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REDUCED NUMBER OF SECRETAGOGIN-CONTAINING NEURONS IN THE DEVELOPING CORTEX IN DOWN-SYNDROME

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Brain developmental disorders can be due to cell migration deficit, a process which is characterized by a bewildering complexity of signaling molecules and factors. We have recently identified secretagogin, a calcium-sensor protein, in neurons of rodent rostral migratory stream and its human foetal equivalent where it regulates enzyme externalization to digest the extracellular matrix, thereby promoting forward neuroblast migration. We now explored a large population of secretagogin-containing cells in the developing cortex, where cells migrate in radial or tangential orientations in the different layers of the cortical plate.Of note, secretagogin is practically missing in the adult human neocortex. Secretagogin-expressing cells were numerous during midgestation but their density further increased at the early third trimester to robustly decrease before birth.In addition to this temporal scale, we analyzed both frontal and temporal lobes in spatial detail to find that secretagogin-immunoreactive cells were especially numerous in both the marginal and subventricular zones. In aborti with Down-syndrome, we identified a robust decrease of secretagogin-cell density, compared to controls, in the marginal zone and cortical platewith a preserved temporal profile throughout pregnancy.No significant changes were seen in either the temporal or the quantitative profile of secretagogin-distribution in the subventricular zone.When normalized to total cell number, however, we detected a relative secretagogin cell loss only in the cortical plate. We suggest that secretagogin-containing neurons are critical regulators of the human neocortex with a mechanism remaining to be explored.

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NEUROSERPIN EXPRESSION DURING HUMAN BRAIN DEVELOPMENT AND IN ADULT BRAIN REVEALED BY IMMUNOHISTOCHEMISTRY AND SINGLE CELL RNA SEQUENCING

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Neuroserpin is a serine-protease inhibitor mainly expressed in the CNS and involved in the inhibition of the proteolytic cascade. Animal models confirmed its neuroprotective role in perinatal hypoxia-ischaemia and adult stroke. Although neuroserpin may be a potential therapeutic target in the treatment of the aforementioned conditions there is still no information in the literature on its distribution during human brain development. The present study provides the detailed description of the changing spatiotemporal patterns of neuroserpin focussing on physiological human brain development. Five stages were distinguished within our examined age range which spanned from the 7th gestational week until adulthood. In particular, subplate and deep cortical plate neurons were identified as the main sources of neuroserpin production between the 25th gestational week and the first postnatal month. Our immunohistochemical findings were substantiated by single cell RNA sequencing data showing specific neuronal and glial cell types expressing neuroserpin. The characterization of neuroserpin-expression during physiological human brain development is essential for forthcoming studies which will aim to discover its function in pathological conditions, such as perinatal hypoxia-ischaemia and stroke in adult human.

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ESTIMATION OF VISUAL ACUITY USING STEADY-STATE VISUAL EVOKED POTENTIAL IN PRETERM AND FULL-TERM INFANTS

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It is well known that the latency of visual evoked potentials (VEPs) decreases during visual maturation. In case of checkerboard pattern stimulation, the stimulus pattern size that evokes the largest amplitude response is gradually shifted towards higher spatial frequencies. In the present study, we have assessed the electrophysiological visual acuity of healthy preterm (n=7) and full-term (n=18) infants using the steady-state VEP method at 10 Hz. The presence of significant responses was confirmed by T2circ statistics. We have determined the minimum separabile by plotting the amplitude of checkerboard pattern reversal evoked potentials as a function of check sizes (3.5'-60'), and then extrapolated the fitted regression line to the zero amplitude. In accordance with the previous literature,visual acuity improves intensively during the first few months after birthas indicated by decreasing minimum angle of resolution from 17' at 4 weeks to 4' by 4 months of age and approximates the adult value of 1' by 10 months of age. By analyzing the results of the preterm and full-term groups with respect to the postnatal and corrected ages (i.e. the age calculated from the expected birth date),we found similarityin both cases, thereforethe maturation of visual acuity is presumably influenced by genetic factors and visual experience as well.

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EVOLUTIONARY DIFFERENCES IN THE IMMUNOREACTIVITY OF GFAP IN VERTEBRATES

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From the first studies it was generally accepted that anti-GFAP reagents mono-orpolyclonalones, raised against human oranyothe mammalian GFAP react the GFAP of anyvertebrates, although the group of Stafford and Shehab already claimed that the results got in gold fish brains were improved when the immunizing agent was also fish brain. Our former reactions applying Boehringer monoclonal anti-GFAP were successful on chicken, turtles, caiman, carp, goldfish, sterlet, shark and skatebrains. Recent studies, however, revealed differences in the spectra of anti-GFAPs of different origins. Novocastra, eBioscience and BioSB anti-GFAP-s proved to be effective in the brains of, and several turtle, snake and lizard species, dwarf caiman *(Paleosuchus*) aswellas birds (chicken and pidgeon. These reagents, however, were not effective in fishes, representating either ancient types (bichir, sterlet, gar, eel and butterflyfish) ornewones (carp, cruciancarp, goldfish, bleak, bream, catfish, minnows, trout, pike, perch, pumpkinseedfish, cichlids). All the three anti-GFAPs were monoclonal, raised by mouse cells of Clone GA5. Oppositely, the polyclonal rabbit DAKO anti-GFAP ease effective in the fishes listed but not in the above mentioned reptiles and birds. In the case of Chondrichthyes (squalomorph and galeomorphsharks, skates and rays and theholocephalan Callirhinchus) there was no difference betwen the anti-GFAP reagents. These results suggest systematic differences in theGFAP epitopes in the different main clades of vertebrates.

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COMPARATIVE STUDY ON ASTROGLIAL ARCHITECTURES OF SELECTED BRAIN AREAS IN MORE AND LESS ADVANCED ACTINIPTERYGII

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Present study investigates the astroglial architecture in representatives of groups emerged before Teleostei (Brachyopterygii, Chondrostei, Ganoidei), older teleost groups (Osteoglossiformes, Anguilliformes), and newer groups (Ostariophysi: Cypriniformes, Siluriformes; Euteleostei: Salmoniformes, Perciformes) in the most . Astroglia was visulized with immunoperoxidase reacton against GFAP. In different brain regions characteristic astroglial architecture was recognizable. In the telencephalon the eversion, a characteristic feature of actinopterygian brains, resulted in a fan-shaped re-arrangement of radial glia in every species. In the cerebellum the granular and molecular layers were identified due to their plexiform and Bergmann-like glial systems. In the optic tectum the glial fibers were arranged radially, perpendicular to the surface and indicated layered structure. However, both the cerebellar and the tectal glial architectures showed species-specific features. Whereas in some species the Bergmann glia appeared as dense as in mammals, in others they were represented only by scarce fibers. In the optic tectum we also found two main types: i) the glial fibers spanned its whole thickness and a rich colleteral network was found; ii) the fibers were visible only in a subpial zone without rich side-branches; The layered structure was analysed with ImageJ program on 1-1 photos in 2-2 individuals of 6 species. The proportion of immunopositive areas in percentiles were measured in 6 equally wide arbitral zones in each photo. These preliminary results suggest that in actinopterygians the glial architecture has undergone considerable modifications during evolution in the most important brain areas (tectum, cerebellum).

CHARACTERIZATION OF FUNCTIONAL SUBGROUPS AMONG GENETICALLY IDENTIFIED CHOLINERGIC NEURONS IN THE PEDUNCULOPONTINE NUCLEUS

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The pedunculopontine nucleus (PPN) is a part of the reticular activating system which is composed of cholinergic, glutamatergic and GABAergic neurons. Early electrophysiological studies characterized and grouped PPN neurons based on certain functional properties (i.e. the presence or absence of the A-current, spike latency, and low threshold spikes). Although other electrophysiological characteristics of these neurons were also described, systematic assessment of these properties and correlation of them with morphological markers are still missing.

In this work, we conducted electrophysiological experiments on brain slices of genetically identified cholinergic neurons in the PPN. Electrophysiological properties were compared with rostrocaudal location of the neuronal soma and selected morphometric features obtained with *post hoc* reconstruction.

We found that functional subgroups had different proportions in the rostral and caudal subregions of the nucleus. Neurons with A-current can be divided to early-firing and late-firing neurons, where the latter type was found exclusively in the caudal subregion. Similar to this, different parameters of high threshold membrane potential oscillations showed characteristic rostrocaudal distribution as well. Furthermore, based on our data we propose that high threshold oscillations rather emerge from neuronal somata and not from the proximal dendrites.

We demonstrated the existence and spatial distribution of functional subgroups of genetically identified PPN cholinergic neurons, which are in accordance with differences found in projection and *in vivo* functional findings of the subregions. Being aware of functional differences of PPN subregions will help the design and analysis of experiments using genetically encoded opto- and chemogenetic markers for *in vivo* experiments.

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DIVERSITY OF DENDRITIC SPIKES UNDERLYING COMPLEX SPIKE BURSTING IN CA3 PYRAMIDAL CELLS

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Complex spike bursts (CSBs) represent a characteristic firing pattern of hippocampal pyramidal cells (PCs) and can provide instructive signal for new place field formation. CA3 pyramidal cells display location selective activity and often fire CSBs in vivo. The potential dendritic mechanism and synaptic input patterns underlying these events in CA3 PCs have not been well elucidated. For this purpose we used whole cell current-clamp recordings combined with two-photon calcium imaging and glutamate uncaging in CA3PCs in acute hippocampal slices. We found that the general propensity of CA3PCs to produce CSBs was much higher than that of CA1PCs, and we observed a large heterogeneity among CA3PCs in their bursting ability upon somatic or synaptic stimulation. The propensity of producing CSBs varied along the proximodistal and radial axis of CA3. HCN and K_v2 channels differentially regulated CSB propensity in different subregions of CA3. On the other hand, morphology of the dendritic arbor was only a weak indicator of electrophysiological phenotype.

Our results indicate that the synaptic and dendritic mechanisms generating CSB vary across PC types, and are regulated in a wide dynamic range in individual neurons, suggesting different roles of these events in location selective firing and ensemble dynamics of CA3 such as pattern separation and completion during associative learning and memory.

THE M-CURRENT IS A POTENTIAL SYNCHRONIZER OF MESENCEPHALIC CHOLINERGIC NEURONS

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The pedunculopontine nucleus (PPN) is a cholinergic part of the reticular activating system, which provides cholinergic and non-cholinergic fibers to several subcortical areas. Cholinergic PPN neurons also receive cholinergic inputs, which inhibits the M-current, a voltage-gated potassium current. In the present work, we investigated the presence, subunit composition and functional roles of the M-current in cellular, network and behavioral levels.

Cellular electrophysiological experiments were performed on midbrain slices and thalamus-midbrain blocks, as well as activity wheel test was done on KCNQ4 knockout mice and wild type littermates.

M-current was only present on the cholinergic neurons. Inhibition of the M-current decreased spike frequency adaptation, whereas M-current activators increased it. High threshold membrane potential oscillations were almost completely inhibited by blockade of M-current. M-current activators increased its activation threshold and slightly reduced its amplitude. Optogenetic activation of LDT cholinergic neurons led to M-current inhibition of the PPN cholinergic neurons. Paired recordings of uncoupled neighboring PPN cholinergic neurons revealed that the M-current inhibition decreases the level of spontaneous synchronization between them.

The activity cycles of KCNQ4 knockout mice changed in a significantly different way responding to changes of light-darkness cycles compared to wild type littermates.

One can conclude that the M-current is a hallmark of the cholinergic neurons in the PPN. The channel responsible for M-current is partially, but not exclusively, formed by KCNQ4 subunits. The M-current of PPN cholinergic neurons seem to participate in neuronal synchronization and thus in regulation of PPN activity and sleep-wakefulness cycles.

DIRECT RECORDINGS FROM SMALL STRUCTURES OF MOSSY FIBERS

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Hippocampal mossy fibers (axons of granule cells) are ideal models for investigating fundamental presynaptic functions because their exceptionally large axon terminals (LMFBs) are easily accessible to electrophysiological studies. However, the majority of mossy fiber boutons are much smaller (sMFBs); their size is in the range of the typical cortical excitatory axonterminals and they were unattainable for direct recordings. In addition to the differences in their size and morphology, LMFBs and sMFBscontribute differently to the synaptic output of dentate gyrus granule cells. Although the electrical behavior of the LMFBs is well characterized, generalization of that knowledge to the entire mossy fiber axon is difficult because of the bouton-level morphofunctional heterogeneity.

After optimization of the recording conditions, we managed to perform patch clamp recordings from sMFBs, LMFBs and from the axonal shaft and asked the question whether theirdifferent physiological functions are reflected at the level of their intrinsic signaling.

We found no major difference in the action potential shape along the mossy fiber. The activity-dependence of spike shapes were also surprisingly similar in the different mossy fiber elements,suggesting that spike-shape dynamics do not underlie the different short-term plasticity in mossy fiber boutons.

Furthermore, outside out patch recordings revealed substantial amounts of sodium and potassium currents in all types of mossy fiber structures, which support local action potential generation.

The similar action potential properties of the small and large mossy fiber compartments verify that even these fast properties can be reliable compared by direct recordings from small axonal structures.

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OREXINERGIC NEUROMODULATORY ACTIONS MODIFY OCCURRENCE OF SLOW INWARD CURRENTS ON NEURONS IN THE PEDUNCULOPONTINE NUCLEUS

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Orexins are neuromodulatory peptides of the lateral hypothalamus, which regulate important homeostatic mechanisms including sleep-wakefulness cycles. Orexinergic actions stabilize wakefulness by acting on nuclei of the reticular activating system (RAS) including the pedunculopontine nucleus (PPN). Orexinergic actions in the PPN on cellular level comprise of the development of a tonic inward current or depolarization; mediated by calcium- and mixed cationic conductances, as well as occurrence of noisy background currents and an increase of excitatory postsynaptic current frequency and amplitude.

It was previously shown that serotonergic, cholinergic and cannabinoid actions on the PPN can elicit various responses including depolarization and hyperpolarization. Independently from it, astrocyte-dependent and NMDA receptor mediated 'slow inward currents' (SICs) were regulated in a way related to the previous SIC activity.

In the present project, we investigated orexinergic neuromodulatory actions on SICs of PPN neurons and their relationships with tonic currents by using slice electrophysiology on preparations from mice. We demonstrated that -in contrast with several other neuromodulatory actions and in line with literature data- orexin almost always elicited a tonic inward current. Independently from the tonic currents, actions on SICs were also detected which resembled to other neuromodulatory actions: if SICs were almost absent on the neuron, orexin induced an increase of the charge movements by SICs, whereas if SIC activity was abundant on the neurons, orexin exerted inhibitory action on it.

This finding might strengthen the theory that an astrocyte-dependent neuromodulatory action exists in the PPN, which uniformly responds to several different actions and sets a certain low level of 'random' neural activity.

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LIPOXYGENATION OF THE ENDOCANNABINOID 2-AG IN SPINAL ASTROCYTES

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The endocannabinoid 2-arachidonoylglycerol (2-AG) inhibits neurotransmitter release in the spinal dorsal hornby activating type 1 cannabinoid receptor (CB1), and its effects are terminated by monoglycerol lipase (MGL), which yields arachidonic acid (AA). However, lipoxygenases (LOX) convert 2-AG and AA into proinflammatory leukotrienes. Since2-AG and leukotrienes induce calcium transients in astrocytes,a cannabinoid-leukotriene conversion in the spinal dorsal horn may influence neurotransmission and alsogliotransmission.

Here we investigated the lipoxygenation of 2-AG and AA in spinal glial cells, and its effect on astroglialcannabinoid signaling.

The expression of 5-LOX was confirmed in the spinal dorsal horn and alsoin cultured astrocytes.Leukotriene B4 and 2-AG induced calcium transients in astrocytes, but LTB4 proved to be more potent and more efficacious. Whereas the CB1 inverse agonist AM251 prevented 2-AG-induced calcium transients, the pan-LOX inhibitor NDGA increased calcium signals in astrocytes, andthe inhibition of LOX potentiated the effects of2-AG on the intracellular calcium concentration.Application of NDGA on spinal cords of GFAP-GCaMP transgenic mice also increased spontaneous calcium signals in astrocytes located in the superficial laminae.

Our results suggest that spinal glial cells express 5-lipoxygenase thatconverts AA and 2-AG into leukotrienes. Since the inhibition of this process increases astroglial calcium signaling, it is very likely that LOX plays role in the termination of 2-AG (and probably arachidonic acid) signalingin spinal astrocytes.

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SYNAPTIC COMMUNICATION BETWEEN PYRAMIDAL CELLS AND PERISOMATIC INHIBITORY CELLS IN THE MOUSE PREFRONTAL CORTEX

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Perisomatic inhibitory cells are in a position to effectively control the pyramidal cell activity. Despite the fact that numerous studies have investigated the microcircuit operation in the prefrontal cortex, a cortical region that plays a crucial role in higher-order cognitive functions, it is still largely unknown how the distinct subtypes of perisomatic inhibitory interneurons and pyramidal cells communicate with each other. Here, we examined the features and dynamics of synaptic communication between pyramidal cells and perisomatic inhibitory interneurons, namely, parvalbumin (PV)+ and cholecystokinin (CCK)+ basket cells andPV+chandelier cells in the prefrontal cortex.

Brain slices containing the prefrontal cortex were prepared from mice expressing EGFP and DsRed under the Pvalb and Cck promoter, respectively, and dual whole-cell recordings were obtained from fluorescent protein-expressing interneurons and pyramidal cells. First, we compared the passive and active membrane properties of interneurons and noticed significant differences in many single-cell features. Second, we compared the properties of uEPSCs and found that PV+ basket cells received larger and faster synaptic events than CCK+ basket cells from neighboring pyramidal cells. Third, we contrasted the uIPSC features and observed that PV+ basket cells gave rise to the largest synaptic events onto pyramidal cells. Forth, we examined the short-term dynamics of synaptic transmission and revealed target-selective differences.

Our results show that PV+ basket cells in the prefrontal cortex receive larger synaptic excitation and give rise to larger synaptic inhibition onto local pyramidal cells than the other two types of perisomatic inhibitory cells, suggesting that the three interneuron types contribute to circuit functionsin different manners.

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INPUT-OUTPUT FEATURES OF PERISOMATIC INHIBITORY INTERNEURONS IN THE MOUSE PREFRONTAL CORTEX

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Prefrontal cortex plays a pivotal role in several higher-order cognitive functions. Despite a significant number of studies that have been addressed to clarify the principles of neural operation in this brain region, its microcircuit organization is still poorly understood. Here, we examined the input-output features of two types of basket cells expressing parvalbumin (PV) or cholecystokinin (CCK) in the mouse prefrontal cortex.

Using whole-cell patch clamp technique, we recorded and intracellularly labelled interneurons expressing fluorescent proteins in slice preparations that were prepared from the prefrontal cortex of mice expressing EGFP and DsRed under the Pvalb and CCK promoter, respectively. This targeted patching allowed us to selectively investigate the features of the preferred interneuron types that were *post hoc* morphologically identified. We observed that the two basket cell types differed significantly in their morphological appearance. Cluster analysis distinguished two CCK-containing basket cell categories and three PV-expressing basket cell groups based on the dendritic and axonal arborization. Basket cells in all categories preferred to target the perisomatic region of pyramidal cells. In addition, we identified PV-expressing chandelier cells that morphological features differed from PV-expressing basket cells.

Our results suggest that the three perisomatic inhibitory cell types substantially differ in dendritic and axonal arborizations. The variability in the input-output features may support a distinct, layer-dependent function they play in circuit operation.

FUNCTIONAL EVIDENCE FOR INSULIN AND GLP1 ACTION ON NEOCORTICAL NEUROGLIAFORM CELLS

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Insulin is present in the healthy brain and disturbances in insulin signaling have been linked to aging, obesity, diabetes and Alzheimer's disease. Insulin and the incretin glucagon-like peptide 1 (GLP1) delivered to the brain is therapeutically promising against mild cognitive impairment and Alzheimer's disease. We showed previously that insulin is expressed and released from GABAergic neurogliaform cells in the cerebral cortex of the rat. In addition, insulin is known to regulate local microcircuits through the modulation of tonic inhibitory GABAergic currents.

First we asked whether the action of GLP1 receptors can be detected in insulin containing neurons. Functional expression of GLP1 receptors was confirmed with whole cell patch clamp electrophysiology showing reversible effect of GLP1 on neurogliaform cells.The effect of GLP1 on neurogliaform cells was prevented by pre-treatment with the GLP1 receptor specific antagonist exendin3(9-39) and was absent in hypoglycemia. Next we assessed insulin action in the inhibitory microcircuit and made paired recordings of postsynaptic layer 2/3 pyramidal cells and presynaptic neurogliaform cells. Application of external insulin increased the amplitude of neurogliaform to pyramid IPSCs leaving the paired pulse ratio unchanged. However, unitary inputs from fast spiking basket cells and regular spiking internerurons to pyramidal cells was unchanged upon insulin application.

We provide evidence for functional expression of GLP1 receptors in neurons known to release insulin in the cerebral cortex. Hyperglycemia activates the function of GLP1 receptors in neurogliaform cells suggesting that endogenous incretins and therapeutic GLP1 receptor agonists might have effects on these neurons similar to that of pancreatic beta cells. In addition, insulin arriving to cortical microcircuits rearranges inhibitory inputs to pyramidal cells by selectively enhancing the effect of neurogliaform cells.

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ECCENTRICITY DEPENDENCE OF ELECTRICAL SYNAPSE DENSITY IN THE CAT RETINA

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Introduction: The functional roles of electrical synapses have been studied extensively at the local level but it is not known how their contribution to neuronal inputs changes across the retina.
Objective: We hypothesized that if the relative contribution of electrical synapses remains constant across the retina, their density should decrease with eccentricity together with the densities of most cell types.
Methods: As a test, we mapped the expression pattern of the major neuronal gap junction protein, connexin-36 across the retinal layers and at a range of eccentricities (n=20) using fluorescence immunohistochemistry in the cat retina.Areal density and volume distribution of connexin-36 plaques were measured using Imaris and Fiji image processing tools.
Results: Connexin-36 plaques were found in both synaptic layers in all retinal areas, with the highest density in the ONsublamina of the inner plexiform layer.Here, plaque density decreased with eccentricity (r=–0.65; p=0.002) together with the density fall-off of AII amacrine cells, which weidentified by their calretinin positivity. No significant correlation was found in other layers. Furthermore, the mean plaque volume was correlated with eccentricity (r=–0.70; p=0.001)only at the level of the inner nuclear layer, where AII amacrine cells are known to be connected by electrical synapses.
Conclusions: We identified two patterns of gap junction distribution in the retina: one that is eccentricity dependent and is linked to AII amacrine cells and another, eccentricity independent pattern. The latter pattern suggests that in certain retinal networks, the number of electrical synapses per cell increases with eccentricity.

