compound amplitudes during the high-frequency train (by 180±8%) and shorter burst were similarly effective (319±34% after 4-7 APs at 150Hz). However, the augmentation was not permanent as the amplitudes returned to their initial levels within 60 seconds and in postsynaptic pyramidal cells and other GABAergic neurons the effect of the bursts only resembled classical post-tetanic-potentiation. Consistently with the cell type specificity of the amplification, the feedforward inhibition from single granule cells onto CA3 pyramidal cells is enhanced after single presynaptic bursts, as detected by the increased probability and strength of disynaptic inhibitory events. The average probability of the feed-forward inhibitory events at single MF spikes increased ~2.5 fold (from 13±3% to 33±5%) 1-8.5s after single MF bursts (6 or 15 AP at 150Hz) and interestingly the average probability was larger than what we found during the burst (19±2%).Changes of the short-term plasticity of the connections onto GABAergic cells suggest the involvement of presynaptic mechanisms following the single bursts. Furthermore, the results of the experiments where the initial release probability was changed by varying the extracellular calcium concentration also indicate a presynaptic mechanism. Interestingly, several observations suggest that the vesicle priming is saturated following single presynaptic bursts in MF synapses onto a selected population of GABAergic cells, which play crucial roles in the feed-forward inhibition between the DG and CA3 regions. These results indicate the feed-forward network between the DG and CA3 are efficiently rearranged after single physiologically relevant MF bursts.

3D engineered neural tissue from patient-derived induced pluripotent stem cells as a tool for modelling Alzheimer's disease

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Induced pluripotent stem cells (iPSCs) provide a useful tool to study the pathophysiology of neurodegenerative diseases in a patient-specific way. In this study fibroblasts and mononuclear blood cells isolated from genetically and clinically well-characterized patients with Alzheimer's disease (AD) and from healthy controls were reprogrammed into iPSCs. These cells were induced to become neural precursor cells (NPCs) and subsequently differentiated into neural cells. Using an air-liquid interface based, scaffold-free system we produced 3D engineered neural tissue (3D ENTs). After 6 and 8 weeks of differentiation an in-depth analysis was performed to characterize the 3D cultures. We studied the cellular composition of the 3D ENTs by the qualitative and quantitative expression of marker proteins (IHC, qPCR and WB), the morphology of the generated neurons and their spine density (Golgi-Cox-staining) as well as their functionality in terms of calcium signaling (calcium uptake assay) and electrophysiological properties (multi-electrode array, MEA).

The presence of glial cells and various neuronal subtypes including cortical neurons was confirmed by gene expression analyses. These data demonstrated a homogenous composition of 3D ENTs formed by NPCs, neurons, astrocytes and oligodendrocytes. The generated neurons had a simple uni- or bipolar morphology with spines on their dendrites. MEA recordings and calcium imaging confirmed spontaneous firing activity, another evidence for the presence of functional synapses. Most importantly, the comparison of 3D ENTs derived from AD patients with those of healthy controls allowed the verification of the disease phenotype. 3D ENTs derived from AD patients produced more extracellular Aβ42 than healthy controls as measured by ELISA. Furthermore, immunostaining analysis revealed more Aβ deposits in diseased cultures than in control ones. In conclusion, these findings demonstrate the suitability of 3D ENTs to examine the pathophysiology of neurological and psychiatric disorders such as Alzheimer's disease that may be also employed in drug development.

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A novel level of functional diversity within the hippocampal CCK-expressing interneuron class

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Quantitative analysis of demyelination in a mouse model of multiple sclerosis with coherent anti-Stokes Raman scattering microscopy

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Degradation of myelin sheath is the cause of neurodegenerative diseases, such as multiple sclerosis (MS), but a clear mechanistic understanding of myelin loss is missing. To study the process of myelin breakdown we induced demyelination with the widely used oligodendrocyte apoptotic agent cuprizone. We applied coherent anti-Stokes Raman scattering (CARS) microscopy – a nondestructive label-free method to image lipid structures in living tissue – to monitor myelin loss and this novel method confirmed earlier results showing a brain region dependent myelin destructive effect of cuprizone (genu of corpus callosum > forceps minor of corpus callosum > anterior commissure > cerebral cortex > cerebellar peduncles). In addition, high resolution in situ CARS imaging revealed myelin debris forming lipid drops alongside myelinated axons fibers. We quantified the

amount of debris with a custom-made software for segmentation and 3D reconstruction and found brain region and cuprizone dose dependent accumulation of lipid drops in correlation with the thickness of myelin around axons. Thus, CARS microscopy is a potent tool for quantitative monitoring of myelin degradation at unprecedented spatiotemporal resolution during oligodendrocyte apoptosis in living tissue. The automimmune initiation of MS has been recently questioned by proposing that the immune response is a consequence of oligodendrocyte degeneration. We speculate that accumulation of lipid drops around degrading myelin might be instrumental in triggering subsequent inflammatory processes.

Modulation of interictal-like and spontaneous population activity by microsurgical intervention in rat brain slices

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Epileptic reorganization of GABAergic inhibitory interneurons in rodent models of absence epilepsy

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Multiple theories were proposed to address the pathomechanisms involved in the generation of spike-and-wave discharges (SWDs) characteristic of absence epilepsy. Detailed investigations of the rat models of the disease – Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and Wistar Albino Glaxo (WAG/Rij) – confirmed the presence of a cortical epileptic focus in the S1 somatosensory cortex, in which a local breakdown in GABAergic inhibition and abnormal pyramidal neuron morphology may contribute to seizure initiation. SWDs generated here propagate rapidly and invade other cortical and subcortical areas. We aimed at exploring potential epileptic reorganization associated with recurrent pathological excitation in two functionally distinct classes of GABAergic inhibitory interneurons in the GAERS and WAG/Rij models, compared to non-epileptic controls (NEC) and normal Wistar (WISK) rats. Interneuron-specific and perisomatic inhibitory interneurons expressing the calcium-binding proteins calretinin (CR) and parvalbumin (PV), respectively, were examined using light microscopy in two regions: the S1 somatosensory cortex and the dentate hilar subregion of the hippocampus. GAERS, WAG/Rij and WISK displayed a significantly lower density of CR-positive interneurons in

the dentate hilus when compared with NECs. In addition, GAERS showed the same pattern in the infragranular layers of the S1 cortex. PV-positive interneurons were markedly reduced in density in the hilar regions of GAERS and WAG/Rij, but not in WISK. In the S1 region, the two epileptic strains showed significant differences compared to NECs, but in opposing directions: GAERS were characterized by higher PV-positive cell densities, whereas WAG/Rij rats had fewer PV-positive interneurons. Our results suggest significant epileptic reorganization in GAERS and WAG/Rij rats. The loss of CR-expressing interneurons involved in the synchronization of dendritic inhibition of principal neurons may lead to network hyperexcitability and lower the seizure threshold. The sparse PV-immunopositive network characterizing the cortical focus of WAG/Rij rats may as well indicate an impairment of GABAergic inhibition. On the other hand, the increased number of S1 PV-labeled interneurons of GAERS might indicate a mechanism to compensate for the epileptic activity. However, PV-immunostaining may be altered by Ca2+ ion influx into the cells, causing uncertainty in the determination of the number of PV-containing neurons. The reorganization of interneuron circuitry in the hippocampus shows some similarity to temporal lobe epilepsy.

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Region-specific adenosinergic modulation of the slow wave 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, somatosensory and visual cortices, respectively, of urethane-anesthetized rats to examine the direct effect of adenosine on UP and DOWN states.

Cortical field potentials (LFPs) were recorded by a 16-pole vertical electrode array inserted nearby 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. In the visual cortex, adenosine modulated the SCR with fast kinetics on the LFP level, although in this case the power of beta activity didn't follow the increment of delta power. Delta power increment was based on increased frequency of state transitions and increased height of UP-state associated slow waves in both regions. 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. Intrinsic mechanisms may be responsible for the differencies in the kinetics, which may enable restorative processes to take place with varying time length. Adenosine may not only be responsible for the induction of recovery sleep, but may shape the region-specific properties of local sleep.

Protein kinase D regulates homeostatic plasticity in hippocampal cell cultures

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Homeostatic plasticity stabilizes the properties of neuronal circuits by adjusting the responsiveness of postsynaptic neurons according to the strength of the trigger inputs. Chronic tetrodotoxin (TTX) treatment precludes the generation of action potentials and eliminates spike-evoked synaptic transmission. Within 48 hours of TTX treatment, neurons adapt to reduced synaptic input by increasing the surface amount of excitatory neurotransmitter receptors. This phenomenon is called homeostatic upscaling, but the underlying regulatory cascades are still far from understanding.

Protein kinase D (PKD) is a serine/threonine kinase, which is highly expressed in CNS neurons. It has been proven that PKD promotes neuronal plasticity via regulating actin dynamics within dendritic spines. However, in non-neuronal cells PKD also plays a role in Golgi-to-cell-surface transport processes by controlling the biogenesis of specific transport carriers. The aim of our study is to investigate whether PKD regulates the intrinsic biophysical properties of neurons during homeostatic plasticity through regulating plasma membrane localized ion channels.

Homeostatic upscaling was evoked in primary dissociated mouse hippocampal cultures by 48h TTX treatment, while endogenous PKD functions were blocked by a specific PKD inhibitor (kbNB 142-70). Neuronal responsiveness was measured by the whole-cell current clamp technique and IV protocols were analyzed. Cells were labelled with biocytin to allow further characterization of the recorded cells. Glutamic acid decarboxylase (GAD) 65 and 67 immunoreactivity was used to define GABAergic neurons, while glutamatergic neurons were characterized based on chicken ovalbumin upstream promoter transcription factor-interacting proteins 2 (CTIP2) and prospero homeobox protein 1 (PROX1) expression.

According to our results, 48h of TTX treatment did not change cell viability or resting membrane potential, but significantly increased T-current mediated postinhibitory rebound firings in the recorded neurons. The addition of PKD-specific inhibitor completely abrogated this effect, indicating that T-type Ca-channel expression (CaV3) is upregulated during homeostatic upscaling in a PKD-dependent manner. Interestingly, long term kbNB 142-70 treatment selectively increased the frequency of H-current mediated voltage sags in the recorded neurons, suggesting increased activity of the hyperpolarization-activated

cyclic nucleotide-gated (HCN) channels. Importantly, this effect was independent from the TTX treatment.
Our results already show that PKD selectively modulates the occurrence of certain currents and the excitability and firing properties of neurons and participates in homeostatic upscaling. Analyses at the mRNA and protein levels are currently in progress to define whether PKD directs transcriptional, transport or modulatory role in case of CaV3 and HCN channels.
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Optogenetic characterization of subiculothalamic connections: Novel function of limbic TRN in head-direction system?

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As part of the limbic system, the anterior thalamic nuclei (ATN) are abundantly and reciprocally connected with the hippocampal formation with parallel recurrent loops, and have the capacity to profoundly shape hippocampal spatial and mnemonic information processing. Cells in the postsubiculum and anterior thalamus discharge as a function of the animal's head direction (HD), but independent of its behavior and location in the environment. The thalamic reticular nucleus (TRN) controls thalamic throughput of sensory information through feedback inhibition. Neurons in the dorsal part of TRN also receive postsubicular projections, yet the role of inhibition in the thalamic HD system remains largely unexplored despite its potential importance for attractor models. TRN could provide a potential source of lateral inhibition among the HD neurons in the ATN.
We used both anatomical and functional techniques to determine the nature of subicular afferents of the TRN. Retrograde and anterograde tracers were injected into the limbic TRN and postsubiculum, respectively to validate the previously described projections. Channelrhodopsin and Archeorhodopsin-expressing viruses were then injected into the postsubicular complex, and in vitro and in vivo electrophysiological recordings were performed three to four weeks after injection. Using in vitro acute brain slices we found that the postsubicular glutamatergic neurons discharge reliably upon optical stimulation trains and that the limbic TRN and thalamic neurons are synaptically connected to them. Furthermore the postsubicular thalamic projections show different synaptic properties in comparison to the well-studied corticothalamic pathway. In vivo in urethane anaesthetized animals we showed that trains of laser stimulation trigger repetitive firing in the dorsal TRN neurons. Freely moving in vivo data suggest that postsynaptic potentiation in the TRN and ATN shows a state dependent behavior: upon resting state the neurons follow the stimulation train precisely but not during active state.