INHIBITING INNER RETINAL SIGNAL TRANSMISSION RESULTS IN SUSTAINED-TO-TRANSIENT SWITCH IN RETINAL GANGLION CELL LIGHT RESPONSES

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As the sole action potential producing neurons in the retina, retinal ganglion cells (RGCs) are responsible for collecting and forwarding information gathered from the visual field towards the brain through the optic nerve. Considering the many possible aspects (e.g. contrast, colour, movement) this visual code carries, the understanding of ganglion cell firing patterns and the underlying mechanisms becomes markedly important. In this study, we examined the temporal characteristics of RGC light responses with an emphasis on response transiency. We recorded the electric activity of mouse RGCs using a microelectrode array while we interfered with inner retinal synaptic transmission by blocking GABAergic inhibition (picrotoxin, PTX) and gap junction mediated signalling (meclofenamic acid, MFA). We found that inhibiting GABA-erg transmission and blocking gap junctions can both affect the light response of RGCs, with said effects most commonly resulting in a significant decrease of response duration that reduces transiency values and causes a switch from sustained to transient characteristics. This suggests that inner retinal connections (especially between amacrine and ganglion cells) are likely to be the most important factor in determining response transiency, contrary to previous suppositions.

LOSS OF INHIBITION TURNS PHYSIOLOGICAL HFOS INTO PSEUDO-SYNCHRONOUS PATHOLOGIC HFOS

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Sharp wave associated ripples represents the highest frequency (~200 Hz) oscillatory activity in the healthy hippocampus, while pathological fast ripples during epileptiform events appears at even greater frequency (250 Hz<) during epileptic population events. Here we explored the different underlying mechanism of the generation of physiological ripples (RIP) and pathological fast ripples (fRIP).

Using focal pharmacological interventions, we show that during RIPs inhibition is intact and synchronized IPSPs contribute much more to local field potential (LFP) generation than population spikes of synchronously firing cells. During fRIPs inhibition is compromised and only synchronized pyramidal cell action potentials contribute to LFP generation.

Dual LFP and loose-patch paired recording of PV+ basket cells (PVBC) and pyramidal cells revealed that: 1) the firing of PVBCs loses its ripple synchronization when network activity shifts from RIP to fRIP generating state, 2) pyramidal cells starts to fire stereotypic bursts when PVBCs stop firing at the peak of fRIPs due to depolarization block, 3) loss of synchronizing inhibition is accompanied by the loss of multiunit correlation and HFO wavelet coherence among multiple LFP recording locations, 4) finally we show that pyramidal cells have highly stereotypical burst pattern during fRIPs events and their firing starts with high temporal precision when inhibition collapses.

In summary, while during RIPs inhibition organizes synchronous firing among distant locations, fRIPs emerge when perisomatic inhibition, and thus inhibitory synchronization collapses and pyramidal cells start to fire their stereotypic bursts at the same time, resulting in pseudo-synchronous activity.

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GRID-LIKE HEXAGONAL PATTERNS OF DENDRITIC BUNDLES IN THE RODENT ENTORHINAL CORTEX

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How this geometrically aligned firing pattern arises is still a continuous debate. One entorhinal cortex specific cytoarchitectonic feature is the patch-like islands in layerII, which has been recently theorized to play a major role in forming grid-cell firing.
In our present study, we have imaged the entire entorhinal cortex in CLARITY treated brains and detected the location of stellate, layerII and layerIII pyramidal cells both in mice and rats. We found that cell islands occur only in the most dorsal part of the MEC and only in mice. However, patchyness arises from dendritic bundles of both layerII and layerIII pyramidal cells. With the help of light and electronmicroscopical techniques we also revealed that the patches of layerIII apical dendrites receive excitatory inputs from the presubiculum. The presubical axons largely prefer the spines of layerIII pyramidal cells over the layerII pyramidal cells. Importantly, presubiculum conveys both head-directional and grid-cell firing information, therefore we suggest that the grid-cell firing in layerIII might be formed in a parallel manner with the layerII grid-cells.

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GLUCOSE-MONITORING NEURONS IN THE CINGULATE CORTEX OF THE RAT. - MICROELECTROPHYSIOLOGICAL STUDY

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In the present experiments, we aimed to characterize the responsiveness of glucose monitoring (GM) neurons in the anterior cingulate cortex (ACC) of anesthetised adult male Wistar and Sprague-Dawley rats. Extracellular single neuron activity changes were recorded by means of multibarreled glass microelectrodes to a) microelectrophoretic application of glucose and neurochemicals (glutamate, noradrenaline, dopamine), b) intraoral gustatory stimulations c) intragastric infusions, and to d) intraperitoneal glucose loads. During these experimental sessions, the actual blood glucose levels where constantly measured by handheld semiautomatic glucometer.

Approximately eleven percent of the the tested neurons responded to microelectrophoretical administration of D-glucose, and both facilitatory (glucose-receptor, GR, 80%) and inhibitory (glucose-sensitive, GS, 20%) neurons were identified. Twice as many GM cells, compared to the non-responsive (glucose-insensitive, GIS) neurons, changed their discharge rate to microiontophoretically administered chatecholamines. The activity changes of cingulate cortical GM neurons were slower but more lasting, and the responsiveness greatly depended on the momentary blood glucose level, which phenomenom was furher confirmed during the intraperitoneal glucose administrations. The GM cell taste responsiveness of was higher than that of the GIS units. 20% from all of the tested neurons displayed distinct responses to intragastric infusion of chemicals.

This uniqe responsiveness of GM neurons to microelectrophoretically administered D-glucose so far was not observed in other GM neuron containing brain areas, thus, this finding suggest an intimate and complex involvement of the cingulate cortical chemosensory neurons in the organization of feeding and metabolism associated regulatory processes.

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PKD CONTROLS ENDOCYTIC AMPAR TRAFFICKING IN THE SYNAPTIC MEMBRANE

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The protein kinase D (PKD) family of serine/threonine kinases affects dendritic development and maintenance, intracellular transport as well as the modulation of transmembrane receptors. Earlier, we have shown that inhibition of PKDimpairs spatial memory formation and reduces LTP in CA1 hippocampal neurons in mice. In the present study, we investigateda potential role of PKD in the regulation of AMPA receptor turnoverin the synaptic membrane.

We used embryonic hippocampal neuronal cultures of CD1 miceand blockedPKD activity usingthe selective inhibitor CRT0066101. Surface expression of the AMPAR subunit GluA1 was assessed throughsurface biotinylation assays and Western Blot analysis upon agonist-induced internalization. Short-term inhibition of PKD activity significantly increased the amount of GluA1on the cell surface indicating that PKD is required for proper endocytic turnover of GluA1.This finding was further corroborated by antibody feeding experiments followed by quantitative microscopicevaluationwithin the PSD of dendritic spines.

To test the dynamics of PKD-mediated AMPA receptor loss from the synapses, we expressed super-ecliptic pHluorine tagged GluA1 (SEP-GluA1) in the neurons. Photobleaching SEP-GluA1 in the dendritic spines of living cells followed by the analysis of fluorescence recovery revealed that inhibition of PKD activity significantly increased the recovery half time of the surface GluA1 signal.

Taken together our results indicatea role for PKD in promoting activity-dependent endocytosis of AMPA receptors.

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ION-CHANNEL AND RECEPTOR SUBUNIT DIFFERENCES BETWEEN FAST SPIKING AND PYRAMIDAL CELLS OF PREFRONTAL CORTEX

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Deep sequencing of fast spiking and pyramidal cells of the prefrontal cortex (PFC) after physiological verification of cell types resulted 19.000 transcripts (20 million reads, more than 100-fold coverage, 84 cells). All harvested cells were tested in a step gradient depolarization paradigm and the shape and location of the cells in the PFC layers were monitored. Because of the large number of “0” values in the sequencing results, we developed a normalization process particularly adjusted to the single cell transcriptomics data and selected at least tenfold differences in the transcriptomes between the two classical cell types. Bioinformatics analysis revealed such high differences in ion-channel and receptor subunits. Since the transcription level differences of the mRNA of a particular protein indicate protein level differences as well, we assume that some of the highly transcribed ion-channels might be novel targets for antidepressant development. This is supported by the fact that the classical cell-type specific markers Vglut and Gad1/2 showed elevated transcription levels in their respective cells.

In summary, we demonstrated that single cell sequencing of electrophysiologically selected and morphologically intact neurons is useful in novel and cell-type-specific drug target discovery that is envisioned to support drug discovery efforts for treatment of mental illnesses.

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NOXIOUS STIMULATION EXCITES BOTH PRINCIPAL NEURONS AND INTERNEURONS IN THE BASOLATERAL AMYGDALA COMPLEX IN VIVO

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During Pavlovian fear conditioning animals learn to associate a neutral cue with an aversive unconditioned stimulus. In spite of the fact that this paradigm is one of the most studied learning processes, our knowledge is very limited about how aversive stimuli like electrical shocks affect single-cell activities. Here, we examined the effects of noxious stimuli on the firing of neurons in the basolateral amygdala and tested the involvement of cholinergic receptors in the change of neuronal activity.

Using juxtacellular recordings followed by labeling of recorded neurons in anesthetized mice, we investigated the alteration of firing rate of *post-hoc* identified neurons caused by noxious stimulation. We observed that electrical stimulation delivered to the paw increased the spiking rate in the majority of principal neurons as well as fast spiking interneurons. Next, we tested the involvement of cholinergic receptors in the electrical shock-induced changes of the firing. We found that in two out of 6 cases the local application of a cholinergic receptor antagonist eliminated the shock-related increase in spiking. Lastly, using optogenetics in amygdala-containing slice preparations we determined that excitation of cholinergic fibers caused outward currents in a portion of principal neurons, while inward currents in a group of fast spiking interneurons.

Our data show that noxious stimuli mainly excite neurons in the basolateral amygdala and this effect, at least in a group of fast spiking interneurons, can be mediated by cholinergic receptors. Thus, our results suggest that aversive information may be conveyed into the amygdala circuits through cholinergic afferents.

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SPROUTING OF PARVALBUMIN-IMMUNOREACTIVE AXONS IN THE DENTATE GYRUS OF TEMPORAL LOBE EPILEPSY WITH DIFFERENT ETIOLOGIES

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Temporal lobe epilepsy (TLE) is often related to hippocampal sclerosis (HS), malformation of cortical development (MCD), although infew cases, MRI cannot detect morphological alteration. Previous studies have showna decreasednumber of parvalbumin-immunoreactive (PV-IR) cells in HS, however, the number of axo-somatic synapses wasnormal. In our present study, we examined PV-IR cells and axons in the dentate gyrus (DG) of surgically resected tissues of therapy-resistant TLE patients with different etiologies.Based on MRI, HS, HS+MCD, MCD, and MR-negativepatient-groups were formed. Neurons expressing PV and NeuNwere examined with light and transmission electron microscopes (TEM).

PV-IR cells were observed mostly in subgranular location in the dentate hilus in controls, in MCD and inMR-negative cases. In HS and HS+MCD groups, a decreasednumber ofhilar PV-IR cells and an increased number of ectopic PV-IR neurons were detected in the dentate molecular layer. In HS and HS+MCDgroups an extensive networkof PV-IR axons were visible in the molecular layer, probably due to sprouting. Sprouting of PV-IR axons were frequently observed in patients with infant febrile seizure. No significant correlation was observed between sprouting of PV-IR axons and location of PV-IR cells, and with dispersion of granule cells. TEM examinations proved that PV-IR axon terminalsterminatein the molecular layer on distal dendritic shafts and spines of granule cells.Our results indicate alteration of target profile of PV-IR cells in HS, and the role of infant febrile seizure in the pathomechanism of this process.Support: NAP2.0 (2017-1.2.1-NKP-2017-00002), 20765-3/2018/FEKUTSTRAT.

NEUROTROPHIN RECEPTOR DYNAMICS IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common neurodegenerative disorder but its mechanism is still unknown. Changes in the receptor dynamics on the plasma membrane are essential to receptor function. However, whether receptor dynamics are affected in disease conditions is unexplored. In our study we applied a novel approach to investigate the neurotrophindynamics in AD.We measured diffusion parameters ofneurotrophin receptors in human living neurons usinghuman induced pluripotent stem cells (hiPSCs) derived from PSEN1 mutant AD patients and single molecule detection technology.

We have found that $p75^{NTR}$ dynamics is different in AD and healthy neurons, while the dynamics of TrkA receptors are unchanged($D=0.2\ \mu m^2/s$). The diffusion coefficient of $p75^{NTR}$ was significantly smaller in AD neurons compared to control($D_{control}=0.18\ \mu m^2/s$; $D_{AD}=0.28\ \mu m^2/s$). We have also found that $A\beta_{1-42}$ administration increased the diffusion coefficient in control hiPSCs ($D=0.58\ \mu m^2/s$). Our results suggest that $p75^{NTR}$butnotTrk Asignaling has a significantrole in AD and it is unlikely that this difference is due to toxic Aβ accumulation.

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EXAMINATION OF THE OXIDATIVE STRESS AND THE TUMOR NECROSIS FACTOR (TNF)-A-PRODUCTION IN HEPATIC ENCEPHALOPATHY

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Hepatic encephalopathy (HE) is one of the most serious complications of liver disease or portosystemic shunt. Hyperammonaemia, manganese, bacterial lipopolysaccharides (LPS) and oxidative stress represent the major etiopathogenetic factors in HE. Cerebral oxidative stress and neuroinflammation have key role in HE, but the precise mechanism is not fully understood. While oxidative stress induced by ammonia and manganese is mainly attributed to astrocytes, neuroinflammation is primarily caused by microglia, however astrocytes are also able to produce cytokines. The separate examination of these two cell types is needed, however most in vitro models are not suitable to perform it. In our experiments, the HE-relevant factors (ammonia, manganese, LPS) were examined regarding to both the oxidative stress and the TNF-α-production of astrocytes in two distinct in vitro HE models. Primary rat astrocytes were purified by eliminating the microglia either with a special chemical treatment or with shaking of the cultures. Absence of microglia was confirmed by immunocytochemistry. Having treated with the above factors, TNF-α-production and the oxidative state was assessed by ELISA and a fluorescent method, respectively. Our results showed that only the manganese was able to cause significant oxidative stress in astrocytes. TNF-α-production was significantly increased in neither of the in vitro models. Our findings dispute other results regarding to the oxidative stress in HE and suggests that astrocytes do not contribute to the TNF-α-production during HE.

HUMAN INDUCED PLURIPOTENT STEM CELLS INDUCE MORPHOLOGICAL AND FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY

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Spinal cord injury results in irreversible tissue damage followed by very limited recovery of function. In this study we investigated whether transplantation of human induced pluripotent stem cells (hiPSCs) into the injured rat spinal cord is able toinduce morphological and functional recovery.

hiPSCswere grafted intraspinallyor intravenously one week after a thoracic (T9) spinal cord contusion injury performed in Fisher 344 rats. Control animals underwentcontusion injury without hiPSC transplantation. Locomotor analysis of the injured animals was performed by BBB-test and our detailed kinematic analysis system. Two months after the transplantation the retrograde tracer Fast Blue (FB) was applied distal to the injury to determine the extent of axonal sparing/regeneration.

Grafted animals showed significantly better functional recovery after contusion injury. Morphologically, the contusion cavity was significantly smaller in grafted animals than in controls. The amount of spared white matterwas significantly greater in grafted animals. Retrograde tracing studies showed statistically significant increase in the number of FB-labelled neurons in different segments of the spinal cord, in the brainstem and in the sensorimotor cortex.The extent of regeneration and functional improvement was inversely related to the amount of chondroitin-sulphate molecules around the cavity and to the astrocytic and microglial reactions in the injured segment.

These data suggest that grafted hiPS cells are able to induce morphological and functional recovery after spinal cord contusion injury.

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INVESTIGATION OF POTENTIAL NEUROANATOMICAL CHANGES IN SCHIZOPHRENIA-LIKE WISKET RATS

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Schizophrenic patients,besides the well-known behavioral alterations, show several neuroanatomical and morphological abnormalities in the brain including the reduction of gray matter in many brain regions. To enhance the face validity of a schizophrenia rat model (Wisket), control and Wisket brain samples were histologically analyzed.
Two experimental groups of male rats were studied: naive WISTAR rats without any treatments (n=3) and WISKET rats (n=3) after postweaning social isolation and subchronic ketamine treatment for 15 days at the age of 4-7 weeks. At the age of 5 months Chresyl-violet staining was performed on frozen cut coronal brain sections. The slides were photographed with light-microscope (Zeiss) equipped with image-capture software. Images of sections were analyzed using ImageJ. At given anatomical levels cortical thickness, area of the hippocampus and brain ventricles were measured. Quantitative analysis was also applied regarding the extent of the lateral ventricles and structure of the hippocampus.
The Wisket animals showed decreased cortical thickness in the prefrontal regionandstructural alterations of the pyramidal layer in the CA3 hippocampus region was also observed. At the level of the anterior commissure the enlargement of the lateral ventricles was also revealed in Wisket rats.
The histological analysis confirmed the similar neuropathological alterations observed in schizophrenic patients. These results enhance the face validity of our schizophrenia animal, thus Wisket rat substrain seems to be an appropriate model for simulating either behavioral or morphologicalalterations.

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CHARACTERIZATION OF MOLECULAR CHANGES IN THE DORSOMEDIAL PREFRONTAL CORTEX OF SUICIDE VICTIMS

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Recent studies revealed that two human regulatory brain systems, the resting state (RSN) and salience networks (SN) are critical elements in psychiatric disorders, especially in mood disorders. Different lines of investigations indicate the involvement of neurotrophins and other neuroplastic molecules in depression and suicidal behavior. To address the involvement of these genes within RSN and SN networks, their levels were determined in relation to depression in suicide victims in different compartment of these two networks. Among the examined cortical regions, the dorsomedial prefrontal (DMPFC), frontopolar and temporal cortices were selected for further examination. Subjects were assigned to three groups: normal control, depressed suicide victims, and suicides without any known signs of chronic depression. We found that the mRNA levels of brain-derived neurotrophic factor (BDNF) and coronin-1A (CORO1A) were increased, while the expression of glial fibrillary acidic protein (GFAP) was reduced in the DMPFC, frontopolar and temporal cortices of suicide victims. The mRNA level of calcium/calmodulin-dependent kinase II (CamkII) was elevated in the DMPFC, but reduced in frontopolar and temporal cortices of suicide victims. Significant differences were found in the expression of tropomyosin receptor kinase B (TrkB) and glucocorticoid receptor (NR3C1) in the DMPFC of depressed and suicide victims compared to control. These data suggest the involvement of these genes in depression, specifically, their roles in the DMPFC, one of the key member of the RSN network.

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A STRUCTURAL EVALUATION OF SOME KYNURENIC ACID ANALOGUES AND THEIR ELECTROPHYSIOLOGICAL EFFECTS

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A tryptophan metabolite kynurenic acid (KYNA), as an excitatory amino acid receptor antagonist is an effective neuroprotective agent in the case of excitotoxicity, which is the hallmark of brain ischemia and several neurodegenerative processes. However, its use as a neuroprotective agent is limited because it rarely crosses the bloodbrain barrier (BBB). This is why BBB-penetrant KYNA derivatives became the focus of research. During the past fifteen years, our research group has developed several neuroactive KYNA derivatives, some of them proved to be neuroprotective in preclinical studies.

In this study, an evaluation of some neuroprotective derivatives with divergent molecular characters is presented, together with their most typical effects on a monosynaptic transmission in the CA1 region of the hippocampus of rat. Their effects on the basic neuronal activity (on the field excitatory postsynaptic potentials: fEPSP) were studied using in vitro hippocampal slices in physiological and ischemic circumstances, respectively. KYNA and its some derivatives (SZR-72, SZR-105) in 200 μM concentration proved to be inhibitory, while other derivatives (SZR-73, SZR-104) with the same concentration had the opposite effect on fEPSPs. Interestingly enough, KYNA has a Janus-faced effect on fEPSPs; in the micromolar range it has an inhibitory effect, while in nanomolar concentrations it has a facilitatory effect. Derivatives with facilitatory effects (in 200 μM) were able to delay significantly the total loss of synaptic transmission in ischemic circumstances. Ideas on possible relations between molecular structures and their physiological effects are discussed.

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LESION-INDUCED SECRETOME OF NEUROECTODERMAL STEM CELLS PROMOTES FUNCTIONAL RECOVERY AFTER SPINAL CORD IN JURY

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Spinal cord injury results in irreversible tissue damage followed by limited recovery of function. We have earlier proved that grafted cells of the neuroectodermal stem cell line NE-GFP-4C induces significant functional recovery supported by neuroprotection and extensive axonal regeneration. The grafted cells produced GDNF, IL-6, IL-10 and MIP-1a. We hypothetise that intraspinaldelivery of these factors into the injured spinal cord may be able to induce as good morphological and functional recovery as the grafted neuroectodermal stem cells.

A contusion injury was performedat the level of Th11vertebra. The delivery of secretome started 7 days after injuryeither via osmotic pumps or transfected fibroblast directly into the lesion cavity. Animals in the control groups received saline or fibroblast. In the positive controls NE-GFP-4C cells were grafted intraspinally. Locomotor analysis of the animals was performed through the use of the BBB-test and a detailed kinematic analysis system. The extent of supra- and propriospinal axonal sparing/regeneration was determined by retrograde tracing with Fast Blue.

The factors adminestered through the use of osmotic pump or transfected fibroblast induced significantly improved functional recovery compared to controls. Larger number of retrogradely labelled neuronsfound in the treated animalssuggestedimproved axonal connections. The extent of the contusion cavity andthe amount of spared white and graymatter were significantly greater in the treated cords. These results suggest that the secretome-based treatment is a promising putative therapeutic approach to treat spinal cord injury.

ANOXIC DEPOLARIZATION INDUCES GLUTAMATE EXCITOTOXICITY AND CAUSES CELL DEATH IN RAT BRAINS LICES

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Glutamate excitotoxicity and cellular calcium overload are the central mechanisms of neuronal damage after ischemic stroke. Anoxic depolarization (AD) induces extensive extracellular glutamate elevation and serves as the hallmark of ischemic cell death. Our aim was to examine the role of NMDA receptors in AD evolution and the AD-related excitotoxic cell damage. AD was induced by oxygen-glucose deprivation (OGD) in rat coronal brain slices (350 µm, n=13). AD occurrence was confirmed by simultaneous monitoring of local field potential (LFP) and extracellular glutamate concentration using enzymatic biosensors. NMDA receptors were pharmacologically blocked by a non-competitive NMDA antagonist; MK-801 (100 µM). The AD caused infarct volume was determined by using triphenyl tetrazolium chloride (TTC) staining.