Rhythmic persistent firing of neurogliaform interneurons in human neocortical slices

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Persistent firing is a form of activity-induced ectopic action potential generation which has been recorded in several GABAergic interneuron classes from the hippocampus and the neocortex of rodents in vitro and in vivo. In order to assess potential species specific aspects of persistent firing, we performed whole cell patch clamp recordings of layer I interneurons in rat and human neocortical slices. Interneurons in the layer I of the human neocortex were capable of establishing persistent firing induced by the same paradigm used in rodents. Furthermore, we found that identified human neurogliaform cells show persistent firing rhythmically reoccurring in the frequency range of slow oscillations. This bistable persistent firing state was absent in rodent neurogliaform cells or in human non-neurogliaform interneurons showing a single persistent firing episode following onset. We hypothesize that rhythmic persistent firing in neurogliaform cells might contribute to the generation of slow oscillations in the human neocortex.

Effects of visually guided finger tapping on power spectral properties of resting-state brain activity

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During the last decade increasing number of studies has been published on the importance of understanding the neural mechanisms of resting-state brain activity, however, our knowledge about the relationship of resting-state and task-related brain activity is still limited. The aim of this study was to investigate the effects of visually guided motor actions on power spectral properties of resting-state brain activity.

Twenty healthy young adults participated in the experiment. As a motor task subjects had to perform finger tapping with their right or left index fingers, while the pace of tapping was determined by a visual cue. To assess the effects of the motor task on resting-state brain activity, three minutes long eyes-closed resting periods were considered before (BEF) and after (AFT) the motor task. EEG was recorded by a high-power system with 128 channels and 2048 Hz sampling rate. Preprocessing of the recordings included a multi-stage semi-automatic elimination of artefacts and scalp current density transformation. Relative power spectrum in the [0.5 30] Hz frequency range was obtained by the FFT algorithm after applying a Hanning window on artifact-free two seconds long data segments. Statistical comparison of power spectra of BEF and AFT resting conditions was carried out by cluster-based permutation testing. Significant differences were found posteriorly in the 7-25 Hz frequency range with a predominance of the right occipito-temporal region. Lower alpha (8-10 Hz) power was significantly higher in the AFT as compared to the BEF condition. The corresponding negative cluster showed a bilateral occipital, occipito-temporal topography. In contrary, higher alpha and beta oscillations up to 25 Hz were significantly higher in the

BEF condition. This effect was localized in the occipital and right occipito-temporal brain regions.
We revealed significant effects of visually guided finger tapping on the relative power of resting-state alpha and beta oscillations in posterior brain regions. These results are in agreement with previous findings suggesting decrease of dominant alpha frequency as a result of physical and mental fatigue.

Tracking Gestalt-related inferences of long-range horizontal connections in the cat primary visual cortex.

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Primary visual cortex (V1) is the first level of the visual processing in which neurons are assumed to describe a "primal sketch" of the visual scene, i.e. the light intensity distribution that falls on the retina. According to David Marr's hypothesis (1982) visual primitives, which include edges, bars, blobs, and termination primitives possess attributes such as orientation, contrast, length, width and spatial position among others. It is also assumed that visual cortical neurons can exploit the spatial frequency component of these primitives suggesting that they carry out a dual analysis: a contour based responses to edges and another of texture based responses to spatial frequency components. Recent anatomical and physiological investigations have implicated that the construction of the „primal sketch" is likely to be carried out according to rules established by early Gestalt-psychology. Accordingly, the type of interaction is determined by the geometry of the arrangement within the visual field. Our goal is: (i) to unravel how the organization of input and output signatures of neurons such as orientation and direction preference are arranged within the visual scene and (ii) how long-range connections of visual cortical neurons can contribution to the „primal sketch". Since all these features should be multiplexed onto the 2D projection of the visual space a detailed retinotopic representation, i.e. retinotopic map of the visual cortex, is necessary to obtain.
Here we used optical imaging of intrinsic signals for detecting high resolution retinotopic representation of the visual cortex. To this, visual stimuli were displayed in narrow windows extending 30 deg at length placed parallel to the horizontal and vertical meridian and subtending 0.7 deg at width. Each stimulus window contained moving square wave luminance gratings of 0.6 cyc/deg spatial frequency drifting at 1 Hz temporal frequency. Activity maps acquired to visual stimuli were treated with low- and high pass filters and subjected to time-frame analysis.
Interpolation of single condition activity maps showed a smooth representation of the visual space along both azimuth and elevation and conformed to earlier data using electrophysiological recordings (Tusa et al, 1978; Albus, 1975). The retinotopic mapping paradigm introduced here allows a rapid and efficient detection of cortical representation of visual space that will be used for studying the visual field representation of single cell connections.

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An open source toolkit for combining neurophysiology and rodent behavior

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Neurons in the brain communicate with action potentials timed at millisecond precision. Therefore, mechanistic insight about function often comes from veridical recording of these action potentials. For instance, understanding how neurons represent specific information about external stimuli and internal variables requires registering neuronal action potential firing while animals are engaged in different behaviors. Moreover, to have sufficient statistical power, animals have to repeat these tasks time and again. Thus behavioral neurophysiology has a dual objective: train animals on a behavioral task of interest and record their neuronal activity while they perform the task.

Importantly, the millisecond timing of neuronal firing has strong implication on the scope of behavioral tasks. One can only hope to extract specific information carried by spike timing if behaviorally relevant events of the task, like cue stimuli, go and stop instructions, reward and punishment delivery are under the same precision of temporal control.

Standard experimental setups capable of achieving this are not in routine use. We present an affordable, modular, open source system capable of flexible behavioral task design and execution, submillisecond precision hardware control, combined neural recordings and optogenetic stimulation. Key components of this system are (i) custom designed, modifiable environments for mouse behavior; (ii) open source microcontroller-based behavior control equipment (BPod, designed by Joshua I Sanders); (iii) open source data acquisition system based on Intan technology (open ephys designed by Joshua H Siegle and Jakob Voigts) (iv) open source computer vision based position tracking (Bonsai, desgined by Goncalo Lopes); (v) open source pulse generator (PulsePal, designed by Joshua I Sanders); (vi) light source (laser or LED) and a light coupling system for optogenetic stimulation; (vii) freely available Matlab software package for storing and analyzing combined behavior/electrophysiology/optogenetics experiments (CellBase, developed by Adam Kepecs and Balázs Hangya). How this setup operates will be demonstrated on a simple associative learning task. We believe this system will be a useful tool for a broad community of neurophysiologists.

Synchronous Population Activity in the Hippocampal CA3 region and Dentate Gyrus, In Vitro

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Hippocampal sharp wave-ripples (SPW-Rs) and dentate spikes from the dentate gyrus described for freely moving rats occur during slow wave sleep and behavioral immobility and thought to play an important role in memory formation. We investigated the cellular and network properties of these events with simultaneous laminar multielectrode in a rat hippocampal slice model, using physiological bathing medium. The electrode array was placed on the surface of the hippocampal slice, perpendicularly to the granule and pyramidal cell layer. Spontaneous population activities were generated in the dentate gyrus and CA3 region of slices prepared from the temporal hippocampus of young rats, in vitro. These events were characterized by a local field potential gradient (LFPg) transient, increased fast oscillatory activity and increased multiple unit activity (MUA). They were apparently similar to synchronous population bursts previously recorded in rodent hippocampal slices, considered as in vitro models of SPW-Rs. The synchronous population activity recorded in the dentate gyrus was termed dentate wave in rat. CSD analysis, which estimates transmembrane currents in the local neuronal population confirmed that SPW-Rs were locally generated in each of the DG and CA3 region with conserved intrahippocampal connections. Simultaneous recordings (n=23) indicated that the waves were often synchronized in the DG and CA3 (n=8), although propagation was observed from the CA3 and DG also (n=15). This suggests that the information transmission among the CA3 region and the DG consists of multidirectional processes.

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

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

In this work we present multiple 3D, acousto-optical, two-photon laser-scanning technologies to monitor neuronal activity at different scales with genetically-encoded calcium indicators in a near-cubic-millimeter scan range (up to 700 × 700 × 1,400 μm3),

with a high scanning speed (up to 1 Mhz), with high (<500 nm) resolution in the center core, and less than 1.9 x 1.9 x 7.9 μm3 resolution throughout the whole scanning volume.
We used volumetric random-access calcium imaging of spontaneous and visual stimulation-evoked activity from hundreds of neurons. We applied 3D trajectory scanning technique to measure multiple dendritic segments in vivo with motion compensation possibility. Finally we introduced an expansion of trajectory scanning technique to monitor the activity from more than 100 cells in vivo while obtaining information for motion correction.

Graph Theoretical Analysis Reveals: Women's Brains Are Better Connected than Men's

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Deep graph-theoretic ideas in the context with the graph of the World Wide Web led to the definition of Google's PageRank and the subsequent rise of the most popular search engine to date. Brain graphs, or connectomes, are being widely explored today. We believe that non-trivial graph theoretic concepts, similarly as it happened in the case of the World Wide Web, will lead to discoveries enlightening the structural and also the functional details of the animal and human brains. In the present work we have examined brain graphs, computed from the data of the Human Connectome Project, recorded from male and female subjects between ages 22 and 35. Significant differences were found between the male and female structural brain graphs: we show that the average female connectome has more edges, is a better expander graph, has larger minimal bisection width, and has more spanning trees than the average male connectome. Since the average female brain weighs less than the brain of males, these properties show that the female brain has better graph theoretical properties, in a sense, than the brain of males.

Investigation of the circadian and homeostatic aspects of sleep-wake regulation 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. On the other hand, unequal distribution of different sleep and wake phases during day and night proves the daily rhythm of sleep. 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 one-week habituation time the circadian rhythm seemed to be shifted by 6-7 hours, in accordance with previous reports. In contrast, daily rhythm of sleep and wakefulness was

not completely eliminated as reported by others. 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.

Analysis of acoustically and electrically evoked auditory cortical responses in the cat

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Changes of auditory cortical evoked potentials depending on vigilance state are well known. However, underlying thalamocortical mechanisms are still elusive at the neural circuit level. We investigated the simultaneously recorded changes of the click and wideband noise evoked responses in the medial geniculate body (MGB) and in the auditory cortex and also studied the cortical evoked responses induced by single pulse electrical microstimulation of the MGB. Recording electrodes were chronically implanted to the surface of the auditory cortex. A 24 channel linear array multielectrode was implanted into the medial geniculate body (MGB) for recording and microstimulation. Local field potentials, single and multiunit activity (MUA) were recorded and analyzed. Position of the MGB electrodes was verified by recording tonotopic responses and post mortem histology. The configuration of the auditory cortical evoked potentials induced by MGB microstimulation was similar to those elicited by click stimuli but with shorter latency. In slow wave sleep the amplitude of the early components of the click evoked cortical response was not suppressed and the late phase was prevailed by large amplitude positive wave and decreased unit firing. Similarly, late components of the cortical responses evoked by MGB microstimulation displayed increase both in amplitude and latency. Paired presentation of acoustic and electric stimuli with different delays revealed mutual modulation of both early and late cortical evoked components. We have analyzed the amplitudes of responses evoked by the paired stimulations and found that the superposition of evoked potential components is not linear. This non-linearity depends on the distance between the two stimuli in the paired stimulation and also on the vigilance state of the animal. The same phenomenon was observed when double click or double electric stimuli were presented.