MK-801 treatment decreased AD amplitude (9.30 ± 0.54 vs. 15.62 ± 5.08 mV; MK-801 vs. control). In contrast, MK-801 enhanced the glutamate peak in response to AD (95.12 ± 78.8 vs. 71.35 ± 40.7 µM; MK-801 vs. control) and delayed AD onset (807.26 ± 294.1 vs. 497.75 ± 151.67 s; MK-801 vs. control). Furthermore, NMDA antagonism enhanced OGD-related infarct volume (99.98 ± 0.01 % vs. 58.67 ± 36.78 % MK-801 vs. control).

Taken together, NMDA receptor blockade failed to prevent AD occurrence or to reduce the profound glutamate release during AD. Based on these findings, we propose that alternative NMDA receptor independent glutamate release mechanisms operate in brain ischemia. The complexity of the pharmacological profile of glutamate excitotoxicity thus warrants further research.

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TNF-αPRETREATMENT OF EMBRYONIC SPINAL CORDS IMPROVES THE OUTCOME AFTER VENTRAL ROOT AVULSION IN JURY

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Transplantation of embryonic spinal cord into a motoneuron depleted spinal cord induces reinnervation of the denervated muscles by the host and graft motoneurons. Here we studied if TNF-α pretreatment of the spinal cord grafts is able to improve the outcome of the transplantation procedure.

Embryonic spinal cord pieces, derived from 13-day-old SD-GFP rat embryos were grafted into the spinal cord of adult female Sprague-Dawley rats. Animals received untreated or TNF-α pretreated grafts after lumbar 4 ventral root avulsion and reimplantation. Control animals had only their ventral root avulsed and reimplanted.

Electromyography proved that grafted animals displayed significantly improved locomotor parameters compared to the controls. Moreover, the ankleflexor muscles (eg. extensor digitorum longus – EDL) and the extensor (soleus) muscle showed improved coordination and higher numbers of motor unit action potentials (MUAPs) in animals that received TNF-α pretreated grafts as compared with the animals grafted with untreated embryonic tissue. Retrograde labelling from the EDL muscle showed significantly greater numbers of reinnervating motoneurons in both grafting paradigms compared to the control animals, without significant difference between animals that received TNF-α pretreated or untreated grafts. Grafts received abundant serotonergic innervation likely to contribute to the coordinated movement pattern seen in these animals. Anterograde tracing applied both into the graft or the host spinal tissue revealed strong reciprocal connections between the graft and the host cord.

The study proved that the grafted embryonic tissue is able to integrate both morphologically and functionally into the host environment and TNF-α treatment improves the integration of grafts.

SYNAPTIC FUNCTIONAL DISTURBANCES LEADING TO C1Q DEPOSITION IN HEALTH AND DISEASE

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Mounting evidence supports the role of the microglia-derived local complement system in selective elimination of synapses. This mechanism of synaptic pruning relies on the deposition of C1q to particular synapses that triggers their selective microglial engulfment via a yet not fully deciphered mechanism. Complement-dependent synaptic pruning is fundamental during early brain ontogenesis, and possibly in adult synaptic plasticity. Overactivation of the microglia-complement axis has been repeatedly implicated in neurodegenerative and neuropsychiatric disorders ranging from Alzheimer's disease to schizophrenia. To get a better insight into the functional impairments underlying synaptic C1q-tagging, we employed an unbiased proteomic strategy. Fluorescence-activated sorting of C1q-tagged and untagged synaptosomes from healthy mice and a mouse model of Alzheimer's disease were conducted followed by proteomic comparisons. Our results pointed out the role of synaptically activated, local apoptotic-like processes in C1q-tagged synapses that was verified on human brain specimens as well. Triggering of the apoptotic-like machinery and the concomitant synaptic C1q deposition could beelicited by lower synaptic strength as we demonstrated in a model of sensory deprivation. We unveiled excessive mitochondrial oxidative stress in C1q-tagged synapses in the mouse model of Alzheimer's disease. Moreover, we observed a striking enrichment of septin proteins,which are known to isolate individual dendritic spines via creating a molecular barrier. Overall, our results revealed the importance of apoptotic-like processes in synaptic C1q deposition and gave insights into the functional impairments that characterize the tagged synapses in neurodegeneration. This work was supported by grants

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EVALUATION OF ELECTROPHYSIOLOGICAL DATA OF KAINATE INDUCED CHRONIC EPILEPTIC EVENTS IN MICE

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Interictal spikes are a common type of neuronal activity registered in epilepsy, believed to be caused by cells in healthy brain tissue surrounding the epileptic focus. Interictal spikes are first observed during epileptogenesis and then between spontaneous seizures. Because of their increased prevalence prior to seizures, they were considered to hold a predictive function, but recent findings also link them to cognitive impairments, a defining characteristic of the disease. Despite this significant finding, albeit being highly investigated, the mechanisms behind interictal spikes are still largely unknown. Our aim is to investigate the network activity behind interictal spikes and to identify the cell types that generate it using a combination of *in vivo* two-photon calcium imaging and electrophysiology. In order to accomplish this, we used the kainate model of temporal lobe epilepsywith cortical kainate microinjection to make two-photon imaging more suitable. Here we present our electrophysiological findings of the epileptiform events following kainate treatment.Four to six weeks after injection, an epidural electrode and local measuring electrodes were implanted into the mice. In the recorded ECoG and LFP data, interictal-like epileptiform activity and seizure-like events were observed. Based on our preliminary results, we found a correlation between the kainate induced spontaneous chronic epileptiform events and the dose of the kainate treatment; these results were also supported by immunohistochemical analysis. Our results demonstrate the suitability of cortical kainate injection to investigate interictal spikes and also lay the foundation for subsequent two-photon imaging measurements.

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MODELLING THE NEUROPATHOLOGY OF MUCOPOLYSACCHARIDOSIS TYPE II WITH DISEASE-SPECIFIC HUMAN INDUCED PLURIPOTENT STEM CELLS

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Mucopolysaccharidosis II (MPS II) is a lysosomal storage disorder with progressive multisystem involvement and limited life expectancy, caused by impaired function of the iduronate 2-sulphatase (IDS) enzyme. Knowledge about its neuropathology is limited due to the unavailability of human material, although the neural symptoms are currently incurable. Therefore, our aim was to establish an *in vitro* human model to study its central nervous system-related pathomechanism and to provide a platform to screen alternative therapeutic products. The disease phenotype was confirmed in the established MPS II-iPSC lines and their differentiated counterparts by IDS enzyme activity and quantitative glycosaminoglycan assay. In the presence of mitogens, MPS II NPCs showed significantly decreased self-renewal capacity, although, their cortical neuronal differentiation potential was similar to that of healthy controls. Additionally, major structural alterations in the ER and Golgi complex, accumulation of storage vacuoles, and increased apoptosis were observed in the MPS II samples. The disease-specific phenotype was more pronounced in GFAP+ astrocytes, with increased LAMP2 expression but unchanged in their RAB7 compartment. Based on these finding we hypothesise that lysosomal membrane protein carrier vesicles have an initiating role in the formation of storage vacuoles. A novel human MPS II disease model was established which recapitulates the *in vitro* neural phenotype of the disorder. Our system provides unlimited amount of disease-relevant cell types and offers a good platform for further study of MPS II pathophysiology or for drug testing and gene therapy studies.

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PRONOUNCED HETEROGENEITY AMONG THE ACTIVITY OF VARIOUS NEOCORTICAL NEURONS DURING ABSENCE SEIZURES

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Although absence seizures are usually considered benign, some recent evidence indicates that in a substantial number of cases comorbidities including learning disabilities and other neurological and psychiatric conditions can develop. Spike and wave discharges (SWDs), a hallmark of absence epilepsy, are generated in the cortico-thalamo-cortical system. In rodent models of absence epilepsy we previously showed that cortically derived feed-forward inhibition in the thalamus plays a key role in generating SWDs, but the activity of cortical neurons is less well characterized. We have now recorded the activity of regular spiking neurons (RS) and fast-spiking interneurons (FSI) in the infragranular layers of the peri-oral region of the somatosensory cortex, i.e. the cortical initiation site and somatosensory thalamus, using either multi-site (up to 64 channels) silicone probes in freely moving GAERS rats or glass pipettes in head-restrained awake GAERS rats. As a group RS, neurons do not show a change in their activity at seizure onset or offset. However, some RS neurons show a sharp increase in firing while others a sharp decreased firing during seizures. In contrast, thalamocortical neurons show very little heterogeneity in their peri-ictal firing. Importantly, a subset of FS neurons show a decrease in their activity seconds before seizure onset. On a faster time-base, the relationship of cortical, but not thalamocortical neurons to individual cycles of the SWD also shows marked heterogeneity. Ongoing experiments aim to reveal the identity of these various subsets of neocortical neurons and understand the cellular and network mechanisms involved. These results highlight the importance of heterogeneity in neocortical circuits in the generation of absence seizure paroxysmal activity.

NIMODIPINE DELIVERY WITH PH RESPONSIVE NANOPARTICLES TARGETED TO THE ISCHEMIC AREA IN THE RAT BRAIN

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Nimodipine, an L-type Ca^{2+}-channel blocker was shown to be neuroprotective,and vasodilator through vascular smooth muscle cell hyperpolarization. Nanoparticles are emerging carriers of drug delivery, which can be constructed to release drugs in response to specific signals. We propose that, in the ischemic tissue acidosis can be a relevant signal that could be utilized for the initiation of drug release from nanoparticles. Our aim was to design and test a novel treatment strategy for cerebral ischaemia resting on pH-sensitive nanoparticles containing nimodipine, in our *in vivo* global cerebral ischaemia model.

Anesthetized Sprague-Dawley rats (n=18) were used. After washing a suspension of chitosan nanoparticles (d<20 nm) with or without nimodipine on the exposed brain surface, both common carotid arteries were permanently occluded to create global forebrain ischaemia. Augmenting the ischaemic insult, spreading depolarizations (SDs) were elicited by 1M KCl. Local field potential, cerebral blood flow (CBF) and tissue pH-variations were recorded from the cerebral cortex.

Ischaemia-induced tissue acidosis initiated nimodipine-release from nanoparticles, which was confirmed by the significant elevation ofbaseline CBF (29.3±6.9 % vs. 47.8±23.7 %; nanoparticle only vs. nimodipine associated to nanoparticle). Nimodipine significantly shortened the duration of both SD itself (76.2±17.2 vs. 48.1±23.3 s), and the associated tissue acidosis (138.3±66.1 vs. 65.5±20.2 s), moreover it enhanced the SD-related hyperaemia (2368.0±1324.7 vs. 4604.4±2572.3 %*s).

Our results show that the delivery of nimodipine targeted to the ischemic nervous tissue with pH sensitive nanoparticles is a feasible approach to attenuate secondary brain injury mechanisms such as SD.

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EFFECT OF NATURALLY DEPOSITED AMYLOID-BETA ON THE HIPPOCAMPAL NETWORK

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Alzheimer's disease is a progressive neurodegenerative disorder, which is characterized by amyloid-beta (Aβ) depositions, microglia activation, astrogliosis, dystrophic neurites and aberrations in different neurotransmitter systems.A new type of genetically modified mouse strain, APP-NL-F mice,contain a naturally mutated human amyloid precursor protein (APP) and generate amyloid-beta driven only by the endogenous mouse promoter,to mimic the human symptoms caused by Aβ effect alone.We found that Aβ was strongly expressedin hippocampus, primarily in layers targeted by the perforant path. Block-face scanning electron microscopy allowed the 3D investigation of amyloid plaques that were surrounded by dystrophic neurites. These neurites belonged to many different types of neurons. Because GABAergic cells were reported to be key players in AD, we determined the number of hippocampal parvalbumin (PV) and somatostatin (SOM) interneurons and their connectivity. Surprisingly, we found that the number of PV and SOM interneurons remained unchanged and virally traced septo-hippocampal PV-positive and cholinergic fibers, which often suffer losses during AD, did not show any difference even at 24-month of age. However, PV positive synapses on axon initial segments of pyramidal cells were significantly larger compared to wild type mice. These results show that the natural promoter driven APP gene activation resulted in Aβ production that had no serious network effects, but it caused localizedaxonal deformations and significant synaptic changes.

ALTERATIONS IN MOTONEURONAL CALCIUM LEVEL AFTER LONG-TERM TREATMENT WITH SERUM FROM SALS PATIENTS

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Background: Different pathological processes lead to motor neuronal degeneration in sporadic amyotrophic lateral sclerosis (sALS). Our aim was to understand the effect of chronic treatment with serathat contains anti-motoneuronal antibodies ofsALS patients on calcium homeostasis, neuronal survival and motor function.
Methods: Balb/c mice were injected intraperitoneally for 75 days with sera from healthy individuals (n=3) and patients with sALS (n=6). During the passive transfer isometric muscle strength of their limbs was measured. Number of motor neurons in the spinal cord was measured with the disector method. Intracellular calcium was quantified with geometrical statistics in the spinal cord and in the axonterminals of their *musculus interosseus* samples.
Results: Isometric muscle strength significantly decreased after long-term inoculation of sera from sALS patients. The number of motor neurons were also significantly decreased in the cervical ($p<0,001$) and lumbar ($p<0,001$) spinal cord. Significant increase of intracellular calcium could be documented in the cervical ($p<0,001$) and lumbar ($p<0,001$) spinal cord and in the axonterminals of their forelimb($p<0,001$) and hindlimb ($p<0,01$). Ultrastructural alterations of perikarya and neuromuscular synapses were observed.
Conclusion: Functional regression, motor neuronal loss and elevated intracellular calcium level confirm the motor neuronal degeneration in this model.Such long-term treatment provides a feasiblerepresentation of sALS, as no genetic mutations were observed in the patients. However, it is reconcilable with the transgenic animal modelswidely used in pharmacotherapeutic tests. Therefore this model could begive us further understanding of motoneuronal degeneration in ALS.

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ULTRASTRUCTURAL CHANGES INDUCED BY ACUTE BLOOD SERUM INOCULATION OF ALS PATIENTS WITH GENETIC MUTATIONS

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Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disorder, primarily affecting motor neurons. The genetic screening of a subpopulation ofHungarian ALS patients revealed mutations on the superoxide dismutase 1 (SOD1) and chromosome 9 open reading frame 72 (C9ORF72) genes. Our aim was to determine the importance of different neuropathological aspects in each of these mutations, such as intracellular calcium level elevation, mitochondrial ultrastructural alterations and survival of motor neurons.

Balb/c mice were inoculated intraperitoneally (1 ml/day) for 2 days with blood serum of ALS patients with identified mutation or sporadic ALS patients without mutation (n=3-6/group). Non-treated and healthy serum treated groups were used as controls. Lumbar spinal cord and muscle samples were used for electron microscopic examination of motoneuron somas and neuro-muscular synapses, respectively. Motoneuronal survival was also quantified on lumbar spinal cord samples.

Acute passive transfer of the blood serum of ALS patients induced significant decrease in the number of motor neurons in the spinal cord, elevation of intracellular calcium level and increase of lipofuscin vesicle volume. Motoneuronal loss and calcium level changes were most prominent in C9ORF72 mutation. Furthermore, partial mitochondrial volume decreased in SOD1 point mutations.

Our previous experiments demonstrated the central role of intracellular calcium level elevation in ALS. Our current findings proved that different mutations induced similar morphological alterations, but to different extent. The extensive loss of motoneurons and changes in intracellular calcium level suggest that C9ORF72 mutation might present a more progressive phenotype.

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OPERA SINGING: UCOVERING THE NEURAL BASIS OF SINGING EXPERTISE USING FMRI

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Introduction:
During singing the same anatomical vocal tract structures define different qualities of voice. Amateur singers use their congenital or “natural” voice instinctively. Conversely, an opera voice can only be obtained after many years of learning and practicing, resulting in precise adjustments of respiratory, laryngeal, and articulatory muscle groups that are linked to a particular sound quality. The most salient differences between these two types of singing are the vibrato, the timbre, and the perceived loudness.
Hypothesis:
We hypothesized that mastering opera singing technique leads to highly specialized brain representations of the specific vocal functions, which can be dissociated from those involved in natural singing. We aimedat uncovering the neural bases of singing expertise by examining vocal production and voice perception in opera singers.
Design:
We designed and implemented behavioral tests (Music expertise questionnaire, vocal range, singing accuracy, voice quality dichotic working memory test) and fMRI paradigms (Sensory and motor task-related) enabling investigation of vocal production and voice perception in opera singers using newly developed, highly controlled natural and operatic voice stimuli.
Results:
Our first results revealed that listening to operatic singing leads to fMRI patterns of brain activity in the targeted sensorimotor regionsthat differ from those observed during perception of singing with natural voice or control sinusoid melodies.
Conclusion:
Ourfindings suggest that neural processes of singing expertise in natural and opera singing can be dissociated and characterized using functional MRI.

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SPINAL NEURONAL UPREGULATION OF P2X4 RECEPTOR IN CFA EVOKED CHRONIC IN FLAMMATORY PAIN

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Long-term and intense noxious stimulation related to chronic inflammation lead to central sensitization and plasticity within the superficial spinal dorsal horn. It is widely accepted, that interleukins play a pivotal role in spinal pain processing. By a purinergic receptor mediated action, the activation of nociceptive primary afferents and the consecutive release of ATP induce considerable increase of interleukin-1βeta within the spinal dorsal horn contributing to the hyperexcitability of the neural circuit. Accumulating evidence suggests that the P2X4 receptor may be one of the major key mediators that are involved in the cytokine secretion including IL-1β. Our knowledge is moderately scanty regarding the role and expression of P2X4 in chronic pain conditions. Thus, in the present experiment we investigated the expression and distribution of this receptor in the spinal dorsal horn of adult male Wistar rats suffering in chronic inflammatory pain evoked by unilateral plantar injection of complete Freund adjuvant (CFA). Single immunoperoxidase reactions revealed a substantial P2X4 receptor expression within the lamina I-II of the spinal gray matter following CFA injection which was further validated by Western blot analysis. The cellular distribution of P2X4 was examined by using double- and triple immunofluorescent labelings. Beyond the colocalisation of P2X4 receptor with various interneuronal and primary afferent markers we observed abundant increase of the purinergic expression on excitatory but not inhibitory axon terminals in CFA model compared to control.

COMPLEX EXAMINATION OF THE AUDITORY SYSTEM IN PACAP-DEFICIENT MICE

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide with widespread cytoprotective, neuroprotective and regulatory functions. It is present in several sensory organs, also in the inner ear and auditory pathway. It has antiapoptotic effects against oxidative stress in inner ear cell cultures.

In our study male wild-type (WT) and PACAP-deficient (KO) mice were compared with auditory brainstem response test (ABR); with c-Fos immunostaining in the nuclei of the auditory pathway; and with protein profile analysis from cochlear duct lysates.

KO mice showed higher hearing thresholds at lower frequencies and lesser amplitude and shorter latency values at higher frequencies compared to WT mice. Staining of c-Fos showed decreased neuronal activation in the cochlear nuclei of KO animals, however, there was no difference in other nuclei of the auditory pathway compared to WT mice. Endostatin, acidic FGF, osteopontine, BLC, CD54, PF4, TF, DPPIV, IGFBP-2, Serpin F1 and CXCL12 were in detectable amount from the lysates of cochlear ducts, but we did not find any significant difference between WT and PACAP KO animals.

We showed the impairment of hearing functions in the absence of PACAP at both lower and higher frequencies, which was affirmed by the lower neuronal activation in the cochlear nuclei. However, there were no changes at molecular level in the protein composition of the inner ear cochlear duct lysates. We assume that in the KO mice there is an accelerated aging process which results in an early hearing loss of the animals.

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LAYER-SPECIFIC SINGLE NEURON – LFP CORRELATIONS AND CAUSAL FUNCTIONAL CONNECTIVITY DURING MULTIMODAL MISMATCH DETECTION TASK

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Mismatch negativity is a well established yet simple paradigm to examine interactions between low-level sensory input-processing and higher level functions such as predictions or multimodal integration.
In this work, we investigated single unit activity, its layer-specific correlations with the power of Local Field Potential (LFP) and causal connections between the recording sites in the neocortex during a multimodal mismatch paradigm. Parallel to combined auditory (pitch) and visual (orientation) stimulations, LFP signal and unit activity was recorded by two 32-channel Michigan type microelectrode arrays implanted in primary visual cortex (V1) and the multimodal area (AL) of mice.
We found various response types of single units, which increased their spiking frequency in response to visual, auditory or bimodal mismatch to stimuli both in V1 and AL.
Layer-specific correlations between single unit activity and LFP power in alpha and gamma frequency bands revealed top-down bottom-up effects.
Also, extending the convergent cross mapping (CCM) our causality analysis shows that CCM can be utilized to determine functional causal interactions in specific frequency regimes of LFP.
Our study provides insight into multimodal predictive coding at the correlated single neuron and mesoscale levels of cortical activity across layers and areas.

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CYTOKINE EXPRESSION OF REACTIVE ASTROCYTES IN CHRONIC INFLAMMATORY PAIN

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Several studies investigated the role of astrocytes in chronic inflammatory pain within the central nervous system. Upon activation spinal glial cells produce inflammatory cytokines, which influence neuronal functions leading to central sensitization and enhanced pain states. The most prominent representative of proinflammatory cytokines is interleukin-1beta (IL-1β). Our earlier data showed that the spinal astrocytes are the main source of IL-1β in the chronic phase of inflammatory pain, in addition astrocytes also express the ligand binding IL-1RI unit of the IL-1 receptor. The IL-1β ligand acts on its neuronal and astrocytic interleukin-1 receptor type 1 (IL-1RI) leading to cell-type specific responses.

In the current study inflammatory pain was induced by intraplantar injection of complete Freund adjuvant (CFA). To selectively investigate the role of astrocytes primary cultures were produced from spinal cords of C57BL6 wild type and IL-1RI deficient mice. For the activation of astrocytes IL-1β stimulation was applied.

In the spinal cord of CFA-injected mice we observed a significant increase of IL-1β level on post injection day 4 which correlates with the nociceptive test results. By using cytokine array method, we demonstrated a significant increase in the expressional level of several proinflammatory cytokines in the supernatant of astrocyte cultures upon IL-1β stimulation.Furthermore, we validated the expressional changes of these cytokines by Western Blot, ELISA and Immunohistochemical experiments.

Our data show that IL-1β triggers a cascade of astrocytic cytokine release, which probably further increase neuron-glia and glia-glia interaction.