Protein kinase D regulates neurotransmitter receptor turnover in hippocampal neurons

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Synaptic plasticity is known to underlie memory formation and learning. Synaptic strength is modulated in an activity-dependent manner via altering the actin network in dendritic spines or by regulating neurotransmitter receptor recycling in the postsynaptic membrane. It is proven that protein kinase D (PKD) promotes neuronal plasticity via regulating actin network remodeling in dendritic spines. PKD also controls the integrity of Golgi apparatus and mediates the somatodendritic distribution of transferrin receptor. Our aim is to investigate whether PKD modulates neuronal plasticity via neurotransmitter receptor turnover, as well.

PKD activity was modified genetically or pharmacologically, either via overexpressing a constitutively active PKD mutant or by the application of a selective PKD inhibitor. Fluorescently labeled transferrin uptake was analyzed to follow receptor-mediated endocytosis in cultured hippocampal neurons. Endosomal compartments were identified with EGFP-tagged Rab endosomal marker proteins. Kinetics of transferrin receptor endocytosis and colocalization between internalized transferrin molecules and Rab positive vesicles were analyzed by confocal microscope. Our results show that PKD has an effect on the early steps of endocytosis, directs vesicular trafficking at the plasma membrane and stimulates the recycling endosomal system.

To extend our investigations on PKD-regulated neurotransmitter receptor turnover, the relative amount of the AMPA receptor GluA1 subunit within the plasma membrane was analyzed in relation to modulating endogenous PKD activity. Cell surface biotinylation was used to selectively precipitate plasma membrane inserted GluA1 via labeling its extracellular region with membrane impermeable biotin. During antibody feeding, anti-GluA1 antibody was added to living neurons for a short period. After fixation, postsynaptic areas were highlighted by Shank2 immunoreactivity and the surface-bound anti-GluA1 signal was analyzed by confocal microscopy. Our results indicate that long-term inhibition of PKD decreases, whereas short-term PKD inhibition increases the amount of plasma membrane inserted postsynaptic AMPA receptors. Increased PKD activity, on the other hand, reduces surface expression of GluA1 subunit in the dendrites. Thus, PKD presumably regulates rapid neurotransmitter receptor endocytosis directly at the plasma membrane while it also affects the secretion and plasma membrane insertion, albeit with a slower kinetics.

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GABAA receptor subunit content of hippocampal somatic and axon initial segment synapses

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Hippocampal pyramidal cells (PCs), expressing different GABAAR subunits, receive compartment-specific GABAergic inputs from distinct interneurons. Previous immunolocalization experiments suggested input-specific differences in the distribution of α1 and α2 subunits in perisomatic synapses of hippocampal PCs. In contrast, functional data demonstrated similar decay time constants of IPSCs evoked by three different perisomatic region-targeting interneurons (i.e. axo-axonic cells and PV positive and CCK-containing basket cells), indicative of a rather homogenous perisomatic synapse population. Recently, with highly sensitive immunolocalization techniques (SDS-FRL and antigen retrieval immunohistochemistry) it was found that virtually all somatic synapses contain the α1 and α2 subunits. However, the relative abundance of these two subunits in individual perisomatic synapses in the hippocampus remains unknown. By using the face-matched mirror replica technique we show that the number of immunogold particles for the α1 and α2 subunits shows positive correlation in the CA3 and CA1 PC AIS and somatic synapses, and that the density ratio of immunogold particles for the α1 and α2 subunits is similar. In addition, we show that the β1, β2 and β3 subunits are ubiquitously present in the perisomatic synapses of hippocampal PCs. Based on the α and β subunit content of the synapses, there is no indication of multiple AIS and somatic synapse populations in the CA3 and CA1 PCs. We conclude that GABAAR subunits are uniformly distributed in the perisomatic synapses of hippocampal PCs.

Localization of connexin 43 gap junctions and hemichannels on tanycytes of adult mice

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Tanycytes are special, glial cells lining the lateral walls and the floor of the third ventricle behind the optic chiasm. These cells play important role in the regulation of neuroendocrine axes and energy homeostasis.

To determine whether tanycytes communicate with each other via connexin43 (Cx43) gap junctions, tanycytes were loaded with lucifer yellow (LY) through patch pipette. In all cases, LY filled larger group of tanycytes. Wall of blood vessels were also labeled through their connection with tanycyte processes. Cx43-blocker carbenoxolone inhibited the spreading of LY from the patched cell.

Using immunocytochemistry, the highest density of Cx43-immunoreactive spots was observed in the cell bodies of α-tanycytes. In lower density, Cx43-immunoreactivity was

also observed in β-tanycyte cell bodies. The processes of both types of tanycytes were surrounded by Cx43-immunoreactivity. At ultrastructural level, Cx43-immunoreactivity was present among tanycyte cell bodies in all tanycyte regions, however, gap junctions were more frequent among the α-tanycytes. Cx43-immunoreactivity was frequently found on the ventricular surface of tanycytes.
Cx43-immunoreactivity was also observed on the contact surface formed between two tanycyte endfeet processes and between tanycyte endfeet process and axon varicosity in the external zone of the median eminence and capillaries in the arcuate nucleus and median eminence.
Our results suggest that gap junctions are present not only among tanycytes, but also between tanycytes and the axons of hypophysiotropic neurons. In addition, Cx43 hemichannels may facilitate the transport between tanycytes and extracellular fluids, like the cerebrospinal fluid, extracellular space of the median eminence and the circulating blood.