SPIKE LFP PHASE-COUPLING IN THE SUPERIOR COLLICULUS OF THE ANAESTHETIZED CATS

Zsófia Giricz

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The superior colliculus (SC) of the midbrain is the origin of the tectofugalmultisensory system in the mammalian brain. Although several studies addressed the electrophysiological properties of the SCneurons, the connection between the low frequency oscillations (LFP) andthe spiking activity of the single neuronsis still poorly understood.

Extracellular multiscale recordings were performed using 64-channel platina-iridium microelectrodes in two halothane-anaesthetized, paralyzed, artificially respiratedcats. The background neuronal activities (without any sensory stimulation) were analysed. The spikes were sorted with Klusta software package.The phase-coupling of the single SC neurons to different frequency bands (theta (4-8 Hz), alpha (8-12 Hz), beta (13-30 Hz), gamma (31-50 Hz))was statistically analyzed with Rayleigh uniformity test.

The majority of the recorded SC neurons(888/956; 93%) possessed phase-coupling in at least one of the investigated frequency bands. In the alpha- and gamma-band oscillations revealed the similar preferred phase in both cats. The coupling tobeta-band oscillation was also similar in the two cats but without any preferred phase of the local oscillations. On the other hand, the direction ofthephase-coupling to theta oscillations was not uniform in the two animals.

Our results demonstrated thatthe background activity of the SC neurons is strongly phase-locked in both cats. Similarly, high number of phase-locked neuronswere found in both animals. However further investigation is necessary to clarify the influence of the sensory signals on thephase-coupling of theSC neurons.

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HIGHLY SPECIALIZED FRONTAL CORTICAL CONTROL OVER THE ANTERIOR THALAMIC RETICULAR NUCLEUS

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Thalamic relay nuclei establish reciprocal and topographical connections with the GABAergic thalamic reticular nucleus (TRN). The entire TRN receives inputs from the layer 6 (L6) of the neocortex. L5 cortical output, which otherwise exerts profound control over thalamic activity, was thought to reach thalamus without innervating TRN thus without an intrathalamic feed forward inhibitory component. Here we report a novel L5 pathway to a well-defined sector of the TRN arising exclusively from the frontal cortices.

Conditional viral tracing in the L5-specific Rbp4-Cre mouse revealed dense, topographically organized projection in the antero-ventral TRN following injections to frontal but not to parietal cortical areas. Compared to L6 synapses L5 synapses showed distinct ultrastructural properties at the electron microscopic level and functional differences in *in vitro* electrophysiological experiments. While optogenetic activation of both L6 and L5 synapses elicited EPSCs in TRN cells, they differed in their short term plasticity and AMPA/NMDA ratio. Optogenetic activation of L5 cells *in vivo* in anesthetized miceelicited action potentials in anterior TRN cells with short latency and high fidelity. The magnitude of the response (number and frequency of action potentials) gradually increased with increasing number of recruited L5 cells converging on the same TRN neuron. This raises the possibility that the thalamic feed forward inhibition via the L5-TRN pathway is sensitive to elevated cortical synchrony levels in the frontal corticothalamic system. The data suggest powerful and temporally precise cortical control by the stronger coupling of TRN cells to the ongoing cortical oscillations in frontal circuits.

NEUROINFLAMMATION AND SENSITIZATION ARE MEDIATED BY INTERLEUKIN-1 IN A MODEL OF COMPLEX REGIONAL PAIN SYNDROME

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Complex Regional Pain Syndrome (CRPS) is a chronic pain condition developing after small injury and characterized by severe pain accompanied by hypersensitivity, swelling, autonomic alterations of the skin. Although the ethiology is unknown, it is caused by peripheral and central nervous systems damage or disfunction and/or immune response against sensory nerve-derived antigens. The therapy is still unsatisfactory, therefore, there is a great need to identify the pathophysiological mechanisms.
Here we investigated neuroinflammatory mechanisms in a passive transfer-trauma translational mouse model of CRPS, where small plantar skin-muscle incision was performed in female C57Bl/6 mice treated daily with purified serum-IgG from patients with long-standing CRPS or healthy volunteers. The role of the intereukin-1 (IL-1) pathway in using IL-1alpha/beta- and microglia-specific IL-1 knockout (KO) mice, as well as IL-1 receptor-antagonist anakinra in comparison with the glucocorticoid prednisolone.
CRPS IgG significantly increased and prolonged swelling and hyperalgesia of the incised paw compared to healthy human IgG. Following short-lasting paw inflammation in all groups, CRPS mice displayed sustained microglia and astrocyte activation in the of the spinal dorsal horn and pain-related brain regions, indicating central sensitization. Full deletion of IL-1, perioperative and later anakinra treatment, but not prednisolone abolished transferred CRPS symptoms and neuroinflammation. Microglia-specific IL-1 deletion inhibited hyperalgesia increase in the early phase suggesting that neuroinflammatory mechanisms contribute to the CRPS phenotype.
We conclude that persistent CRPS-related pain is mediated by autoantibody-induced neuroinflammation and central sensitization. Our results highlight novel therapeutic use for IL-1 receptor antagonists, such as anakinra, to prevent or treat CRPS.

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EVIDENCE FOR THE IN VIVO ANALGESIC EFFECT OF SPHINGOMYELINASE VIA LIPID RAFTS

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Transient Receptor Potential ion-channels, such as TRP Vanilloid 1 and Ankyrin repeat domain 1 (TRPV1, TRPA1), are expressed in nociceptive primary sensory neurons. Capsaicin, low pH,noxious heatactivate TRPV1. Irritant molecules (allyl-isothiocyanate, formaldehyde) activate TRPA1.Lipid rafts are defined as liquid ordered plasma membrane microdomains rich in cholesterol, sphingomyelin and gangliosides. Sphingomyelinase (SMase) decreases membrane sphingomyelin by hydrolization. The aim of the present study is to examinethe*in vitro*actions, andthepotentialanalgesiceffect of SMase in *in vivo*mousemodels.

The effect of SMase was analysed on isolated trigeminal (TG) neurons by measuring agonists-induced Ca^{2+}-transients with ratiometric technique, and on TRPV1-,or TRPA1-expressing CHO cells by measuring ^{45}Ca-uptake. We investigated the mechanonociceptiveand thermonociceptive threshold of the animals in RTX-induced thermal-, mechanical hyperalgesia, and formaldehyde-evoked hyperalgesia model.The analgesic effect of SMase was also measured in capsaicin-evoked acute nocifensive response („eye-wiping") test.

The results show, that theSMase treatment diminished the percentage of responsive cells, and the magnitude of Ca^{2+}-transientsin TG neurons, and decreased the ^{45}Ca-uptake on receptor-expressing CHO cells.SMasetreatment significantly reduced the RTX-induced thermal, mechanical and formaldehyde-evoked hyperalgesia and the capsaicin-evoked eye-wiping movements.

Our*in vitro* and *in vivo* results suggest that the hydrophobic interactions between the TRP-channel and lipid raft interfaces modulate the opening properties of these channels and therefore, targeting this interaction might be a promising tool for drug developmental purposes.

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COMPARATIVE IDENTIFICATION OF VISUAL-MOTION COMPUTATIONS IN CATS AND MICE VIA GENETICALLY TARGETED OPTICAL CIRCUIT ACCESS

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Direction-selective (DS) responses have been measured in LGN cell bodies and axon terminals of mice, suggesting that retinal DS cells could contribute to the computation of direction selectivity in the cortex. Whether and what form of cortical direction selectivity across visual areas is linked to retinal direction selectivity remains not well understood. To answer this question, we disrupted retinal DS using two independent genetic approaches and recorded neuronal responses to visual motion in V1.

In addition to our mouse work, we are also exploring motion computations in a species with frontally looking eyes, cats. We aim to translate genetic methods perturbing neuronal computations into non-rodent species. We are testing different AAVs to achieve cell- or layer-specific labeling in the retina and cortex of cats.

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LAYER SPECIFIC MOLECULAR HETEROGENEITY OF THE EXTRACELLULAR MATRIX IN THE OLFACTORY BULB

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The olfactorybulb (OB) consists of thefollowinglayers:olfactorynervelayer (ONL), glomerularlayer (GL),externalplexiformlayer (EPL), mitralcelllayer (MCL),internalplexiformlayer (IPL) andgranulecelllayer (GCL). The neuronal network of OB is continuously reorganized throughout life. These processes require high degree of neural plasticity. Molecules of extracellular matrix (ECM) play an important role in the synapse remodeling. The major components of the ECM are hyaluronan (HA), lecticans including aggrecan, brevican, neurocan, versican and glycoproteins e.g., tenascin-R (TN-R) and link proteins (e.g. HAPLN1).

The present study describe the distribution of HA, TN-R and HAPLN1 in the OB in rat.

The experiments were performed on adult Wistar rats. HA was labeled using biotinylated Hyaluronan Binding Protein. TN-R and HAPLN1 were detected with antibodies. Double fluorescent labeling was made using neurofilament or MAP2 antibodies in combination with HAPLN1.

HA reaction showed only moderate staining intensity in the OB except the GL and EPL. The TN-R reaction was almost negative in the entire GL. The strongest reaction was visible in the IPL. The HAPLN1 reaction was present in the GL. The MAP2 and neurofilament antibodies showed positive reaction among the HAPLN1 stained areas. The strongest staining was visible in the EPL.

According to our results, the expression of the examined molecules shows a layerspecificstainingpattern. The organization of these molecules may be related with the high degree of synaptic plasticity in the OB.

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CAPSAICIN-INDUCED TRPV1 CHANNEL ACTIVATIONIS MODULATED BY OXYTOCIN IN CULTURED DORSAL ROOT GANGLION NEURONS

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Aim: The cellular and molecular mechanisms of the supposed antinociceptive effects of oxytocin (OT) on primary sensory neurons expressing the transient receptor potential vanilloid type 1 (TRPV1)/capsaicin receptor were examined.

Introduction: It has been revealed that OT plays an important role in pain modulation through oxytocin receptors (OTR) expressed by neurons in the spinal cord and perikaryaof dorsal root ganglion (DRG) neurons.This study was designed to examinewhether OT is involved in TRPV1 nociceptor ion channel functioning through OTR signalisation in cultured DRG neurones.

Materials and methods:DRG cultures were prepared from spinal ganglia of adult male Wistar rats (n=9) weighing 250-310 g.Neurons responsive to capsaicin were identified by using the Co^{2+}-uptake assay.

Results: In the presence of 1 μM capsaicin 38±8.56 % of cultured DRG neurons exhibited staining after incubation in the cobalt uptake assay buffer. Administration of OT or OT co-administered with Atosiban10 min prior to the capsaicin challenge failed to affect the proportions of cobalt-labelled neurons significantly (35.78±4.87 and 31.51±7.96%, respectively). However, administration of oxytocin (1 μM) for 3 days resulted in a significant decrease in the proportion of neurons showing cobalt staining (28.45±4.2), $p<0.05$, n=9), which was prevented by co-administration of Atosiban.

Conclusion: Our results using the Co^{2+}-uptake assay indicate that OT may modulate the activation of the TRPV1 nociceptive ion channel in cultured DRG neurons. It is suggested that the modulatory role of OT effected through OTRs may bear of significance in the nociceptive and local regulatory/sensory-efferent functions of chemosensitive primary sensory neurons.

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FORM, SYNAPSES AND FUNCTIONAL TOPOGRAPHY OF A NEW CELL TYPE IN THE VISUAL CORTEX

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Here we report the morpho-functional features of a novel type of neurons in the cat visual cortex which has spine-free dendrites but assumed to be excitatory rather than inhibitory. This neuron was selected from a large pool of intracellularly labelled cells based on its large cell body and numerous long dendrites giving rise to stellate morphology. The main axon gave off long-range horizontal axons up to 2.8 mm in layers 5/6 before entering the white matter. 3D-reconstruction revealed that the axon provided en passant boutons (n=1192) and terminal boutons which were uniformly distributed without obvious spatial clustering. Dendritic length, surface and volume were at least 3 times larger than corresponding parameters of any known excitatory and inhibitory neuron types in layers 2-4 and 6 in the cat visual cortex. Quantitative electron microscopy of labelled boutons representing proximal and distal parts of the axon showed that for both categories the postsynaptic targets are chiefly dendritic spines (78%) and less frequently dendritic shafts (22%) of other excitatory neurons. GABA immunopositive dendrites represented a minority of the targets (4 of 9 dendrites tested). 3D-EM reconstruction of the boutons (n=28) showed a complex shape. On average, the boutons had 01.39 μm^3 volume and 7.51 μm^2 surface are. Superimposing the axonal field onto the orientation map obtained using intrinsic signal optical imaging showed that the majority of connections prefer oblique orientations rather than iso-orientation.

The results obtained for this novel cell type suggest an integrating role of a broad range of synaptic inputs onto the dendrites before sending out the output to a broad range of excitatory targets in the same layer and to other cortical and subcortical regions.

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CHANGES OF THE DIPEPTIDYL PEPTIDASE 4 EXPRESSION IN DIFFERENT CELL TYPES IN INFLAMMATORY CONDITIONS

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DPP4 is an integral membrane glycoprotein expressed on many cell types. As a serine protease, DPP4 cleaves dipeptides from oligopeptides and proteins containing proline/alanine in the penultimate position. DPP4 is also known as cell surface antigen CD26 on T-lymphocytes and receptor for Coronaviruses. We showed previously that spinal inhibition of the DPP4 resulted in a strong antihyperalgesic and anti-inflammatory effect. DPP4 immunoreactivity was found on neurons and glial cells in the spinal cord and its expression increased significantly in astrocytes during inflammation or microglia in neuropathy. The main goal of this work was to study the involvement of DPP4 in different inflammatory processes at cellular level. The changes of the DPP4 expression were examined in three inflammatory model systems using immunocyto/histochemistry and Western blot analysis. Astrocyte cell culture was treated with interleukin-1β or bacterial lipopolysaccharide to mimic inflammatory events. Gradual time-dependentincrease of the DPP4 immunoreactivity and protein level were detected in astrocytes. DPP4 expression was also studied in normal and Freund adjuvant treated peritoneum (mesothelial cells) or in healthy and inflamed tooth pulps following cavity preparation. Increased DPP4 expression was observed on mesothelial cells after 3 and 5 days of the treatment. Similar increase of the DPP4 expression was detected in pulp cells (fibroblasts) after 1 day and in odontoblasts after 8 days of cavity preparation. Electric stimulation of the teeth causing sterile inflammation didn't result in changes in DPP4 expression. Our data suggest that DPP4 expression changes are related to TLR involved inflammatory processes.

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TASTE REACTIVITY ALTERATIONS AFTER INTERLEUKIN-1β MICROINJECTION INTO THE CINGULATE CORTEX OF THE RAT

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Previous studies revealed various roles of the primary cytokine interleukin-1β (IL-1β) in the regulation of feeding. The cingulate cortex - part of the forebrain limbic circuitry - , in the focus of our presentresearch,is known to be involved in the central regulation of feeding and metabolism, in general and specific food-associated motivational processes, as well asin the hedonic evaluation of relevantstimuli.
In our present series of taste reactivity test experiments, the involvement of cingulate cortical IL-1β mediated mechanisms was examined in the taste sensation of adult maleWistar rats. IL-1β or phosphate buffered saline (controls) were microinjected bilaterally via guide cannulas fixed in position duringprevious stereotaxic operation. The taste reactivity test was performed in a glass cylinder where the animals were placed, and by means of a microinjection pump we administered the taste solutions in their oral cavity via chronically implanted taste cannulas made of polyethylene tubes. Two concentration series of the five basic tastes served as gustatory stimulus solutions. Based on simultaneous video recordings, internationally agreed species specific mimical and postural-locomotor response patterns were ‘off-line’ analyzed. Response rates of ingestive and aversive patternsof the cytokine treated and the control groupssignificantly differed in case of three – two unpleasant and one pleasant - taste qualities. Results indicated involvement of cingulate cortical interleukin-1β mechanisms in the control of motivationally determined taste perception processes.

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COMPLEMENT COMPONENT 1Q SUBCOMPONENT BINDING PROTEIN IN THE BRAIN OF THE RAT

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Complement component 1, q subcomponent binding protein (C1qbp) is a multifunctional protein involved in immune response, energy homeostasis of cells as a plasmembrane receptor, and a nuclear, cytoplasmic or mitochondrial protein. Recent reports suggested its neuronal function, too, possibly in axon maintenance, synaptic function, and neuroplasticity. Therefore, we addressed to identify C1qbp in the brain using in situ hybridization histochemistry and immunolabeling at light and electron microscopic level. C1qbp has a topographical distribution in the brain established by the same pattern of C1qbp mRNA-expressing and protein-containing neurons with the highest abundance in the cerebral cortex, anterodorsal thalamic nucleus, hypothalamic paraventricular (PVN) and arcuate nuclei, spinal trigeminal nucleus. Double labelling demonstrated the presence of C1qbp in neurons but not in glial cells in the brain. Furthermore, not all neurons express C1qbp, for example, in the PVN, magnocellular neurons selectively contain C1qbp. Further double labelling suggested the mitochondrial localization of C1qbp in the brain, confirmed by correlated light and electron microscopy at 3 different brain regions. Post embedding electron microscopy also suggested uneven C1qbp content of mitochondria in different brain areas but also within single neurons. These data suggest a specific function of C1qbp in the brain related to mitochondria, such as energy homoeostasis of neurons.

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CORTICAL LAYER 6 MODULATES ON-GOING NETWORK STATE

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Cortical layer 6 is the source of an excitatory projection to the primary thalamic areas, the function of which is still debated. Here we tested thehypothesis that L6 corticothalamic feedback functions as a modulatory system in the thalamus and can induce a state change in the thalamocortical system, similarly to other modulators.

We used chronically implanted NTSR1-ChR2 transgenic mice, in which thalamically projecting layer 6 neurons selectively express channelrhodopsin, to measure the cortical local field and unit responses elicited by optical stimulation of the corticothalamic feedback.

Repeated pulse-like L6 stimulation elicited spindles during deep sleep, while under desynchronized and more synchronized epochs no evoked spindles were observed.Using longer stimuliwe found that tonic activation of L6 feedback can elicit state change in the thalamocortical network in a dose- and state dependent manner. During stage II. sleep, low intensity L6 stimulation eliminated sleep spindles, but retained delta activity, transforming the network to a stage III. sleep-like state. Higher intensities, however desynchronized thalamocortical activity, with a corresponding drop in delta- and sigma-, and an increase in gamma LFP power. During deep sleep, low intensity corticothalamic activation produced little or no effect, while high intensity L6 stimulation induced desynchronization, similar to that during light sleep. The spatial extent of the state change was limited, both low and high intensities acting only locally.

We conclude that corticothalamic feedback can indeed act as a local modulator in the thalamocortical system.

THE ROLE OF SECRETIN SIGNALING IN THE REGULATION OF GNRH NEURONS

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Gut hormones are good indicators of the metabolic state of the body, which is important for normal reproductive functions. These hormones are able to enter the central nervous system (CNS) and interact with the hypothalamo-pituitary-gonadal (HPG) axis. Secretin is a member of this family, but its effect on GnRH neurons has not been examined yet. In order to have more information about its action in the neuroendocrine regulation of reproduction, *in vitro* electrophysiological experiments were carried out on GnRH neurons of GnRH-GFP male mice. Application of secretin (100 nM) significantly increased the frequency of the spontaneous postsynaptic currents (sPSC) and that of the miniature postsynaptic currents (mPSC) in GnRH neurons. Resting membrane potential became depolarized after secretin treatment. Frequency of evoked action potentials also increased. The secretin triggered elevation of the frequency of mPSCs was prevented by using secretin receptor antagonist and intracellularly added G-protein coupled receptor blocker GDP-β-S, supporting the involvement of secretin receptor (expressed in GnRH neurons) in the mechanism. RT-qPCR study confirmed the expression of secretin receptor gene (Sctr) in GnRH neurons. Changes in the frequency suggest presynaptic alterations which in case of GnRH neurons in males often leads to changes in the $GABA_A$ input. Intracellular application of nNOS blocker NPLA attenuated the excitatory effect of secretin. PKA blocker KT-5720 also eliminated the stimulating effect of secretin on GnRH neurons. These data suggest that secretin acts on GnRH neurons via cAMP/PKA/nNOS retrograde signaling pathway modulating their GABAergic input.

ELEVATED LEVEL OF GLUCAGON-LIKE PEPTIDE-1 RECEPTOR IN THE DORSOMEDIAL HYPOTHALAMIC NUCLEUS OF DIABETIC PATIENTS

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We previously showed that glucagon-like peptide-1 (GLP-1) and prolactin-releasing peptide (PrRP) of brainstem origin activate neurons located in the dorsomedial hypothalamic nucleus (DM) and contribute to satiation in rats. A previous study reported decreased expression of GLP-1R in the paraventricular (PVN) and infundibular nuclei (INF) in type 2 diabetic mellitus (T2DM) patients, and the authors suggested a role of the changes in dysregulation of feeding behavior and glucose homeostasis in T2DM. Now we investigated the topographical distribution of the GLP-1, GLP-1 receptor (GLP-1R) and PrRP in the human DM. GLP-1 and PrRP-fibers are abundant in the DM and their distributions overlap within the nucleus.In situ hybridization probes for GLP-1 receptor were developed for radioactive in situ hybridization histochemistry. We found that a high number of neurons in DM expresses GLP-1R in both human and rat brains. Therefore, we also examined GLP-1R expression in *post-mortem*hypothalamus from five T2DM patients versus five control subjects. An increased GLP-1R expression was found in the DMof the T2DM subjects as compared to controls, while there was no difference in the expression level in the paraventricular nucleus. These findings have been confirmed by PCR techniques, and also byWestern blotting at the protein level. Thus, the special role of GLP-1 in the hypothalamic food intake regulation that was reported in rats, can be implied also in human hypothalamus, especially in the DM.

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CENTRAL NESFATIN-1/NUCB2 RESISTANCE IN IMPAIRED GLUCOSE HOMEOSTASIS

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Nesfatin-1, the secreted fragment of nucleobindin'2 (NUCB2) prohormone has recently been linked to the regulation of glucose homeostasis. Additionally, significance of brain-derived NUCB2/nesfatin-1 has been emerged.The mechanism of action is unclear. The prevalence of type 2 diabetes mellitus is higher in adult individuals born with intrauterine growth retardation. Contribution of central nesfatin-1 signalling to this condition has not been investigatedyet.

To model intrauterine growth retardation we used male intrauterine protein restricted (PR) rats, and comparedthemwith normally nourished (NN) mates.PR conditions caused a delay in the embryonic development of hypothalamic NUCB2 neurons, however no difference in NUCB2 expression was observedat birth. The birthweights of PR pups were lower than that of NN rats, butthis difference has vanished by the time of later studies. In the 12-week-old PR rats a marked increase in NUCB2 mRNA expression was found in the hypothalamus, accompanied by central nesfatin-1resistance. Parallel, impaired glucose tolerance and insulin sensitivity has appeared. Oppositely, NN rats centrally treated with nesfatin-1 for one week performed betterin glucose and insulin tolerance tests than vehicle-treated, without signs of developing nesfatin-1 resistance. Acute central nesfatin-1 injection decreased the number of fasting-activated (Fos+) neurons in NN, but not in PR rats,only in the arcuate nucleus.

We conclude that altered development of hypothalamic NUCB2 neurons leads to adult onset of central nesfatin-1 resistance in PR rats that may contribute to the development of impaired glucose homeostasis. The arcuate nucleus probably have a critical role in this process.

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DISTRIBUTION AND ULTRASTRUCTURALLOCALIZATION OF THE GLUCAGON-LIKE PEPTIDE-1 (GLP-1) RECEPTOR IN THE BRAIN OF RATS

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GLP-1 by acting on its receptor (GLP-1R) in the CNS inhibits food intake and regulates energy homeostasis. The distribution of GLP-1R mRNA in rat brain has been investigated, but little information is available about the subcellular localization and the distribution of the GLP-1R protein.
Immunocytochemistry was performed to determine the localization of GLP-1R protein in the brain. Very dense network of GLP-1R-immunreactive (IR) perikarya and/or processes were observed in the lateral septal nucleus, median preoptic area, hypothalamic paraventricular nucleus, arcuate nucleus (ARC), median eminence (ME), amygdala, tuberomammillary nucleus, area postrema (AP) and the NTS. Ultrastructural examination of GLP-1R-immunoreactivity in the ARC, ME, AP, NTS showed GLP-1R immunoreactivity in association to the membrane of perikarya, dendrites and axonal profiles. Large number of GLP-1R-IR perikarya and dendrites were observed in the ARC. In this nucleus, numerous GLP-1R-IR axons were also observed establishing both symmetric and asymmetric type synapses. In the external zone of the ME, GLP-1R-immunoreactivity was observed on axon terminals terminating around capillaries. In the NTS, GLP-1R-immunoreactivity was primarily observed in axons. In the AP, GLP-1R-immunoreactivity very densely labeled perikarya completely ensheeting these cells.
In conclusion, in this study we provide a detailed map of the GLP-1R-IR structures in the CNS. Our data demonstrate that in addition to the perikaryonal and dendritic distribution, GLP-1R is also present in axonal profiles suggesting the presynaptic action of GLP-1. The presence of GLP-1-IR profiles in the circumventricular organs suggests that peripheral GLP-1 may act in these brain regions.

THE ROLE OF THE MC3- AND MC4 RECEPTORS ON UROCORTIN1 NEURONS IN THE EDINGER-WESTPHAL NUCLEUS

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The role of the urocortin 1 (Ucn1) expressing neurons in the centrally projecting Edinger-Westphal nucleus (cpEW) hasalready been confirmed in the regulation of the energy homeostasis and in stress adaptation response. Morphological and functional experiments showed the presence of orexigenic and anorexigenic peptides and their receptors in the cpEW. The role of the hypothalamic melanocortin (MC) system in the control of energy-balance is also wellknown, however itsinteraction with cpEW-Ucn1has not yet been tested.

Here hypothesized that a) nerve fibers containing the ligands of the MC system [i.e. alpha-melanocyte-stimulating hormone (alpha-MSH), agouti-related peptide (AgRP)] innervate the cpEW. We also anticipated that b) MC receptors (i.e. MC3R and MC4R) co-exist with Ucn1.We put forward that c) MC4R expression is affected by 2 days fasting period, in addition,the alteration of d) environmental temperature affects MC3R expression in Ucn1 neurons. We also hypothesized that e) local treatment with MC receptor agonists and antagonists affects the energy homeostasis.

Our results show, that the cpEW-Ucn1 neurons express both MC3R and MC4R, and,they alsoreceive alpha-MSH and AgRP immunoreactive afferentation. Upon fasting, the immune-density of Ucn1, MC4Rand that of alpha-MSH nerve fibers decreased, while that of AgRP increased. Ucn1-MC3R expression was affected by the change of environmental temperature. Intra-cpEW administration ofalpha-MSHincreased the metabolic rate that was reversed by MC4R antagonist. MC3/4R antagonist decreased the core temperature.

Based on these data, we conclude that the MC system influencesthe energy balanceat least in part through cpEW-Ucn1 neurons.

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RAPID EFFECT OF ESTRADIOL ON DIFFUSION DYNAMICS OF GLUTAMATE RECEPTORS

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Changes in diffusion properties of glutamate receptors (AMPAR and mGluR1) play pivotal role in synaptic plasticity. Among many factors, controlling synaptic plasticity, gonadal steroid 17β-estradiol (E2) is an essential factor. In spite of the well established genomic effect of E2 on synaptic plasticity little if any attention has been given to the rapid action of E2 on the glutamate receptor diffusion. Using our single molecule live cell imaging technique we examined the rapid effects of E2 on the lateral diffusion of AMPAR and mGluR1 molecules in the plasma membrane of neurons differentiated from PC12 cells. Single AMPAR or mGluR1 molecule trajectories were individually tracked and analyzed both on the membrane of soma and neurits and mean square displacement as well diffusion coefficient (D: μm^2/sec) was determined. Both AMPAR and mGuR1 molecules showed restrictive and area specific movements along the neurites and somas with multimodal trajectories. The administration of 100pM and 100nM of E2 evoked a rapid, dose-dependent effect on the D_{AMPAR} molecules with limited or no effect on the D_{mGluR1} molecules. This effect was mimicked by G1, membrane estrogen receptor agonist. Blocking the polimerization of cortical actin network abolished the effect of 100pM E2 suggesting an essential role of cytoskeleton in the rapid effect of E2. Our findings provide first evidence that E2 rapidly alters membrane diffusion of glutamate receptors in living neurons possibly through the membrane estrogen receptor. Our resultssuggest that E2 can rapidly tune the synaptic plasticity via altering the surface movement of AMPAR and mGluR1 receptors.

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EXPRESSION OF NUCB2 AND ITS FRAGMENTS IN INTACT RAT AND IN INFLAMMATORY RAT MODEL

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NUCB2 is a central inhibitor of food intake. It is also expressed in the periphery, where alterations in its expression was associated with the metabolic status and with tumor formation. The 47.44 kDa peptide is theoretically cleaved within the cells into three fragments; nesfatin-1, -2 and -3 (9.61 kDa, 10.11 kDa and 27.72 kDa, respectively). The N-terminal fragment nesfatin-1 is secretable, and responsible for the known biological activities. Cleavage of NUCB2 is therefore a key element to its function both centrally and peripherally.

In the present study we compared expression of NUCB2 and its derivates in different rat organs by Western blot method. Analyses were performed using control and lipopolysaccharide (LPS) treated rats. Antibodies against both the N- and the C-terminals of NUCB2 were applied.

NUCB2 prohormone was demonstrated by both antibodies in all types of investigated tissues. The presence of 9.61 kDa nesfatin-1 was not confirmed. The smallest band with the N-terminal antibody appeared at approximately 14 kDa. However, cleavage of NUCB2 between nesfatin-1 and -2 was demonstrated by the C-terminal antibody in certain organs (brown fat, stomach, lung, heart), where nesfatin-2 and 3 together (around 38 kDa) was present. LPS treatment seemed to diminish the expression of NUCB2 in the testis and in the brown adipose tissue and to elevate the expression of the 14 kDa product in the lung and plasma.

We conclude that NUCB2 is widely expressed in rat organs and the dynamics of its processing is tissue-specific. Posttranslational modification of the nesfatin-1 is suggested.

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OPTOGENETIC INHIBITION OF CALRETININ POSITIVE CELLS IN THE DORSOMEDIAL THALAMUS INFLUENCES SLEEP BEHAVIOR

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The unique anatomical connections and physiological activity of the calretinin positive (CR+) neurons in the dorso-medial thalamus (DMT) strongly suggest that these neurons have key role in collecting and transferring the ascending arousal signals to the forebrain. Previouslywe haveidentified, that graded optogenetic stimulation of DMT/CR+ cells can generate biological relevant arousal patterns (transient alternation of sleep rhythms, microarousals or permanent arousal). However, it is still unclear, whether DMT is necessary to maintain wakefulness. Here, using optogenetic manipulations together with polysomnographic recordings, we investigated, how the inhibition of DMT/CR+ neurons influences wakefulness in naturally behaving mice.

We monitored the behavior in the first two hours of the light phase (subjective night), when mice gradually switch from wakefulness to sleep, with and without selective optogenetic inhibition of DMT/CR+ cells for 36 minutes. We found that during inhibition, the amount of movement and EMG activity was significantly reduced, while EEG delta power significantly increased, when compared to the corresponding periods of non-inhibited days. These difference persisted even after switching the laser off. The altered activity could largely be attributed to the significantly shorter sleep onset as a result of DMT/CR+ inhibition. The sleep structure (non-REM and REM bouts) of the animals was not different between the inhibited and non-inhibited cases.

Our data show, that inhibition of DMT/CR+ activity decrease arousal, manifested in reduced locomotion and shorter sleep onset. We propose, that DMT/CR+ activity are necessary for proper wakefulness, and plays a major role in sleep/wake transitions in naturally behaving conditions.

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REPETITIVE MILD TRAUMATIC BRAIN INJURY CAUSES LONG-TERM COGNITIVE IMPAIRMENT IN RATS

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Repetitive mild traumatic brain injury (rmTBI) has been shown to affect cognitive abilities, and it can even lead to chronic traumatic encephalopathy. Rats with rmTBI hold potential as a functional model to study neurocognitive disorders such as Alzheimer's disease.

For inducing rmTBI, a weight-drop injury model was used (described by Marmarou et al. in 1994). In the initial pilot study, we developed a mild TBI in adult rats, with no prolonged effect on cognition and memory.

In the main study, our aim was to develop two rmTBI models, and compare their effects on cognition on the basis of the time interval between the successive injuries. Groups were defined on the basis of the severity of the injury: Sham (repetitive sham operation, no injury), single mild (mTBI – height: 15cm), repetitive mild (rmTBI – 15cm, 5 hits, 24 hours apart), rapid repetitive mild (rapTBI – 15cm, 5 hits, 5 minutes apart) and single severe (sTBI – 150cm) TBI. In the Novel Object Recognition (NOR) test, both rmTBI and rapTBI showed poor performance at 2 weeks post-injury,, while the single mTBI group showed no impairment. At 8 weeks post-injury, the rmTBI group still performed significantly worse than the Sham and mTBI groups, while the rapTBI group recovered. In the Morris Water Maze (MWM) test, at 6-7 weeks post-injury, all TBI groups performed similarly during the acquisition phase, whereas during the probe trial, the rapTBI group performed significantly worse than the Sham group.

Results suggest that rmTBI may prove as the best model for long-term cognitive impairment.

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THE EFFECTS OF BINGE DRINKING AND HANGOVER ON ANXIETYIN MICE

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The aim of the present study was to determine the effects of binge drinking and hangover on anxiety. Hence, male C57BL/6 mice were exposed to drinking in the dark, a classical animal model for binge drinking of alcohol: first the circadian rhythm of mice was changed for 14 days, then their water switched to alcohol of 20% for 4 days (2 hours on the first three days and 4 hours on the fourth day). Previous studies demonstrated that corticotropin-releasing factor (CRF) and Urocortin1 (Ucn1) induce anxiety and locomotor hyperactivity, whereas Urocortin2 (Ucn2) and Urocortin3 (Ucn3) produce anxiolytic and locomotor suppressive actions. Thus, the effects of CRF and the urocortins were also tested: CRF, Ucn1, Ucn2 or Ucn3 was administered intracerebroventricularly (icv) on the 4th and 5th day (immediately and 24 hours after binge drinking). After 30 min the mice were examined for signs of anxiety in an elevated plus-maze test for 5 min. Four days of binge drinking exerted anxiolytic effects in mice which, surprisingly, were enchanced by CRF and Ucn1, but not Ucn2 and Ucn3. In contrast, after one day of withdrawal the mice expressed signs of anxiety which were reversed, also unexpectedly, by CRF and Ucn1, but not Ucn2 and Ucn3. The present study suggests that binge drinking and hangover have different effects on anxiety, which are aggravated and attenuated paradoxically by CRF and Ucn1. However, the impact of the locomotor actions of CRF and the urocortins cannot be fully excluded.

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ACTIVITY OF BASAL FOREBRAIN NEURONS IN A CLASSICAL SUSTAINED ATTENTION TASK

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The basal forebrain (BF) plays key roles in multiple brain functions, including sleep-wake regulation, attention and learning/memory. The BF consists of cholinergic, GABAergic, and glutamatergic neurons. Stimulation of BF cholinergic neurons enhances cortical processing and visual attention performance including the detection of sensory cues, in which projections from the horizontal diagonal band of Broca (HDB) to the prefrontal cortex are especially important.

To test whether neurons of the HDB indeed show activity patterns correlated with attention, we trained mice on the 5-choice serial reaction time task (5-CSRTT), which measures the ability of rodents to sustain spatial attention over a large number of trials. We developed an automated training system, in which mice freely alternated between their home cage and a training cage according to a fixed time schedule. To investigate the firing patterns of HDB neurons during attention; therefore we implanted tetrodes to the mouse HDB. To verify the placement of the implanted tetrodes immediately after the surgery we combined CT and MRI scans.

We found that the automated training greatly improved learning speed. Mice maintained an average accuracy over 80% during recording. A number of neurons in the HDB responded phasically to light cues but decreased their firing rates during nose-poke events when the trial was rewarded. Firing rate changes in the ‘attention period’ preceding cue presentation were less frequent. These data indicate that HDB neurons may have a more distinct role in learning; however, a specific role of cholinergic cells in sustained attention is yet to be tested.

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14-MONTH-OLD INFANTS TRACK OTHERS' LINGUISTIC COMPREHENSION WHEN INTERPRETING AMBIGUOUS SEMANTIC LABELS

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The aim of the present study was to investigate how 14-month-old infants process linguistic labels in an object naming paradigm, in the presence of an adult observer, who has a false belief about the identity of a referred object. Event-related potentials were recorded in order to explore whether infants expect the observer's false belief to be about a specific item or an object category, as an item's label could refer to different exemplars of a category.In half of the trials, the observer and the infant believed that the same object was labelled. In the other half of the trials, unknown to the observer but visible to the infant, the object was switched to another, perceptually distinguishable exemplar from the same category before labelling (e.g., a red apple switched to a green one, labelled "apple"). Based on the results, 14-months-old infants showed a significantly larger frontal positivity in the 300-700 ms time window when the observer had a false belief about a referred object's identity, even though the label was correct for both parties. Thus, infants, as young as 14 months old, seem to track a communicative partner's belief about a referred object's specific identity, and understand that the same semantic label might refer to different exemplars according to one's knowledge.

ACTIVATION OF GABAERGIC CELLS IN THE EXTENDED AMYGDALA DURING MEMORY CONSOLIDATION DISRUPTSFEAR MEMORY EXTINCTION

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Consolidation of fearful memories is canonically attributed to the prefrontal cortex, basolateral amygdala and ventral hippocampal network. Formation of extinction-resistant fear memory and its generalization to other contexts are still poorly understood, despite constituting the core symptomatology of several anxiety disorders. The bed nucleus of stria terminalis (BNST) belongs to the aforementioned neuronal network of interconnected limbic regions, suggesting its integrative modulatory role in fear memory formation and anxiety-related behaviors. BNST consists of mainly GABAergic neurons (vGAT+) expressing different neuropeptides such as corticotrophin-related factor (CRF+) and somatostatin (SOM+), but it is unknown how these cells modulate fear memory processes. Here, we used a chemogenetic strategy to study the involvement of genetically defined BNST cell populations in the consolidation of conditioned fear memory and in the expression of anxiety-related behaviors. We expressed stimulatory and inhibitory DREADDs (designer receptors exclusively activated by a designer drug) in either vGAT+, CRF+ or SOM+ neurons of the BNST. We tested whether stimulation or inhibition of vGAT+ neurons interferes with conditioned fear memory consolidation after cued auditory fear conditioning or affects innate anxiety in the open-field test. Stimulation of vGAT+ neurons enhanced fear recall and caused an anxiogenic effect. Manipulation of CRF+ neurons neither had any effect on fear recall nor anxiety-related behavior. Stimulation of SOM+ cells in the BNST during memory consolidation increased fear generalization and impaired fear extinction. Our results provide insight how aberrant neuronal activity in a subcortical structure could contribute to the emergence of extinction-resistant fear memory genesis.

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GENETIC LACK OF TRPA1 RECEPTOR ATTENUATES THE B-AMYLOID1-42-INDUCED NEUROTOXICITY IN THE BASAL FOREBRAIN CHOLINERGIC NEURONS

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The basal forebrain cholinergic (BFC) neurons in susbtantia innominata and nucelus basalis magnocellularis complex (SI-NBM) play essential roles in learning, they are affected in Alzheimer's disease (AD). Amyloid beta ($A\beta_{1-42}$) accumulates in AD that is toxic to BFC neurons. Transient Receptor Potential Ankyrin1 (TRPA1) receptors are expressed in nociceptive neurons and astrocytes, however their role in degenerative central nervous system diseases is unclear. We aimed to investigate TRPA1 receptors in $A\beta_{1-42}$-induced neurotoxicity.

The presence of TRPA1 receptor on cholinergic neurons and astrocyte was examined by fluorescence immunohistochemistry for TRPA1, choline-acetyltransferase (ChAT) and anti-glial fibrillary acidic protein. 300μM $A\beta_{1-42}$ was injected into SI-NBM of adult male wild type ($TRPA1^{+/+}$) and TRPA1 knockout ($TRPA1^{-/-}$) mice. Cholinergic fibre loss was visualized by acetylcholinesterase, cholinergic cell loss with ChAT immunohistochemistry. Novel object recognition (NOR), radial arm maze (RAM) and Y-maze tests were used to investigate memory loss.

The triple labelled immunohistochemistry revealed TRPA1 expression on cholinergic neurons in SI-NBM region. $A\beta_{1-42}$-injected WT mice showed significant cholinergic cell loss and cholinergic fiber loss. In $TRPA1^{-/-}$ mice $A\beta_{1-42}$-induced cholinergic cell and fiber loss was significantly attenuated. In both NOR and RAM tests significant memory loss was detected in $A\beta_{1-42}$-injected $TRPA1^{+/+}$ mice, but not in $TRPA1^{-/-}$ group.

We demonstrated that TRPA1 receptors play a crucial role in the $A\beta_{1-42}$-induced cholinergic damage in the SI-NBM. Based on our data this receptor might be a promising drug target in the future therapy of AD.

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ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR AGONIST PHA-543613 IMPROVES LONG-TERM MEMORY AND ATTENTION IN AGED RATS

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The α7-nAChR is a promising drug-target for improving cognitive deficits in neurocognitive disorders (NCDs) Aging is the main risk factor for NCDs, and previous studies showed that long-term memory and attention in human subjects and rats may deteriorate with aging. Here, our aim was to investigate the effect of α7-nAChR agonist PHA-543613 (PHA) on various cognitive functions in a natural aging model using laboratory rats. The experiments were carried out with young (5-17 month old) and aged (22-34 month old) male Lister Hooded rats. Long-term memory function of the animals was tested with novel object recognition (NOR) using 24-hour retention interval, and in water maze (MWM) paradigms. Attentional functions were tested with the psychomotor vigilance test (PVT). Effects of PHA was tested at 0.3 to 3 mg/kg doses.

We found that PHA at the dose of 1 mg/kg improved the discrimination index of the aged animals, however the young animals compared to the vehicle treatment. In the MWM aged animals showed significantly worse performance compared to the young animals. Dose-dependent performance improvement was found with the aged animals, but not with young animals. Reaction time and task performance rate of the aged animals were significantly improved by PHA at both doses.

To sum up, these results suggest that using α7-nAChR agonists may potentially reverse aged-related cognitive deficits, hence it can be an effective approach for the treatment of dementia. Furthermore, naturally aging animals may be a useful behavioral pharmacological model for translational research in the future.

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ROLE OF THE DOPAMINERGIC CELLS IN THE MEDIAN RAPHE IN THE BEHAVIOUR OF MALE MICE

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Dopamine; social behaviour; anxiety; aggression; sociability; openfield; median raphe region; mice

According to previous studies the median raphe region (MRR) is known to contribute significantly to social behavior. Beside serotonin there are some dopaminergic neurons in this region. Dopamine is linked to reward and locomotion, but very little has been discovered about its role in the MRR. This experiment was designed to clarify this question. We used pharmacogenetic technology in mice containing Cre enzyme under the promoter of the dopamine transporter (DAT). With the help of adenoassociated virus artificial receptors (both stimulatory and inhibitory as well as control mCherry) was injected into the MRR. Several weeks later 30 min after injection of the artificial ligand (clozapine-N-oxide) locomotion (open field/OF), social behavior (sociability, social interaction/SI), anxiety (elevated plus maze/EPM) and short-term memory (y-maze) were studied. Manipulation of the dopaminergic cells of MRR had no effect on locomotion (OF, closed arm entries in EPM, total arm entries in y-maze). Stimulation of DAT+ cells of MRR decreased social interest (sociability and SI, detectable even 24h later) and increased aggression with a tendency of reduced anxiety and better short-term memory. Stimulation of dopaminergic neurons showed the opposite of what was shown for the whole MRR. Nevertheless, these findings support specific role of MRR dopaminergic cells in social behaviour, anxiety and memory formation.

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THE EFFECT OF UROCORTIN 3 ON CHRONIC MILD STRESS INDUCED ANXIETY

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Urocortin 3 (UCN3), a CRF-related peptide, binds CRF receptor 2 selectively that might be involved in stress coping. UCN3 was found to have an anxiolytic and anti-depressant effect. In the present study our aim was to investigate if repeated central administration of UCN3 is able to reduce the anxiogenic behavior observed in chronic mild stress (CMS).

Wistar adult male and female rats were subjected to a 6-week long regimen of unpredictable mild stress. After intracerebroventricular cannulation, animals received 2 µg/2 µl UCN3 daily for 10 consecutive days. Animal behavior was assessed by open field (OF) and elevated plus maze (EPM) tests. In the OF horizontal and vertical locomotion, immobility time and central activity (time and distance travelled in the center of the arena) were observed. In the EPM we registered the time spent and entries into the open arms.