The influence of gap junctions on the estimate of passive electrical properties of cerebellar Golgi cells

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The passive and active electrical properties, together with the detailed three-dimensional morphology of neurons determine the way in which they integrate their synaptic inputs. The passive electrical properties of many glutamatergic principal cells have been determined, but much less is known about GABAergic interneurons (INs). Interestingly, most INs are richly interconnected with gap junctions (GJs), but how the presence of electrical synapses on INs affects the estimate of their passive electrical properties is largely unknown. In this study, we aimed to determine how GJs affect the estimate of passive electrical parameters of cerebellar Golgi cells (GoCs) with a combination of in vitro dual soma-dendritic patch-clamp recordings, anatomical reconstruction of the recorded neurons and multi-compartmental modelling. Computer simulations demonstrate a large, GJ distribution-dependent variability of the apparent passive electrical parameters of GoCs. For example, setting up 10 random syncytia of GoCs with a central cell coupled to 10 other cells through 20 GJs resulted in large variance (coefficient of variation = 0.49; min = 62 Ω*cm; max = 318 Ω*cm) in a specific axial resistance (Ra) estimate. We also show that the specific membrane capacitance (Cm) of GoCs is 1 μF/cm2, but a single-cell approximation of the membrane voltage responses of syncytium-embedded GoCs produced an apparent Cm value of 2.7 μF/cm2. This can be quantitatively reproduced by embedding of a GoC into a syncytium, where the central neuron is connected to 9 other GoCs with 18 GJs, each having a 1 nS conductance. Our results indicate that Cm and Ra of electrically coupled INs can be considerably different when their values are estimated with single-cell approximation.

Pathological changes of the giant motoneurons in schizophrenia patients

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Schizophrenia is a major mental disorder with ca. 0,8% prevalence in the population. Beside the positive, negative and cognitive symptoms, alterations of the motor function are also present in this disorder, such as eye movement-, fine motor function-, and speech-related disturbances. Studies showed morphological and cytoarchitectural changes in the brain of schizophrenic patients, primarily focusing on the hippocampal, prefrontal and subcortical areas. The primary motor cortex (Brodmann's area 4) is largely connected with the latter regions and has a major role in the execution of movements. Our goal was to investigate any changes in the cytoarchitecture of this area.

Immunohistochemistry was carried out on post mortem samples of 5 schizophrenic and 5 control subjects, using SMI32 and parvalbumin (PV) immunostaining. SMI32 is the non-phosphorylated form of the Neurofilament H, mainly present in pyramidal cells of cortical layers III and V. In addition to the perisomatic interneurons, parvalbumin labels the giant motoneurons of layer V (Betz cells) as well. The cell density and size of the immunostained pyramidal cells, and the Betz cells separately, were investigated in our study. To exclude the possible effect of the medication, Sprague-Dawley rats were treated with antipsychotics chronically (4 weeks intraperitoneal haloperidol or olanzapine). Their primary motor cortex was checked for alterations using the same methods as in human samples.

The human data showed a decrease in cell size and -density of the giant motoneurons. Other pyramidal cells of the primary motor cortex did not show these changes in schizophrenia patients. The results of the animal model did not prove the effect of the chronic antipsychotic treatment on the pyramidal cells of the area, size and density of these cells were not altered. However, area of the striatal region of treated rats showed a remarkable enlargement. The electron microscopic analysis revealed degenerative changes of certain giant motoneurons in the schizophrenia samples. Numerous, asymmetric-like synapses were found on the surface of the giant Betz cells, suggesting an extraordinary perisomatic excitatory input.

Our results providing evidence about the sensitivity of the Betz cells in the human patients can further explain the impairment of the motor functions in schizophrenia.

Nucleus incertus inhibits hippocampal somatostatin-positive interneurons

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Different somatostatin (SOM)-positive interneuron types located in the stratum oriens/alveus of the hippocampus target different somatodendritic compartments of the hippocampal pyramidal cells and, therefore, they can specifically regulate distinct inputs of the pyramidal cells. Hippocampo-septal projection neurons give local collaterals to the proximal dendrites of the pyramidal cells, while also target septal neurons. Bistratified cells target the proximal dendrites of the pyramidal cells, while oriens-lacunosum moleculare (OLM) cells target their most distal dendrites and they innervate bistratified cells as well. These cells may be activated by septal cholinergic and glutamatergic cells, as well as by local pyramidal cells. However, the source of their inhibition that would help to maintain an appropriate level of activation balance is still unknown. The relaxin 3 positive cells of the nucleus incertus (NI), located in the rostral pons, project to the stratum oriens/alveus of the hippocampus. Although many of these cells are known to be GABAergic, a direct incerto-hippocampal GABAergic projection has not been described so far. Using anterograde tracing with adeno-associated viruses in a vesicular GABA transporter-Cre mouse line, we show that relaxinergic fibers arising from the NI are also GABAergic. Confocal and electron microscopic analysis of these fibers showed that they selectively target only interneurons in the str. oriens/alveus of the hippocampus, and SOM positive OLM and hippocampo-septal projecting interneurons are targeted as well. Furthermore, we found that their synapses contain GABAA receptors and their scaffolding protein gephyrin postsynaptically. In vitro electrophysiology combined with optogenetic stimulation of these fibers showed the inhibition of hippocampal SOM-positive interneurons and the attenuation of sharp-wave ripple events. Finally, in vivo physiology in freely-moving mice showed that the selective optogenetic activation of GABAergic cells of the NI significantly reduces hippocampal theta activity. These observations suggest that NI might play an important role in the regulation of hippocampal functions.

Alteration of the GABA-A receptor α1 subunit expression in different types of interneurons of the human epileptic hippocampus

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GABAergic inhibition is known to play a complex role in the pathomechanism of epilepsy. Beside the interneuron loss and fiber reorganization, other factors like changes in the subunit composition of certain transmitter receptors also contribute in the pathological changes affecting the function of the preserved interneurons. In this study we examined the changes of the expression of GABA-A receptor α1 subunit (α1) in different functional types of interneurons.