In the OF test the CMS group showed a decrease in the time spent and distance travelled in the center of the arena, this was partially reversed by UCN3. In the EPM test the CMS animals spent less time in and entered less frequently into the open arms. UCN3 alone did not effect the EPM parameters, but triggered a significant increase in open arm time and entries of the CMS animals.

The current findings, together with previous results, demonstrate that UCN3 exhibits anxiolytic-like effects in the CMS-induced anxiety model and underlie the possible therapeutic potential of CRF receptor 2.

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QUANTITATIVE ULTRASTRUCTURAL ANALYSIS OF NEURONAL MITOCHONDRIA IN THE MEDIAL PREFRONTAL CORTEX OF CHRONICALLY STRESSED RATS

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Chronic stress plays a key role in the pathophysiology of major depressive disorder (MDD). The chronic mild stress (CMS) protocol is a well-established model to mimic depressive like behavior in experimental rats and it is a useful tool to investigate neurobiological changes contributing to the disease. In our earlier studies, we found cellular alterations in the medial prefrontal cortex (mPFC) of chronically stressed rats (1, 2). The redox imbalance hypothesis is a theory that provides explanations for the subcellular biochemical changes in response to stress. Since mitochondria are major producers of various reactive oxygen species (ROS), one could hypothesize that the behavioral stress-induced subcellular oxidative stress may affect the morphology or number of these subcellular organelles. Here, we applied a quantitative electron microscopic analysis to determine the density and morphology of mitochondria in the mPFC of control (n=3) and CMS exposed (n=3) rats. We focused on the infralimbic region of the mPFC and we did a random systematic sampling procedure to make ultrastructural images with a transmission electron microscope (TEM) at 40 000x magnification. Images were analyzed with an unbiased stereology protocol. Circa 3000 EM images were examined and we counted 40000 mitochondria. The morphological parameters were evaluated on 7000 structures. We found no significant differences between the two groups. In sum, our present preliminary data could not reveal any stress-induced morphological changes affecting neuronal mitochondria.

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DEOXYNIVALENOL AFFECTS NEURONAL ACTIVITY IN THE BRAIN REWARD SYSTEM AND INHIBITS MOTIVATIONAL BEHAVIOR IN MOTHERS

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Deoxynivalenol (DON), or vomitoxin, is a mycotoxin produced by Fusarium graminearum and culmorum. Mycotoxins or secondary metabolic products of mould fungi, are micropollutants, which affect human and animal health. The neuronal and behavioural actions of DON were addressed in the present study. In the first experiment, the neuronal activation pattern following intraperitoneal injection of DON into adult male rats at a dose of 1 mg/kg was investigated while control animals received physiological saline solution. Brain sections were labeled for c-Fos immunohistochemistry. DON induced significant c-Fos activation in the accumbens nucleus, the medial prefrontal cortex and the ventral tegmental area, while other brain regions did not show increased c-Fos expression. Further double labeling studies suggested that local interneurons may be activated by DON treatment. The activation pattern suggests that DON influences the reward system of the brain. To assess the behavioural relevance of this activation, we examined the effect of DON on a special goal-directed behaviour, the pupcaring behaviour in mother rats. Pup retrieval latencies were increased by DON administration, and DON-treated mother rats spent less time with nursing suggesting reduced maternal motivation. In agreement with the behavioural inhibition, electrophysiological recording on rat brain slices indicate that in vitro field responses evoked by electrical stimulation also tend to decrease in the nucleus accumbens as a result of DON pretreatment. The data imply that acute uptake of the mycotoxin DON can influence the reward circuit of the brain and exert negative behavioural actions.

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CHEMOGENETICALLY INFLUENCED GABAERGIC NEURONS IN THE PREOPTIC AREA OF MICE AFFECT MATERNAL BEHAVIOURS

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The early mother-infant relationships have long-term effects on the offspring and the mother. The expression of the maternal behaviour in mammals is regulated mostly by the medial preoptic area (MPOA). Previous results of our laboratory suggest that the tuberoinfundibular peptide 39 (TIP39) is involved in the control of maternal motivation. TIP39-containing fibers are abundant in the MPOA. In the present study, we examined the GABA-ergic neurons of the MPOA system regarding their role in maternal behavioural control and their innervation by TIP39-containing terminals as well as their projections from the MPOA. First, we performed immunohistochemistry in mice expressing a fluorescently tagged vesicular GABA transporter (VGAT) and found that many TIP39-containing fibers target the GABAergic neurons in the MPOA. Moreover, we also found that numerous GABA-ergic cells show neuronal activation in mother mice. Then, to determine brain-wide outputs from GABAergic neurons of the MPOA, we used adeno-associated virus (AAV)-MCherry tracing. Most of the brain areas that play an important role in parenting, receive direct inputs from the injected cells. Furthermore, to reveal the function of GABA-ergic neurons in the MPOA on the maternal care we measured spontaneous maternal behaviour and performed pup retrieval test on the VGAT-Cre virgin female mouse stimulated chemogenetically following AAV-mediated DREADD-infection in the MPOA. The results demonstrate that chemogenetical inhibition of GABA-ergic neurons in the MPOA attenuated, while the excitation of these cells intensified maternal behaviour compared to the behaviour of control mice. The results suggest a critically important role of GABA-ergic neurons in the MPOA in the control of maternal responsiveness.

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ROLE OF VESICULAR GLUTAMATE TRANSPORTER 3 POSITIVE CELLS OF THE MEDIAN RAPHE REGION IN SOCIAL BEHAVIOUR

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VGluT3; social behaviour; anxiety; memory; aggression; sociability; open field; DREADD; chemogenetics; median raphe region; mice

Although median raphe region (MRR) is known to be a serotoninergic nucleus with significant contribution to social interaction, but it also contains glutamatergic neurons, characterised by vesicular glutamate transporter 3 (VGluT3) with unknown role.

We used Creand pharmacogenetic techniques (synthetic receptor)in mice to assess the function of VGluT3 in locomotion (open field/OF); social behaviour (sociability; social interaction/SI; resident intruder test/RI); anxiety (elevated plus maze/EPM); and memory (Y-maze; social discrimination/SD, 24h after sociability, the later without CNO (the arteficial ligand) injection).

In OF the inhibitory group moved less. During sociability all groups had intact social interest, and the excitatory group spent less time with the objects and conspecifics. In SD the inhibitory group spent more time with conspecifics without discrimination. Inhibition of synthetic receptor increased friendly social contacts in SI with a tendency in RI test.In EPM the excitatory group entered more oftento the open arm. Y-maze revealed no differences between the groups.

The activity of MRR VGluT3+ neurons wasinversely proportional to friendly social behaviour and decreased anxiety. Pharmacogenetic manipulation has a long lasting effect as seen in SD.

PARENTING-RELATED GENE EXPRESSIONAL CHANGES IN THE BRAIN OF FEMALE ZEBRA FINCHES

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Gene expressional alterations related to parentinghavebeen studied in female rodents. However, in mammals, the role of altered genes can be related to parental care and lactation. To eliminate this ambiguity, here, we compared gene expression in zebra finch (*Taeniopygiaguttata*) between female members of pairs during post hatching period compared with females living in social pairs. Previously, we established that zebra finch brain regions, which contain neurons activated in response to nestlings are located in the hypothalamic and septal regions. Therefore, brain tissue blocks containing these parts of the brain were dissected and investigated. Differentially expressed genes were found by new generation RNA sequencing.Real-time qRT-PCR validated the changes for all 3 altered genes examined.The crystalline mu and the vasotocin-neurophysin genes were up-regulated, while the growth regulation of estrogen binding 1 gene showed down-regulation in parenting condition. In situ hybridization histochemistrylocalized the expression of genes in the brainincluding the preoptic, ventromedial and paraventricular nuclei of the hypothalamus, the nucleus accumbens and the bed nucleus of stria terminalis with gene-specific distribution patterns. In birds some of these areas have already been identifiedin differentaspects of parental care, such as nest building and feeding. Among the 3 genes, only vasopressin has been implicated in parental care. Our resultssuggest that activated neurons in brainregionsinvolved in maternal behavioural controldemonstrate parental alteration in the expression of specific genes. The specific functions of the identified genes need to be investigated in further experiments.

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THE FINESTUCTURE OF EYE MOVEMENTS REVEALS PREDICTIVE INTERNAL MODELS OF DYADIC AGENT DYNAMICS IN 14-MONTH-OLD INFANTS

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Humans employ rich internal models to make predictions about and interact flexibly with the environment. Eye movements have been shown to reflect sophisticated cognitive operations relying on such internal models and their measurement thus potentially offers a window onto how subjects develop and employ their internal models. However, studies of infant cognition typically only use very simple measures of eye movements, such as relative looking times between two discrete targets, which provide limited information about their internal models. Here we used statistically principled maximum likelihood-based methods to analyse the moment-by-moment fine structure of pursuit and saccadic eye movements of 14-month-old infants while subjects viewed dynamic scenes including two, potentially interacting agents. We found that subjects' gaze followed more predictably moving agents with a smaller time lag, consistent with an understanding of dyadic interactions between agents, and these time lags were consistently smaller than those of purely reactive eye movements in response to non-predictable events in the scene. These results provide quantitive evidence for rich internal models of complex, multi-agent scenes in young infants.

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NEURONAL ACTIVITY AND EPIGENETIC CHANGES IN LIMBIC BRAIN TERRITORIES IN THREE HIT THEORY OF DEPRESSION

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Depression encumbers society and healthcare due to its increasing prevalence. Lack of an equivocally accepted animal model may result from largely unknown and possibly heterogeneous pathomechanism. Based on the three hit theory, the coexistence of genetic, epigenetic and environmental stress factors may cause the disease. PACAP heterozygous mice (genetic factor) with history of maternal deprivation (epigenetic model) were exposed to chronic variable mild stress. To support the predictive validity of our model, half of animals was subjected to fluoxetine treatment. FosB, as a marker for neuronal activation and acetylated H3 histone protein, as an indicator of epigenetic changes were examined in 17 brain areas. 11 of 17 investigated territories showed significant changes in FosB expression. The number of suffered hits (5 areas), the treatment (2 regions) and their interaction (4 nuclei) influenced neuronal activity. The ecethyl-H3 histone labeling revealed that both stress and treatment affect the acetylation of this protein in PFC and BST. However, when mice with maternal deprivation history were exposed to these factors, no epigenetic change was observed. In summary, genetic, epigenetic and environmental stressors alone or in interaction set a pattern of neuronal activity that may be partially influenced by standard antidepressant therapy. Maternal deprivation applied in young age causes significant changes in epigenetic sensibility to stress and fluoxetine treatment in stress adaptation centers of the brain. Our results may help to understand what interactions of genetic susceptibility, epigenetic alterations and environmental stress causes depression, and what constellation of these leads to therapy resistance.

NEURAL BASIS OF DISTRACTOR RESISTANCE DURING WORKING MEMORY MAINTENANCE

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Visual working memory (WM) representations must be protected against the interfering distractor stimuli. It is widely believed that this might be accomplished bythe same visual attentional selection processes that are active during visual perception, leading to enhanced and suppressed global visual cortical processing of task relevant and irrelevant information, respectively.However,the validity of this view has been questioned based on theinconsistency of the related experimental results as well as recent theoretical advances. Here we show that WM-content-dependent modulation of EEG responses to distractor stimuli presented during the maintenance period is confined to the later stages of visual information processing, starting around 200 ms, which is inconsistent with the perceptual attentional selection account predicting attentional effects at the earlier ERP components such as N1. Furthermore, the results of our fMRI experiment revealed significant differences in WM-content-dependent modulation of the fMRI responses between specific areas of the same visual cortical face network, the middle fusiform face area (FFA) and the posterior FFA. In fact, when faces as opposed to gratings had to be maintained in WM, fMRI responses to grating distractors were reduced and unaltered in the posterior and middle FFA, respectively, even though response enhancement would be expected in both regions according to the global attentional selection account. Taken together, our findings open a new perspective on WM distractor resistance which involves feedback processes that prioritize and protect information processing in specific visual cortical areas containing the most precise task-relevant WM representations.

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THE BENEFICIAL EFFECTS OF COGNITIVE TRAINING IN CONTROL AND SCHIZOPHRENIA-LIKE WISKET RATS

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Introduction: The cognitive therapy has beneficial effects for several behavioral symptoms in schizophrenic patients. To enhance the face and predictive validities of a schizophrenia rat model (Wisket), the effects of cognitive training was disclosed in control and Wisket animals.

Methods: Four groups of animals were involved in the experiment: trained Wistar and Wisket rats after a 10-day long training in the AMBITUS with different tasks starting at the age of 10 weeks, and their non-trained corresponding. At the age of 14 weeks all groups took part in a 4-day long experiment with a new task. The AMBITUS is a rectangular corridor with 8-8 side-boxes along the both sides of each wall, that is an appropriate device for food-rewarded behavioral testing of rats. It provides automatic registration of the locomotor and exploratory activities, while the eating parameters could be obtained from video records.

Results: The Wisket animals exhibited altered locomotion, exploratory and food collecting activities at the first few days of the training session, revealing impaired motivation and learning ability. Most of the parameters normalized with training, except for the decreased exploratory activity. Tests with a new task after a 2-week delay revealed that both trained groups had long-term improvement in their motivation and cognitive functions compared to the non-trained ones.

Discussion: The results resembles the effects of cognitive behavioral therapy in human schizophrenics providing a significant support for the predictive validity of this substrain as an animal model of schizophrenia.

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INTERLEUKIN 6 DEFICIT RESULTSIN PASSIVE COPING AND DEFENSE

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Introduction: Interleukin 6 (IL-6) is a pleiotropic cytokine,a key member of the pro- and anti-inflammatory pathways. It hasan essential role in CNS function and may contributein several neurological/psychiatric diseases.
Aim: We aimed to examine behavioral changes in IL-6 knockout mice using a battery of behavioral tests.

Method: Sociability and social novelty-, social interaction-, aggression-, elevated plus maze-, Y maze-, shuttle box-, operant conditioning-, startle-, conditional fear-and tail suspension tests were performed.

Results:IL-6 KO was achived by inserting a STOP kodon to the first exon of 4th splice variant by CRISPR-Cas9 in FVBAnt mice. IL-6 deficient mice displayed significantly more defensive behavior during aggression test, visited significantly more arms in the Y maze,failed to escape or escaped with significantly higher latency from the foot shock in the shuttle box test, showed significantly more startle responses, freezed with significantly higher frequency in conditional fear test and were significantly more immobile during the tail suspension test compared to the control group.

Conclusion: IL-6 deficiency alters the behavioral phenotype of micewith passive coping and defense. These findings underscore the importance of cytokines in general and IL-6 in particular in the regulation of behavior.

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BINGE DRINKING AND HANGOVER HAVE DIFFERENT IMPACTS ON DEPRESSION IN MICE

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Corticotropin-releasing factor (CRF) and Urocortin1 (Ucn1) have been implicated in binge drinking of alcohol, anxiety and depression. The aim of the present study was to investigate the impacts of binge drinking and hangover on depression, and the additional effects of CRF and the urocortins (Ucn1, Ucn2 and Ucn3). Therefore, male C57BL/6 mice were exposed to drinking in the dark, a classical method used to investigate binge drinking in animals. According to this method the circadian rhythm of mice was inverted for 14 days, then their water changed to alcohol of 20% for 4 days (2 hours on the first three days and 4 hours on the fourth day). Intracerebroventricular (icv) administration of CRF, Ucn1, Ucn2 or Ucn3 was performed immediately and 24 hours after binge drinking. After 30 min the mice were investigated for signs of depression in a forced swim test that lasted 6 min. Four days of binge drinking induced antidepressant effects in mice that were increased by icv administration of Ucn1, but not CRF, Ucn2 and Ucn3. However, after one day of withdrawal the mice produced signs of depression that were enhanced by icv administration of CRF, but not the urocortins. The present study suggests that binge drinking and hangover have different impacts on depression, which are further emphasized by Ucn1 and CRF, respectively. The participation of CRF receptors (CRF1 and CRF2) in these effects are yet to be determined.

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CRF SYSTEM MEDIATES ANXIETY IN CHRONIC KIDNEY DISEASE

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Chronic kidney disease (CKD) is commonly associated with anxiety, depression and cognitive impairment, the background of which is not yet defined. The aim of the present study was to investigate the effect of CKD on anxiety-like behavior and the expression of stress-related genes: corticotropin-releasing factor (CRF), arginine vasopressin (AVP)and CRF receptor 1 and 2 (CRFR1, CRFR2).

CKD was induced by performing a two-phased 5/6 partial nephrectomy in adult male Wistar rats. The development of CKD was confirmed by measuring serum urea and creatinine levels. The behavior of the animals wasassessed7 weeks after nephrectomy using the computerized open field (OF) and elevated plus maze (EPM) tests. The gene expression of CRF, AVP, CRFR1 and CRFR2 were also determined in anxiety-associated brain regions: amygdala, prefrontal cortex (PFC) and hypothalamus.

Following nephrectomy, the elevated serum urea and creatinine levels confirmed CKD. In the OF test,CKD induced a significant increase in immobility, whereas vertical locomotion, central ambulation and central time were decreased. The EPM test showed a reduction in thenumber of open arm entries and open arm time. The relative gene expression of both CRF and CRFR1 significantly increased in the amygdala and PFC, whereas the upregulation of CRFR2 was detected in the hypothalamus. AVP expression also increased in the hypothalamus, but downregulation was observed in the amygdala.

The results of the behavioral tests are characteristic of anxiety-like behavior, which might be explained by the upregulation of CRF and CRFR1 in the amygdala.

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MAPPING OF THE NEURONAL ACTIVATION MECHANISMS UPON SOCIAL ENCOUNTER IN RAT

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In a previous study, we identified the posterior intralaminar complex of the thalamus (PIL) as a relay station of socially relevant sensory information innervating and activating oxytocin-secreting neuronsupon social encounter.Our present aim was to describe the activation pattern in the whole brain during social interaction in rats and to examine the role of the PIL, and its TIP39-containing cells in the process.

We examined the interactionsbetween 2 familiar and 2 unfamiliar female rats. The activation patterns were determined following direct interaction, and also with the exclusion of physical interaction. The activation of the cells was determinedby the c-Fostechnique.Wealso performed chemogeneticstimulationof the PIL, and anterograde tract-tracing from the area. TIP39-immunreactivity in labeled fibers was examined with double immunohistochemistry.

Significantly higher level of activation upon any social encounter was found in the PIL, the medial amygdala, the somatosensory and infralimbic cortices and the nucleus accumbens, compared with control animals withoutany social interactions. The highest density of fibers projecting from the PIL was found in the mesencephalon, the amygdala and the medial preoptic area. Infralimbic cortex and medial amygdala were activated following chemogeneticstimulationof the PIL.

The results suggest that the PIL may convey socially relevant information to several other brain regions. The PIL and other brain regions activated by social encounter may participate in the formation of social behaviors, contributing to the regulation of itscomplex neuronal mechanism.

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MEDIAL SEPTAL PACEMAKER NEURONS SYNCHRONIZE IN PHASE NOT IN FREQUENCY DURING HIPPOCAMPAL THETA GENERATION

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The hippocampal theta oscillation typically occurs during exploratory behaviours and REM sleep and has been linked to learning and memory. The medial septal region of the basal forebrain has been identified as responsible for theta generation. According to the leading theory, rhythmically active individual ‘pacemaker’ cells, firing at their own frequencies, are synchronized to a common frequency and thus give rise to the hippocampal theta. However, experiments in which multiple septal neurons were recorded concurrently are rare and therefore the mechanisms of septal theta synchronization are still debated. To address this, we aimed to decipher the network mechanisms of rhythm genesis in the medial septal circuit by analysing multiple simultaneously recorded medial septal neurons from an anesthetized rodent model of hippocampal theta oscillation in both rats and mice (sensory stimulation-evoked theta). Additional recordings were performed in awake drugfree mice (spontaneously forming theta). A group of medial septal neurons showed persistent theta rhythmic firing, endowing them with the capability to pace the hippocampus when synchronized. By testing multiple possible ways of their temporal coordination relative to each other we rejected the aforementioned theory of frequency synchronization. On the other hand relative timing of action potentials during the theta cycle supported the notion of phase synchronisation as a powerful basis of theta genesis. To better understand the mechanisms underlying theta generation we also built a minimal network model consisting of theta rhythmic pacemaker units. We have demonstrated that this simple model can capture various features of septal theta genesis.

SIGNAL SHAPE BASED FUNCTIONAL NEURON CLUSTERS FROM TWO-PHOTON IMAGING OF LEARNING MICE

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Novel imaging solutions allow the fast 3D recording of neural networks from all cortical layers with a temporal resolution of up to 100 Hz. Simultaneous acousto-optical scanning of hundreds of cells at cortical depths of over 1 mm with red-shifted calcium indicators makes it feasible to examine the effect of learning in behaviour experiments at single-cell and network scales. Our automatic data analysis workflow involves (i) non-rigid motion correction between deformed scenes of different days, (ii) 3D motion correction of volume scans, (iii) activity-based Ca source and background component detection, (iv) ΔF/F calculation, and (v) cell registration on multiple-day measurements before and after learning. Finally, we present a clustering method based on the shapes of Ca2+ signals (common signal components) and make an attempt to find relation between these groups and the functional clusters of cooperating cells in the network.

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FITTING HAWKES PROCESSES TO MULTI-UNIT ACTIVITY OF AN EPILEPTIC PATIENT

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Hawkes process is a general framework of self-excitationregarding point processes. In the special case of exponential core function self-excitation is characterized by two parameters, volatility and friction. These control the length and density of trains of events (bursts) to occur, but it can be shown that imbalance of these two parameters may lead to the explosion of the process.We note that an additional parameter – mean firing rate – is used. Our aim was to investigate how processes fitted to human multi-unit activity (MUA) change during epileptic activity.

We recorded MUA from a patient with therapy refractory epilepsy using laminar microelectrodes. After applying a band pass filter (500-3000Hz) the signal was thresholded using a median based filter in order to derive series of events from the continuous signal. Hawkes processes were fittedto distinct epochs of the data using maximum likelihood estimation (MLE). Epochs were represented with their parameters acquired during the MLE. Significance of the results was determined by computing the confidence ellipsoid of a given estimate.

We found clear separation between epochs recorded during interictal periods and seizure in the parameter-space. Also, we observed that processes fitted to seizure-containing epochs drifted towards unstable regimes.

DESIGN OF GAP JUNCTION INHIBITORS: IDENTIFYING STRUCTURAL PRINCIPLES OF ASTROCYTIC AND NEURONAL CONNEXIN MODELS

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Gap junctions (GJ) play a key role in connecting astrocytic as well as neuronal networks. Distinguishing astrocytic and neuronal GJ communicationrequires selective and potent inhibitorsfor both the astrocytic connexin 43 (Cx43) and the neuronal connexin 36 (Cx36) isoforms. Specific small molecule inhibitors, however, do not exist to date, selective inhibition of astrocytic GJs can only be achieved by large peptides and antibodies that do not cross the blood-brain barrier. Here, we present a structural approach of delineating crucial extracellular residues in known connexin structures in order to design and discover selective and potent small molecule inhibitors.

The first connexinstructure,obtainedby cryo-electronmicroscopy(Cx43, Unger et al., 1999) provided information only on the helical arrangement and served as a basis for identifying the position of the protein's main chain (Fleishmann et al., 2004). Using the recently determined atomic structure of Cx26 (Maeda, 2009; Bennett, 2016), we identified residue pairs, responsible for stability of hemichannels and full connexins. We found that a ring of residue pairs from the extracellular loops EL1 (Glu 187) and EL2 (Ser72) provide the stabilization link among Cx26subunits. These residues correspond to Ser and Glu inCx43as well. Furthermore, stabilization of the full Cx channel is supplemented by strongly networking EL1 residues.

In parallel, we constructed a rough model of Cx43 transmembrane regions and EL loops,criticalfor connexin formation from hemichannels. We found that residues important from the viewpoint of stability are part of the Cx43-specificGAP26 and GAP27 peptides. Byidentifyinga significantly smaller number of structurally important, channel forming residuesand differentiating between conserved and subtype-specific residues in the Cx43 model, our approachwill serve the drug design process to discoverselective Cx43 inhibitors in the future.

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CHRONIC CALCIUM IMAGING OF NETWORK ACTIVITY IN FREELY BEHAVING MICE WITH MINIATURE FLUORESCENT MICROSCOPE

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Detecting activity of the same neuronal network in freely behaving animals over a long time-course (up to several months) is a major challenge in neuroscience. Cellular level monitoring requires invasive, mechanically stable recording setup which often has the drawback of introducing factors that might destroy the investigated area in the long run. Here, in our pilot study we tested how long the adeno-associated virus infected and GCamP6f expression introduced hippocampal neurons can be monitored with chronically implanted grin-lenses and miniscopes in freely behaving animals. GCamP6f expressing neurons showed normal morphology and Ca dynamics up to 1 year after virus infection and 4 months of grin-lens implantation. The same neuronal elements of the recorded area have been monitored during each recording session. We concluded that miniaturized microscopes are suitable for detecting neuronal network dynamics evolving during learning and aging.

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INVESTIGATING KYNURENIC ACID PRODUCTION AND KYNURENERGIC MANIPULATION ON ACUTE MOUSEBRAIN SLICE PREPARATIONS

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Manipulation of kynurenic acid (KYNA) level through kynurenine aminotransferase-2 (KAT-2) inhibition with the aim of therapy has been the subject of extensive recent research. Although mouse models are of particular importance, neither the basic mechanism of KYNA production and release nor the relevance of KAT-2 in the mouse brain has yet been clarified. Using acute mouse brain slice preparations, we investigated the basal and L-kynurenine (L-KYN) induced KYNA production and distribution between the extracellular and intracellular compartments. Furthermore, we evaluated the effect of specific KAT-2 inhibition with the irreversible inhibitor PF-04859989. To ascertain that the observed KYNA release is not a simple consequence of general cell degradation, we also examined the structural and functional integrity of acute brain slices with biochemical, histological and electrophysiological tools.
We did not find dramatic changes in the viability of the brain tissue after several hours incubation time. HPLC measurements proved that mouse brain slices intensively produce and liberate KYNA to the extracellular compartment, while only a small proportion retained in the tissue. Finally, specific KAT-2 inhibition with PF-04859989 significantly reduced the extracellular KYNA content.
Taken together, these results provide important data about KYNA production and release, and evidence for the first time of the function of KAT-2 in the adult mouse brain. Our study extends investigations of KAT-2 manipulation to mice in a bid to fully understand the function.

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PROBABILISTIC TRACTOGRAPHY SHOWS CORRELATION WITH CORTICO-CORTICAL EVOKED POTENTIALS (CCEP) OVER ICTAL AREAS

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For patients suffering from drug-resistant epilepsy, surgery can be the most effective treatment. The operation is less successful for extratemporal lesions, especially when seizures are not associated with structural malformations. Our aim was to predict the results of CCEP mapping using probabilistic tractography, in order to better describe fiber-tracks and networks participating in the seizure genesis.

Four epileptic patients live with extratemporal epilepsy fulfilledour inclusion criteria;all of them were candidates for epilepsy surgery and were implanted with subdural electrodes prior tosurgical resection. CCEP mapping was performed using bipolar stimulation on every neighboringelectrode pairs while the evoked potentials were recordedon the remaining electrodes(0.5Hz, 0.2ms, 10mA). Ictal EEG was analyzed to find electrodes that were involved in seizure onset and early spread. We selected midpoints between stimulation electrode pairs as seed regions, and every recording electrode was marked as a target region for probabilistic tractography. Probabilistic fiber tracking was initiated from each bipolar stimulation zone to the remaining contacts. The z-score of the CCEP amplitudes and mean connectivity values of probabilistic tractography were correlated between the stimulated and the recording electrodes using bivariate Pearson correlation.

From 126 cortico-cortical stimulationsplaced over ictal areas, 55.2% correlated significantly with the results of tractography. Two patients showed high correlation (75%) and two patients showed low correlation (35.42%).

The combination of these two methods can facilitate to survey the expansivity of the ictal system. Using non-invasive mapping methods, we attempt to substitute invasive surgical investigations to revealthe epileptic network.

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MODELL-FREE UNIQUE EVENT DETECTION IN TIME SERIES

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Outlier detection in time series is a standard part of data processing pipelines and has paramount interest in neuroscience, physics and finance. Outliers can be disturbances such as eye blinking artifacts on EEG recordings but in many cases, anomalous rare events are subject to extreme interest such as the detection of epileptic seizure onsets, gravitational waves or fraudulent activity on online accounts.

In this work, a new outlier detection algorithm is presented to discover a particular class of outliers: unique events. These particular outliers lie on those parts of the state space which has been visited only once by the trajectory. Our algorithm heavily uses nonlinear time series analysis techniques, we apply time delay embedding and compute average time-distance statistics locally from nearest neighbour time-indices in order to detect unique states in the reconstructed state space.
We tested our new method on simulated examples and compared it's performance with a density based outlier-detector. We found that our method showed superior performance according to f1-score and ROC AUC metrics.
We also demonstrate results on intracranial LFP recordings to detect ictal spikes and an epileptic seizure onset.
We demonstrated, that unique event detection is a new and powerful approach to outlier discovery, which adds a new aspect to the existing methods on the field, showed its performance on simulated examples and on LFP datasets. In the light of these results, our method has the potential to find application in many scientific fields, including neuroscience.
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MODELLING ALZHEIMER'S DISEASE WITH 3D ENGINEERED NEURONAL TISSUE DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

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Induced pluripotent stem cells (iPSCs) of genetically and clinically characterized patients with Alzheimer's disease (AD) and controlswere used to study the pathomechanism of AD *in vitro* in a 2D cellular model by our team (Ochalek et al. 2016).These iPSCs were further studied using an air-liquid interface based, scaffold-free system where 3D engineered neural tissue (3D ENTs) were produced. After 10 and 14 weeks of differentiation we performed an in-depth analysis to characterize the 3D cultures. We studied the cellular composition of the 3D ENTs by histochemistry, gene expression and ultrastructural level, which confirmed the presence of NPCs, neurons, astrocytes, and oligodendrocytes myelinating the neurons in the tissue.Gene expression analyses confirmed the presence of glial cells, various neuronal subtypes including cortical neurons and synaptic proteins. Neuronal tissue specific functional assay, the multi-electrode array (MEA) demonstrated the electrophysiological properties of the neuronal circuits building up the ENTs. 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, we detected more Aβ deposits in diseased cultures than in control cultures by immunostaining. Disease related electrophysiological differences were also detected by MEA measurements. 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 for drug development.

SIMULTANEOUS IN VIVO PHOTOSIMULATION AND CALCIUM IMAGING WITH MULTI-3D ACOUSTO-OPTICAL TWO-PHOTON MICROSCOPY

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The widening toolset of optogenetics is one of the major directions of developments in two-photon microscopy, therefore simultaneous stimulation and imaging in 3D is an excellent option to further understand mechanisms in the living brain. Furthermore, compared to electrical recording and stimulation, optical methods enable stimulation and measurement on the dendritic level in flexible spatial and temporal patterns without damaging the living tissue.

Efficacy of two-photon optogenetics is usually limited by the single focus stimulation. Here we describe a novel acousto-optical scanning solution that overruns this limitation by controlling two laser lines with different wavelengths in three dimensions. Switching between the two lasers and scanning patterns happens almost instantaneously making possible to perform photostimulation and Ca^{2+}-imaging in an interleaved fashion. By fine-tuning the scanning parameters, we were able to record every second frame for imaging during stimulation, and following activity even during optical stimulation. As a result, since interlaminar connection is a crucial feature of cortical processing, with this method we can select, image and stimulate neuronal ensembles in a more sophisticated way than with conventional methods, taking us closer to dynamic cortical connection mapping and understanding of the main features of information processing.

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SPATIAL AUTOCORRELATION (MORAN'S I) REDUCES BIAS OF OBJECT DELINEATION IN MICROSCOPIC IMAGES

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A general problem of biomedical image analysis is the unbiased selection of the objects of interest. Current methods are mostly based on subjective selection by the observer or on predefined but arbitrary threshold values. These approaches are user dependent, can be difficult to generalize across different samples and have major problems in case of noisy images. Here we provide a user independent spatial correlation analysis (Moran's I) method that delivers reliable segmentation of objects even with extreme levels of noise and independent of absolute values of image intensity or decisions of the observer. This method does not only consider intensity of pixels but their correlation with their neighbors, too.
In model experiments the local Moran's test selected all pixels of the predefined objects but practically none from the background. Using increasing amount of noise Moran's test avoided selection of background areas whereas threshold-based segmentations selected more and more of the background pixels. The method was also tested on vGluT2 boutons of thalamic VPM nucleus of rats in the first weeks of postnatal development of sensory ascending pathway. It was capable of delineating boutons on normal and modified images with artificially added noise.
Altogether, the Moran's method did not deliver false positive pixels and it selected true positive pixels similar to either threshold based methods or manual delineation. We propose that this method can also be utilized for image segmentation especially on images with low signal to noise ratio, e.g. intrinsic signal imaging or imaging of calcium sensors.

This work was supported by the ERC

EXTRACTING THE NATIVE NEURONAL SIGNALS FROM INHERENTLY DISTORTED RECORDINGS BY MODELLING THE EXPERIMENTAL INSTRUMENTATION

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Electrophysiological instruments, including pipette and amplifier circuits, inevitablydistort patch clamp recorded intracellular signals, especially when the structures are small (such as axons), because their passive parameters are comparable to the instrumental contribution.Consequently, recording instruments not only filter the measured biological signals but also affect the local cellular electrogenesis.To overcome these issues and to retrieve the native signals from small axons we built and tested a model that incorporatesnot only the biophysical parameters of the recorded and reconstructed structure but the experimental instrumentation as well.

The model implemented (1) pipettes asnon-uniform multicompartmentalmodels, which were constrained by the measured original physical parameters, and (2) each element of voltage- and current-clamp circuit, whose contributions were measured by isolating them within the amplifier circuit.

To purify fast axonal signals from instrumental distortions, first, we predicted passive parameters of the axon from the model-corrected voltage-clamp data. Then, we regenerated the distorted current-clamp spikes by adding and tuning simple HH sodium and potassium conductancesto the model. Finally, these simulated conductances predicted native spikes in the reconstructed axon after removing the simulated instrumental components.

Our approach confirmed that instrumentation indeed have significant impacts on recorded signals because the predicted native axonal spikes were (1) considerably larger and faster than the measured signals and (2) similar to the theoretically expected signals. Our observations also suggest that by precisely modelling the recording instruments it is possible to retrieve the native electrical properties of small neuronal structures after their inevitably distorted direct recordings.

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QUANTIFICATION OF CELL DEATH IN LONG-TERM ORGANOTYPIC CULTURE OF THE ADULT HUMAN RETINA

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We developed a culture technique that maintains human adult post mortem retinas in excellent condition for more than three months, enabling research on human retinal tissue that was previously impossible. In this study, we analyzed spatial and temporal characteristics of apoptosis occurring in culture.

Retinal cultures from multiple donors were cultured for up to 10 weeks. The cultures were fixed at different time points and were subjected to TUNEL analysis and immunohistochemistry.

In general, every major cell types survived and all retinal layers were maintained. The rate of apoptosis was low at all ages and remained low during the whole experiment. The initial average apoptosis of 7,663 ±5,216 cells/100 µm at DIV7 decreased to 2,912 ±2,229 by the third week and remained similar to that until the end of the 10th week. At all ages studied, the most intense cell death occurred in the outer nuclear layer. The overwhelming majority of TUNEL-positive cells were calbindin-negative rods. Cones didn't undergo severe apoptosis and a mean density of 4500-5000 cones/mm^2 were measured even in long-term cultures. In the inner retinaapoptosis affected all cell types uniformly, none of them showed disproportionately high levels of cell death.

Our human retinal explant culture system can maintain human retinas in culture for over 3 months while retaining a high level of morphological preservation. The low level of apoptosis and small deviation makes our explant culture system an excellent in vitro model for safety and efficacy testing of drug candidates and retinal regeneration studies.

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MICROGLIA ACTIVATION IN LONG-TERM ORGANOTYPIC CULTURE OF THE HUMAN RETINA

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Purpose: The role of microglia in healthy and diseased brain is an emerging topic in modern neuroscience. Our recently developed organotypic culture system of the post mortem human retina provides a new tool to study microglia in its physiological three-dimensional environment for over 12 weeks. In this experiment we examine gliotic reaction in detail and describe the temporal changes in microglia phenotype.

Methods: Small pieces of freshly isolated human retina were placed on polycarbonate membrane and were cultured in serum-free chemically defined medium for up to 10 weeks. The cultures were fixed at different time points and were analyzed by immunohistochemistry using glia-specific markers.

Results: All retinal layers were maintained, and every major cell-types survived. Both astroglia and Müller cells became hypertrophic and showed an increased GFAP expression. In ex vivo controls the microglia cells were distributed along retinal vessels and populated the inner retina. In culture a rapid change in phenotype occurred. Microglia cells with ameboid morphology appeared in every retinal layer. Parallel to the morphological changes, expression levels of Iba-1 largely decreased, while the expression of CD68 became highly elevated. The expression of lysosomal marker LAMP1 indicated maintained phagocytic function.

Conclusions: The culture method for retinal tissue used in our study provided a suitable model for long-term observation of phenotypic changes in microglial cells. During the culture period, we could observe the activation of the microglia cells and its presence in distinct layers of the retina.

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MODULATION OF NEURONAL ACTIVITIES BY HIGH EFFICIENCY TWO-PHOTON GABA UNCAGING COMPOUND

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Caged compounds are excellent tools to simulate and modulate neuronal activity patterns from subcellular to network level. Although highly efficient excitatory caged molecules have already been synthesized, the development of inhibitory caged molecules for two-photon (2P) microscopy has proven to be difficult.
Quantum chemical models showed that the previously published cage-GABA molecules, such as Ruby-GABA and MNI-GABA, have moderate 2P quantum efficiency beside numerous side effects. Therefore, here we developed a new, more effective caged GABA material - named iDMPO-DNI-GABA for 2P uncaging.
We validated and characterized the iDMPO-DNI-GABA effect with 2P calcium imaging and simultaneous electrophysiological recordings of GCamp6f labelled neurons. We have found that the iDMPO-DNI-GABA is a non-toxic and cellularly very potent caged molecule. Moreover the relative 2P efficiency of iDMPO-DNI-GABA is increased significantly, compared to the previously developed caged-DNI molecule.
Using 2P iDMPO-DNI-GABA uncaging single neuronal activities could be silenced or totally blocked with high efficiency in a well defined spatial and temporal resolution. Finally we simulate pathological neuronal network activities with the application of 4AP *in vitro*. We were able to silence individual cells within the active network during epileptic activities. This novel caged-GABA compound enables us to reproduce and modulate the inhibitory inputs of individual cells or neuronal population.
Taken together, two-photon uncaging of iDMPO-DNI-GABA holds great promise for advancing our understanding of the physiological and abnormal brain activities, therefore it makes possible to develop new therapeutic possibilities for different diseases.

A COMPARATIVE STUDY OF TARGETED GERM-LINE GENOME EDITING METHODS BASED ON CRISPR /CAS9 SYSTEM

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Generation of germ-line transgenic animal with targeted mutation requires a series of time consuming and expensive methods by an advanced researcher. Male pronuclear microinjection of foreign DNA is a classical method for generating transgenic animals. Together with other gene delivery methods including viral and embryonic stem cell mediated gene transfer have several limitations, however. For precise, one-step targeted mutation the use of CRISPR / Cas9 gene editing system can overcome most of the time-consuming steps required for the targeted embryonic stem cell line selection. The aim of or study is to simplify and accelerate the generation of targeted mutations to reveal transgenic mice by using a combination of testicular electroporation and CRISPR system. To compare the effectiveness of the CRISPR system we delivered the elements (Cas9 enzyme, crRNA, trRNA and homologue template) in different forms by testicular electroporation. For the mutation we used a homologue template cassette containing a floxedbicistronicopen reading frame including a marker GFP that was targeting the ROSA26 allele. The integration of the gene was confirmed by PCR. Our improved methodology for generating targeted mutations is a cost and time efficient and can be easily approved by various biomedical researchers.

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FACE PROCESSING DEFICIT IN DEVELOPMENTAL DYSLEXIA

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Current theories suggest overlapping neuronal representations of face and word processing. Recent studies support these theories by showing that recognition of letters and faces is impaired in developmental dyslexia. A few studies revealed that the fusiform face area (FFA) is hypoactive in developmental dyslexia. These studies argue for a domain general, high-level visual impairment in dyslexia in which reading problems are the most salient – but not exclusive – manifestations of the deficit. To explore these claims further, the aim of our research is to assess the perceptual and memory component of face processing in developmental dyslexia.

Methods: In order to measure the perceptual component of face recognition, we applied the Cambridge Face Perception Test (CFPT– Duchaine et al., 2007). The memory component of face processing was assessed by the Cambridge Face Memory Test (CFMT– Duchaine & Nakayama, 2006). Both tests are diagnostics tests of prosopagnosia.

The experimental group consisted of people with diagnosed developmental dyslexia; their mean age was 18.09 (SD=4.50), N=43. The mean age of the control group of normal readers was 28.90 (SD=10.45), N=61.

Our results show that both the perceptual and memory components of face processing show severe impairments in dyslexic group with great individual differences. The adjusted linear model revealed that the perceptual component of face processing and the diagnoses of dyslexia are significant predictors of the memory component of face processing.

Our results support the domain generality of the visual disorder, showing that beyond word processing, other functions represented in the visual ventral stream are impaired as well. The behavioral deficits revealed by our studies urge further studies examining the neural correlates (i.e. the nature of face-processing related N170 component) of face processing in dyslexia.

NEUROCHEMICAL CHARACTERIZATION OF THE LATE BORN NEURONS IN THE SPINAL DORSAL HORN OF MICE

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Complex neural circuits of the spinal dorsal horn (SDH) integrate and transmit diverse somatosensory information from the periphery to the higher brain centres. Neurons in the SDH are derived from the late born neuronal population and differentiate into an excitatory and an inhibitory group. Besides the main neurotransmitters (glutamate, GABA and glycine), they express a set of enzymes, calcium-binding proteins or receptors restricted to distinct subpopulations. Concerning the neurochemical properties, there is a large heterogeneity among the superficial neurons. In contrast, these neurons develop from a relatively homogeneous late born postmitotic neurons. Therefore our aim was to characterize the neurochemical phenotypes of these neurons born within a short time interval revealed by in utero electroporation (IUEP) followed by immunocytochemistry.
The immature migrating GFP-labelled cells had neither excitatory nor inhibitory fates. We found that 19% of the GFP-positive late born neurons express the transcription factor Pax2 that is required for the development of the GABAergic neurons. A small portion of the GFP-labelled cells were calbindin- or calretinin-positive (18-11%) that are mostly excitatory cells in the superficial dorsal horn. Another group of the GFP-positive neurons were also positive for PKC (10%) and NK1 receptor (8%), markers of excitatory neurons with distinct laminar distribution.
Our results indicate that neurons populating the spinal dorsal horn born together in a short time interval from a unique progenitor population differentiate into a large variety of cells that may be due to more extrinsic over intrinsic factors defining their neurochemical, morphological and functional properties.

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SPECIES-SPECIFIC DIFFERENCES IN THE DISTRIBUTION OF GFAP-IMMUNOREACTIVITY IN AVIAN BRAINS

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The earlier studies usually confined to a single species to demonstrate the astroglial architecture of a vertebrate classes with immunostaining against GFAP. The findings were held to be characteristic of (extrapolated to?) the entireclass. The present study compares several avian species in different phylogenetical positions: most ancient Galloanserinae (chicken, quail and duck) as well as basal (pigeon) and crown (finches and parrots, 2-2 species) Neoaves. The immunoperoxidase reactions were performed with monoclonal Novocastra anti-GFAP raised in mouse, on floating Vibratome sections following perfusion with 4% buffered paraformaldehyde. The investigations were focused on the areas which were found to be especially rich in GFAP-immunopositive astrocytes: the ectopallium (formerly 'ectostriatum' in the telencephalon, the deeper zone of the optic tectum and the granular layer of cerebellum, These areas were GFAP-immunopositives in chicken and pigeon but not in the finches, parrots and quail. Therefore, the GFAP-staining did not correspond strictly the phylogenetic correlations.A decreasing tendency was seen during evolution, which probably interferes with the effect of their manner of life.

SEMA3 SIGNALING PLAYS ROLE IN MORPHOLOGICAL FORMATION OF SPINAL DORSAL HORN NEURONS

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The spinal dorsal horn has a highly organized laminated structure, where different laminae show large heterogeneity in both morphological and functional way. By contrast, the spinal dorsal horn neuron originated from only a few different types of progenitor cell. Thus, the microenvironmental factors play major part in formation of neuronal morphology. Secreted semaphorinsplay major role in guidance of axon growth cone and forming the morphology of neurons including spinal cord. Several semaphorin family member molecules are identified involved in the organization of ascending and descending tracts or midline crossing of axons of spinal neurons.The exact morphogenic effect in the spinal dorsal horn hasn't been clarified however.