Surgically removed hippocampi of drug-resistant temporal lobe epileptic patients were examined and compared with control samples. We analyzed the density and synaptic

coverage of the α1-immunopositive interneurons. The coexpression of α1 and calcium binding proteins – parvalbumin (PV) and calbindin (CB) – in interneurons was studied using immunofluorescent double labeling.
In control, α1-positive cells were found throughout the entire hippocampus with largest densities in the CA1 region and the hilus. The density decreased in the hilus of patients with hippocampal sclerosis. In the CA1 region an increased density of immunoreactive cells was found, which may be partially explained by the tissue shrinkage. The percentage of α1-positive-neurons also expressing CB is reduced significantly in the sclerotic CA1. In the hilus, the percentage of α1-positive neurons containing CB increased, and significantly more CB-positive interneuron showed α1-expression. In contrast, a decreased amount of PV-positive cells expressed the α1 subunit in the hilus of epileptic patients. Electron microscopic examination showed that both asymmetric- and symmetric synaptic coverage of α1-positive dendrites was increased in the sclerotic epileptic samples, probably reflecting the sprouting of excitatory pathways and inhibitory cells.
These data suggest pronounced differences of co-expression of ?1 with interneuron-markers in sclerotic epileptic human hippocampal tissue, as well as regional differences between the hilus and CA1 region. Changes in the subunit composition can lead to an altered response to GABAergic inputs and influence the sensitivity to certain drugs.

Expression changes of mitochondrial enzyme citrate synthase in parvalbumin-immunostained inteneurons in the hippocampi of patients with temporal lobe epilepsy

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Dysfunction of mitochondria is well known in several neurological disorders, including different types of epilepsy. There is a limited amount of evidence about changes of mitochondrial enzymes in human epileptic samples, and the available data are controversial. However, citrate synthase being the first enzyme of the citrate cycle may provide information about the metabolic activity of mitochondria in epilepsy.
In our earlier studies we showed the differences of the citrate synthase immunostaining among hippocampal regions, i.e. the elevated density in the epileptic CA2 compared to the control tissue and the inhomogeneous staining patterns within specific subregions in epileptic samples, like CA3 and dentate gyrus. In the present study, we examined the citrate synthase expression in a perisomatic inhibitory interneuron type playing crucial role in the formation of epileptic activity, namely the parvalbumin-immunostained interneurons.
Surgically removed hippocampal samples from patients with drug resistant temporal lobe epilepsy have been examined. Post mortem control samples were obtained from subjects without any sign of neurological disorders. Double fluorescence immunohistochemistry was

used to assess the coexpression of citrate synthase and parvalbumin and the density changes of citrate synthase. Confocal microscopic analyses were carried out. The citrate synthase labeled mitochondria were counted in the hippocampal parvalbumin-positive interneurons. Citrate synthase-immunostaining was present only part of the mitochondria. Interneurons contained significantly more immunostained mitochondria than principal cells. The variation in distribution of citrate synthase-immunostaining of different regions and subjects will be discussed.
This study was supported by the OTKA, Hungary (NN 102802).

Resting-state EEG functional connectivity in newborn infants

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The organization of functional brain networks changes across human lifespan through the mechanism of neuronal growth and plasticity that may contribute to cognitive development. Our study is aimed to analyze functional brain networks by measuring neural synchrony in EEG recordings during quiet sleep of new-born infants. Based on graph theory, macroscopic network organization characteristics were quantified by constructing unweighted minimum spanning trees from EEG data. Clinical biological data corresponding to prenatal influences and general clinical risk factors in both mothers and children were also collected. The functional connectivity within theta and alpha oscillations were found to be strongly predicted by the length of pregnancy. In conclusion the present study demonstrated that the prenatal period functions as a time for maturation when brain functional networks became less centralized.

Comparative Connectomics: Mapping the Inter-Individual Variability of Connections within the Regions of the Human Brain

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The human braingraph, or connectome is a description of the connections of the brain: the nodes of the graph correspond to small areas of the gray matter, and two nodes are connected by an edge if a diffusion MRI-based workflow finds fibers between those brain areas. We have constructed 1015-vertex graphs from the diffusion MRI brain images of 395 human subjects and compared the individual graphs with respect to several different areas of the brain. The inter-individual variability of the graphs within different brain regions was discovered and described. We have found that the frontal and the limbic lobes are more conservative, while the edges in the temporal and occipital lobes are more diverse. Interestingly, a "hybrid" conservative and diverse distribution was found in the paracentral

lobule and the fusiform gyrus. Smaller cortical areas were also evaluated: precentral gyri were found to be more conservative, and the postcentral and the superior temporal gyri to be very diverse.

Synaptic organization of perisomatic GABAergic inputs onto the principal cells of the basolateral amygdala

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Spike generation is most effectively controlled by inhibitory inputs that target the perisomatic region of neurons. Despite the critical importance of this functional domain, very little is known about the organization of the GABAergic inputs contacting the perisomatic region of principal cells (PCs) in the basolateral amygdala (BLA). Here, we determined the number and sources of GABAergic inputs using immunohistochemical- and in vitro single-cell labeling at the light and electron microscopic level. We found that the soma and proximal dendrites of PCs were innervated primarily by two neurochemically distinct basket cell types expressing parvalbumin (PVBC) or cholecystokinin and CB1 cannabinoid receptors (CCK/CB1BC), and that individual PVBCs targeted PCs via more terminals than CCK/CB1BCs. The innervation of the initial segment of PC axons was found to be parceled out by PVBCs and axo-axonic cells (AAC), as the majority of GABAergic inputs onto the region nearest to the soma (between 0-10 μm) originated from PVBCs, while the largest portion of the axon initial segment was innervated by AACs. Detailed morphological investigations revealed that the three perisomatic region-targeting interneuron types significantly differed in dendritic and axonal arborization properties. We found that similar numbers (15-17) of the two BC types converge onto single PCs, whereas fewer (6-7) AACs innervate the axon initial segment of single PCs. Furthermore, we estimated that a PVBC and a CCK/CB1BC may target 800-900 and 700-800 PCs, respectively, while an AAC can innervate 600-650 PCs. Thus, BCs and AACs innervate approximately 10 % and 20 % of PC population, respectively, within their axonal cloud. Our results collectively suggest that these interneuron types are differently embedded into the local amygdalar circuitry in order to fulfill specific functions in network operation during various brain states.

The expression pattern of ECM molecules as a prognostic factor in glioblastoma

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Glioblastoma multiforme (GBM), the most common malignant disease of the central nervous system, has a very unfavorable prognosis with a median overall survival of 16-24 months. The main cause of the poor patient outcome is the extensive invasion of glioma cells to the neighboring parenchyma, causing local recurrence. The extracellular matrix (ECM) is a known to affect tumor invasion and different composition of the ECM in non-tumor brain and glioblastoma has been proven.

This research focuses on the expression level of 20 invasion-related ECM components in 26 GBM fresh-frozen samples using QRT-PCR and proteomic measurements. Expression data and patient survival was then analysed statistically.

It was found that the expression of a single ECM component cannot significantly influence patient survival, instead, the expression pattern of the invasion-related ECM components correlates with patients' survival. Statistical classifiers can distinguish patients with different survival efficiently (positive predictive value: 0.85 for mRNA, 0.89 for proteomic studies, ROC value: 0.775 and 0.875, respectively). In addition, the study has revealed the major role of certain ECM components in the development of invasive character of GBM. mRNA levels of brevican and integrin; protein levels of brevican, cadherin-12, integrin apha-3 besides laminin alpha-4 and beta-1 might be key components in glioma invasion.

Joint assessment of invasion-related molecules' expression creates/provides the invasion spectrum of the tumor that correlates with the survival of GBM patients. Using statistical classifiers permits the adoption of invasion spectrum as a considerably accurate prognostic factor while also gaining predictive information on potential molecular oncotherapeutic targets at the same time.

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No age differences in recovering from the sensory effects of distraction

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Old adults are often characterized as being more susceptible to distraction than young adults. The present study investigated the persistence of a distracted state induced by a rare, unpredictable, but task-irrelevant auditory event in a young (19 to 26 years) and old (62 to 74 years) adult group. We measured the amplitude of the N1 auditory event-related potential (ERP) elicited by task-relevant events following the distracter in various temporal intervals. Because the N1 indexing the sensory registration of auditory events reaches its maximum amplitude when the eliciting sensory event is in the focus of attention, this arrangement allowed to examine the time needed to recover from distraction. Participants listened to a continuous tone with rare, unpredictably occurring, brief pitch glides (glissandos – distracter events). The tone also featured short silent gaps and participants'

task was to press a button every time if they detected a gap. The temporal separation between distracter glides and target gaps could be 150, 250 or 650 ms, and gaps not preceded by other events in the previous at least 3400 ms were also presented. Although the overall N1 amplitudes were markedly lower for old adults, the normalized N1 amplitude functions – showing a decrease with decreasing distracter-target separation – did not differ between the two age groups. In summary, our results suggest that aging does not influence the recovering time from the sensory consequences of distraction.

Implication of microglial fractalkine receptor in hypothalamic control of metabolism

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There is an emerging role of glial cells in sensing and mediating of various homeostatic functions. Microglia, the resident immune cells in the CNS, are highly dynamic and closely associated with neurons and astrocytes. Our aim was to reveal the role of hypothalamic microglia in the regulation of energy homeostasis focusing on the state of negative energy balance (insulin-induced hypoglycemia).

C57Bl6/J mice were fasted overnight and sacrificed 1 hour after intraperitoneal insulin injection (1 IU/kg) in the next morning. Non-fasted and fasted control animals received vehicle injection. Quantitative and qualitative morphometrical analysis of Iba-1 stained serial hypothalamic sections of insulin-injected animals revealed significant activation of microglia in the medial basal hypothalamus, the area that has been implicated in glucose and insulin sensing and regulation of food intake and energy expenditure.

Using c-Fos based functional anatomical mapping strategy we have identified neurons in the hypothalamic arcuate region that became activated in response to insulin-induced hypoglycemia. Histological analysis of double stained material (c-Fos and Iba-1) at light microscopic level found these activated neurons to be surrounded by activated microglia after insulin challenge.

To explore the role of neuron-to-microglia communication in mediation of insulin-induced hypoglycemia, mice that are deficient in fractalkine signaling (CX3CR1gfp/gfp) were injected with insulin or vehicle after fasting. Fractalkine is an immunmodulatory chemokine, which has been shown to play important roles in metabolic disease in both animal models and humans. Within the CNS, fractalkine is synthesized by neurons and involved in the recruitment of microglial cells that possess fractalkine receptor (CX3CR1).

Fractalkine receptor deficient mice did not develop severe hypoglycemia following insulin as did wild type (C57Bl6/J background) littermates. By comparing insulin-induced c-Fos immunoreactivity in these mice, we found special subregions within the hypothalamic arcuate that became distinctly activated in response to insulin injection. Under resting conditions, the number and activation profile of hypothalamic microglia was not different in wild-type (C57Bl6/J) and CX3CR1gfp/gfp animals. However, in response to insulin-induced hypoglycemia, there was a transition shift of microglia from resting to activated morphological phenotype especially in the rostral and middle parts of the arcuate region

and at the level of median eminence in wild type animals. Furthermore, these activational changes in the rostral arcuate nucleus were much less expressed in mice with impaired fractalkine signaling than in the controls.
Our results highlight the role of microglia in general-, and fractalkine signaling in particular, mediating hypoglycemic challenges to the hypothalamic neuronal circuit that regulates energy homeostasis.

Presenter index (S: Oral session; T: mini poster table)