Our primary aim in present study was revealing a detailed developmental map from expression pattern of secreted semaphorins during laminarization and differentiation of spinal dorsal horn using immunohistochemistry and in situ hybridization. Secondly, using dominant negative transgenic technology, we examine the putative function of the semaphorin signalization in spinal dorsal horn neuron differentiation. We found, that migrating or just arrived cells were positive for Sema3A and Sema3F, and these neurons were also positive for semaphoring receptorsneuropilin 1 and plexinA2.Our data are indicating that both neuropilin 1 and plexinA2 aretransmittingthe Sema3 in spinal dorsal horn. Cumulative appearanceof these secreted molecules is indicating that they play role ingrowth of neuronal processes and synaptogenic progresses.

CONSERVED SEROTONERGIC BACKGROUND OF EXPERIENCE-DEPENDENT BEHAVIOURAL RESPONSIVENESS

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Effectively responding environmental threats requires the emergence of stress-induced internal states. Such ability depends on early experiences and, in connection, the adequate formation of central modulatory systems, particularly the development of serotonergic pathways. In the current study we use zebrafish (*Danio rerio*) as a model to unravel the serotonergic background of experience-dependent behavioural responsiveness due to its relatively simple vertebrate central nervous system and robust behavioural stress responses. First, we characterised a highly reactive period during development in which we subjected individuals to social isolation i.e. chronic deprivation of environmental stimuli. Socially isolated fish showed delayed avoidance and sensory responsiveness during novelty challenge compared to socially reared subjects. In line with such decreased reactivity, isolation exerted lower basal and increased novelty-induced whole-brain serotonin content compared to controls. We detected similar stress-induced differences on the level of forebrain limbic structures, e.g. structures homologous to the mammalian amygdala and hippocampus. Acute pharmacological blockade of serotonergic signalling through 5HT1A autoreceptor agonism prevented isolation-induced physiological a behavioural effects as well. Interestingly, the isolation-induced decrease in reactivity was specific to novelty-induced visually-driven challenges. In summary, we found that the absence of adequate stimuli in a sensitive developmental period impairs responsiveness through the emergence of an atypical serotonergic phenotype. Our results support the idea, that serotonergic signalling is one fundamental and ancient channel that transmits early-life information to the adult phenotype, establishing contextually relevant challenge coping.

DISTRIBUTION PATTERN OF THE EXTRACELLULAR MATRIX MOLECULES IN THE DEVELOPING MOUSE BRAIN STEM

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Previousstudies have demonstrated that the molecular and structural composition of the extracellularmatrix (ECM) in the central nervous system undergoes profound transformation during embryonic and early postnatal development.The aim of this study was to detect the changes of staining pattern of different ECM molecules in the developing mouse brainstem by using histochemical(Wisteria floribunda agglutinin (WFA), hyaluronic acid probe (HA)) and immunohistochemical (aggrecan, neurocan, versican (GAG beta), TN-R and HAPLN1)methods.

We found that HA,neurocan and versicanreactions were presentat very early embryonic stage (E13.5)as a diffuse neuropil staining, but the perineuronal net (PNN)composed of these molecules were observed only postnatally (P7). We could not find any aggrecan, WFA or HAPLN1 staining before birth. Postnatally WFA and aggrecan established PNN in the reticular formation and in different brainstem nuclei.Postnatally WFA,aggrecanand HAPLN1 were restricted to the neuropil of some brainstem nuclei, in contrast to HA,neurocan and TN-Rwhich were found throughout the brainstem.

Our results showthat atearly stages of development only a diffuse neuropil stainingis present in the brainstem and the formation of a definitive PNN is recognizable postnatally. We found well developed PNNs in several nuclei of the brainstem in two weeks old animals. We detectedspatiotemporal differences in the distribution of different ECM molecules both in the neuropil and perineuronal net in various brainstem areas. We suggest that the ECM expression pattern appears to be related to the functional maturation of brainstem neural circuits.

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NETWORK EFFECTS OF DENDRITIC INHIBITION IN THE MEDIAL ENTORHINAL CORTEX

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Superficial and deep layer principal neurons in the medial entorhinal cortex (MEC) convey distinct signals into and from the hippocampus, respectively. Their incoming inputs from cortical and subcortical areas, however, largely overlap, which further emphasizes the potential role of specific inhibitory microcircuits in tuning the network activity in different layers. Grid and other spatially modulated cells are abundantly found in layerII but rarely occur in deeper areas, thus we asked whether we see a correlation between layer/principal cell type differences and the nature of their inhibitory inputs. For this, we compared the network effects of the two most abundant interneuronal population: parvalbumin (PV) and somatostatin (SOM) expressing GABAergic cells. ChR2 expression was induced by vector delivery into the MEC of PV-cre and SOM-cre animals. With the combination of optogenetics and whole cell patch clamp techniques we found that PV+ inhibitory interneurons show no target selectivity: they innervate principal cells in all layers. However, the dendritic targeting SOM+ interneurons showed a much larger effect on layerIII-V pyramidal cells than on layerII stellate and pyramidal cells. Moreover, the SOM+ innervation inhibited deep layer cell firing much longer than PV+ inhibition. Juxtacellular and silicon probe experiments on awake mice proved that the optogenetic activation of SOM+ cells in the MEC is able to inhibit the firing of deep layer principal cells for several hundred milliseconds. Our data indicate that dendritic inhibition by SOM+ interneurons might be more influential on non-spatial information processing in the medial entorhinal cortex.

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CHARACTERIZATION OF GENETICALLY IDENTIFIED GLUTAMATERGIC NEURONS IN THE MESENCEPHALIC LOCOMOTOR REGION (MLR) OF MICE

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The pedunculopontine nucleus (PPN) and the neighboring cuneiform (CN) and precuneiform nuclei (PrCN) are parts of the mesencephalic locomotor center. It was recently shown that glutamatergic neurons of these regions have distinct roles in locomotor activity, forming 3 subgroups. In the present project, we sought the possible electrophysiological background of neuronal diversity in the MLR.

To achieve our aims, mice expressing tdTomato fluorescent marker or channelrhodopsin-2 in a vesicular glutamate transporter type 2 (VGlut2)-dependent way were employed, and slice electrophysiology combined with optogenetics, *post hoc* immunohistochemistry and morphological reconstruction was used.

Based on the changes of spike frequency adaptation, action potential amplitude and width elicited by current injections with increasing amplitude, 3 functional subtypes of glutamatergic neurons were found. In group I, there is minimal change in the parameters above with increasing depolarization. In group II, the spike frequency adaptation increases, whereas the action potentials become smaller and slower at the end of the train. In group III, increasing depolarization shortens the duration of the train, which is restricted to the first half –one-tenth of the depolarizing current injection.

In the PPN, 30.2% of all neurons belonged to group I, 21% fell to group II, whereas 48.8% was group III. In the CN, most neurons (85.7%) were in group III, whereas in the PrCN, the majority of neurons belonged to group I (65.2%).

The functional heterogeneity in firing pattern and further cellular electrophysiological properties of MLR glutamatergic neurons might explain their heterogeneous relationship to locomotion.Characterization of functional subgroups among genetically identified cholinergic neurons in the pedunculopontine nucleus

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INVESTIGATION OF THE INTERACTION BETWEEN C1Q AND ITS NOVEL BINDING PARTNERS IN THE CNS

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C1q is the initiator component of the classical pathway of the complement system. Besides its immune functions at the periphery, the complement cascade in the central nervous system participatesin the elimination of synapses during early ontogenesis and inthe dynamic maintenance of synaptic connections in the adult brain via its deposition to certain synapses.Importantly, numerous neurodegenerative disorders, such as Alzheimer's disease,are characterized by severe synapse loss, in which C1q plays a crucial role. Despite its significance, synaptic interaction partners of C1qremain obscure. In this work, we focused on two potential interaction partners, neuronal pentraxin 1 and 2 (NP1/2), based on their homology to pentraxin 3, a well-known C1q-binding protein at the periphery. Our in vitro experiments verified the physical interaction between NPs and C1q;moreover, we demonstrated for the first time that NP1 and 2canactivate the complement cascade through the classical pathway. Their interaction has been further investigated in vivo.Flow cytometry experiments onmurine synaptosomesrevealed thatsynaptically detectable C1q co-localizes with NPs that was also verified by immunolabeling of cortical brainsections. Synaptic location of NPsis not limited to the extracellular side,but they are present intracellularly as well.In summary, our results shed light on novel C1q interaction partners that might be involved in its synaptic pruning-linked function.This work was supported by the National Research, Development, and Innovation Office of Hungary (grants 2017-1.2.1-NKP-2017-00002, FIEK_16-1-2016-0005).

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ELECTRICAL MODULATION OF SPATIALLY SEPARATED GAP JUNCTION COUPLED NEURONS INITIATES RETINAL GANGLION CELL CA^{2+}-TRANSIENTS

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Retinal ganglion cells (rGCs) remain popular choices for neurophysiological investigations, because they are one of the most accessible spiking cells in the brain. Moreover, in vitro retina specimen can be prepared and examined without interfering with the neuronal circuitry. We utilized this model system to explore the correlated rGC activity in the mouse retina. Such an endeavor has been difficult due to the demanding methodological approaches like the combination of visual targeting and paired patch-clamp electrophysiological recordings. Here, we overcome much of these issues by utilizing a Thy1-GCamP mouse line to detect the activity of all rGCs in an extended retinal area. To reveal gap junction (GJ) mediated correlated rGC activity we developed a novel approach, in which an extracellularly applied electrical discharge in cell-attached configuration modulated the intracellular Ca^{2+}-levels of both the target rGCand the GJ coupled rGC partners. This new method allows for: (i) examining the correlated activity of electrically coupled rGC networks in control conditions and under pharmacological interventions, (ii) dye injecting and morphologically characterizing GJ coupled cells in a local RGC assemble and (iii) examining the spike output code of the target cell with and without the coupled network as a response of spatio-temporal modulation of the stimuli.

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SHARP-WAVE-RIPPLES ASSOCIATED CA^{2+} EVENTS IN PARVALBUMIN CONTAINING INTERNEURONS *IN VIVO*

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Sharp-wave-ripple (SPW-R) complexes are typical field potential phenomenain the hippocampus and have important role in memory processes. The SPW-Rs are driven by interaction betweenhippocampal pyramidal cellsand interneurons. According to the literature, during neuronal network activities synaptically activated dendritic segments may participate in the formation of the neuronal engram.Because of the difficulties of imaging and doing electrophysiology together, the mechanisms of *in vivo*dendritic integration during SPW-Rs remain elusive. Nevertheless,the evidence of SPW-R associated dendritic Ca^{2+} signals have not yet been described *in vivo*. Here we investigated dendritic Ca^{2+} responses of hippocampal parvalbumin containing fast-spiking interneurons with two- and three-dimension two-photon microscopy combined with ipsilateral local field potential recordings *in vivo*. We found evidence ofthe existence of dendritic Ca^{2+} spikes during SPW-Rs in awake animals. We also proved the role of the voltage-gated Ca^{2+} channels in dendritic spike occurrences*in vitro*. We showed complex Ca^{2+} events at different subcellular regions of hippocampal interneurons *in vivo*. Through this data, we can understand better themechanisms of hippocampal coincidence detection and the role of interneuronal dendrites in memory formation and consolidation.

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STRUCTURAL CORRELATES OF MODULAR ORGANIZATION OF SIGNAL TRANSMISSION IN PRIMATE SOMATOSENSORY CORTEX

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Axonal connections of cortical columns exhibit patchy distributionsin primate cerebral cortex. The axonal patches represent specific target sites (e.g. columns of similar orientation preference in visual cortex). However, axonal connections also densely distribute outside of patches without any apparent grouping. Instead, theseoutside-patch axons exhibit a radial spread from the origin towards distant target sites.Interestingly both patch and outside-patch axons form axon terminal-like structures supporting a role in synaptic transmission. However, it is not known whether these axons play a similar role in the propagation of activity and dissemination of information within and outside of patches. Toaddress this, morphological properties of reconstructed axons within and outside of patches were compared for intra- and inter-areal connections in the somatosensory cortex of squirrel monkeys.Preliminary findings suggest that axons have similar tortuosity but different bouton density within and outside of patches. Specifically, intrinsic connections within patches exhibit higher bouton density than outside of patches. However, bouton density of inter-areal axons does not differ within and outside of patches. The increased bouton density accompanied by extensive axonal convergence could result in a highly efficient way of signal transmission in terminal arborization patches of the cerebral cortex. In contrast, long range axons outside of patches could provide input to extra classical receptive field and form the structural correlate of cortical plasticity.Supported by NIH NS093998.

SEVERAL IMMUNE SYSTEM MECHANISMS ARE REPRESENTED IN THE TRANSCRIPTOME OF PFC NEURONS

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Deep sequencing of single cells allows to detect molecular footprint of cellular mechanisms composed by low abundance transcripts. Harvesting fast spiking and pyramidal cells of prefrontal cortex (PFC) after physiological verification of cell types resulted 19.000 transcripts (20 million reads, more than 100-fold coverage, 84 cells). Bioinformatics analysis of transcriptomics data revealed that T-cell and B-cell activation pathways as well as MHC-I and MHC-II dependent antigen presentation mechanisms are expressed in neurons. In general, the immune system genes were weakly expressed in neurons. However, 3 cells out of 84, probably being in a particular state of their phenotype, highly represented the above listed mechanisms. We also found some immune system related transcripts, which were represented in more than 50% of the cells. Our present finding suggests that immune system genes are not under suppression in neurons, although, these genes are frequently in off-state. Compared to the observed presence of housekeeping genes (37% in average), the immune system genes are still well represented in our data (19%). The actual state of our study is that we are working on the verification of transcriptomics data at protein level and we also investigate the relationship between these immune system elements and other cell processes. In the theoretical sense, our data support the idea that neurons are able to perform some of the immune system mechanisms or they extensively use the immune system proteins to neuronal functions.

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GLP-1 REGULATES THE POMC NEURONS OF THE ARCUATE NUCLEUS BOTH DIRECTLY AND INDIRECTLY VIA PRESYNAPTIC ACTION

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GLP-1 inhibits food intake and causes weight loss. GLP-1 analogues are successfully used to induce weight loss in humans. GLP-1 exerts these effects at least partly via the POMC neurons of the arcuate nucleus.
Ultrastructural examination revealed that GLP-1R is present in POMC neurons, but also in axons innervating these cells suggesting that GLP-1 regulates POMC neuronsboth directly and indirectly.
In agreement with previous data, Exendin 4 (Ex4) markedly increased the firing rate of all examined POMC neurons (249.86±44.9%; P<0.001; N=20) and depolarized these cells (+4.12±1.7 mV; P<0.001). Inhibition of G-protein signaling by intracellular administration of GDP-β-S prevented the Ex4-induced increase of firing rate, demonstrating that Ex4 has direct stimulatory effect on the POMC neurons.
To examine the presynaptic effects, the influence of Ex4 was studied on the miniature excitatory (mEPSC) and inhibitory postsynaptic currents (mIPSC) of POMC neurons. Ex4 increased the frequency of mEPSCs (160±20.8%; P=0.015) in about 50% (N=7) of the examined POMC neurons (N=15). In addition, Ex4 also increased the frequency of mIPSCs (154.5±8.4%; P=0.002), in one-third (N=6) of the examined POMC neurons (N=19; P=0.002). This effect of Ex4 was not influenced by the intracellular administration of GDP-β-S indicating that GLP-1 has direct stimulatory effect on a population of the inputs of POMC neurons.
In summary, our data demonstrate that GLP-1R is present in axons innervating the POMC neurons. In addition, stimulation of GLP-1 facilitates the effects of the neuronal inputs of POMC neurons via its presynaptic GLP-1R in addition to its direct stimulatory effect.

T-TYPE CALCIUM CHANNELS AND HCN CHANNELS REGULATE 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 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 far from understood. We investigated homeostatic alterations of voltage-dependent membrane currents in primary mouse hippocampal cultures and in organotypic slice preparations upon 48h TTX treatment. Neuronal excitability and physiological properties were analyzed using the whole-cell current clamp technique. Interestingly, a characteristic increase of the depolarizing voltage sag and post-inhibitory rebound was observed. These responses were totally abolished upon the application of specific CaV3 and HCN channel inhibitors, NiCl2 and ZD7288, respectively. Our data indicate that the magnitude of the low-threshold Ca-current associated with the postinhibitory rebound (PIR) and the h-current mediating the voltage sag increased in a homeostatic manner. RT-qPCR and western blot analysis indicated upregulation of CaV3.1 and downregulation of HCN1 gene expression. HCN1 protein levels did not change, while CaV3.1 protein levels increased during TTX treatment. Confocal analysis of organotypic hippocampal slice cultures is in progress to reveal any positional changes in the dendritic localisation of HCN channels. These results indicate that besides AMPA receptors, CaV3 and HCN channels are also specifically regulated during homeostatic plasticity.

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STATIC EXCITABILITY AND SYNAPTIC RESPONSES OF HIPPOCAMPAL NEURONS WEAKLY CORRELATE - A DYNAMIC CLAMP STUDY

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Intrinsic excitability, as one of the most distinctive physiological features of neurons refers to their propensity to generate action potentials in response to depolarizing inputs. This behavior is commonly studied in electrophysiological experiments based on intracellular injection of constant current steps. It is less often addressed how the degree of neuronal excitability as observed in current clamp experiments translate to the firing of the same neurons when they integrate natural inputs. Indeed, neurons typically receive a rapidly fluctuating mixture of excitatory and inhibitory synaptic conductances, rather than a flat current input, during their normal operation. To address this problem, we analyzed firing responses of cultured hippocampal neurons in whole-cell patch clamp experiments and under two stimulus conditions. First, they were driven by current step inputs to obtain their standard input-output functions. Next, we subjected the neurons to simulated synaptic bombardment via dynamic clamp and acquired their dynamic excitability profiles. Remarkably, excitability measures obtained from the two stimulus protocols exhibited only weak correlation. Specifically, one class of hippocampal neurons, referred to as stuttering cells, fired intensely under synaptic bombardment. Conversely delayed spiking neurons exhibited weak responses under dynamic inputs but robust firing under current stimulation. In general, parameters of intrinsic excitability, as measured in current step experiments, yielded low predictive power to estimate the intensity of firing under synaptic inputs. These findings excellently agree with our prior results from computer model simulations and help to gain a better understanding of the functional role of physiological diversity of neurons.

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CORRELATED GANGLION CELL SPIKE BURSTING IS MEDIATED BY ELECTRICALLY COUPLED PRESYNAPTIC AMACRINE CELLS

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Vision, being the most important sensory modulation in humans, accounts for about 80% of the sensory information, hence it is an important area of research. Light information is received by and passed along retinal pathways to retinal ganglion cells (RGCs), where a pattern of action potentials is created to represent perceived visual cues. Some RGCs partake in population coding by which certain visual features are not detected by single cells, but rather encoded by the combined spikes of a group of RGCs. Population coding occurs via spike correlation, a mechanism that heavily depends on signaling through RGC-RGC and RGC-amacrine cell gap junctions. We performed paired extracellular recordings to examine RGC spike correlations in the mouse retina. We found that most RGC pairs with medium time scale (10-50 ms) correlated spiking displayed bursting activity as well. Interestingly, the initiation of bursts displayed a high degree correlation as well indicating that much of the correlated activity was brought about by intraburst spikes, whereas the contribution of solitary action potentials was negligible. We also show evidence that the RGC population in the connexin36 (Cx36) constitutive retina lack both RGC-amacrine cell tracer coupling and bursting activity. Moreover, medium spike correlations as well as spike bursts are highly reduced in RGC pair recordings in the KO animal. These data indicate that presynaptic amacrine cells distribute excitation to electrically coupled RGCs and induces correlated spike bursting.

AUTAPTIC SELF-INHIBITION CONTROLS HUMAN SUPRAGRANULAR BASKET CELL EXCITABILITY DURING COMPLEX EVENT ACTIVITY

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GABAergicautapses are synapse-like self-inhibiting neural connections found in interneurons in various brain areas. However, their role in physiological neuronal activity is still poorly known. Human neocortex, area where higher-order brain functions take place, has a low threshold generating neuronal network activity in physiological conditions. Single pyramidal cell spikes in the supragranular layer trigger neuronal ensemble discharges known as complex events, characterized by mono- and polysynaptic excitation of neurons and their time-locked firing. GABA-releasing parvalbumin-expressing inhibitory basket cells (pvBCs), which play a pivotal role in orchestrating spike timing of various neurons, fire single action potentials with high temporal precision during the events. On the contrary, GABAergicpv+ axo-axonic cells (AACs) are prone to discharge high-frequency spike bursts. We show that pvBCs in non-pathological human neocortex have strong autaptic self-inhibitory connections in the neocortical layer 2/3 where complex events are generated. $GABA_A$ receptor-mediated autapses form contacts at the soma and proximal dendrites and, following a pvBC action potential, reduce the cell excitability on average to 60% from resting condition. Autaptic inhibitory conductance suppresses pvBCs during complex event activity and oppose their firing in high-frequency doublets or bursts. In contrast, AACs lack perisomaticautapses. Thus, perisomaticautapses stabilize pvBC firing frequency through strong self-inhibition. pvBC firing plays a key role in synchronizing neuronal activity in the neocortex and it is likely that autapses stabilize neocortical rhythmic network oscillations. Perisomatic self-inhibition is characteristic of mammalian supragranularpvBCs since we find autapses with similar inhibitory strength in the human and in the mouse neocortex.

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GABA AND GAP JUNCTIONMEDIATED DISTINCT EFFECTS ON RETINAL GANGLION CELL INDIVIDUAL AND POPULATION CODE

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The retinal ganglion cells (RGCs) encode features of the visual scene in the form of action potential trains that are then transmitted to visual centers of the brain. RGCs can perform this task individually or in cooperation with their neighbors, forming individual or population code respectively. We examined how inhibitory chemical synapses of the inner retina as well as RGC gap junction (GJ) signaling contribute to the formation of the RGC individual and population code. We performed Ca^{++} imaging experiments in the*in vitro* Thy1-GCamp mouse retina allowing us to examine all RGCs simultaneously in an extendedretinal area. We found that apharmacolocicalblockade of GABAergic inhibition increased-, whereas the closure of GJs decreased the number of the spontaneous Ca^{++} transients. Interestingly, the two pharmacological interventions had very similar effects on the kinetics of individual light stimulus induced Ca^{++} responses, they both decreased the initiation times and the trial-to-trial variability of responses and increased light sensitivity. However, the GABA blockade increased, whereas closing GJs decreased the amplitudes of stimulus evoked Ca^{++} responses. Moreover, the two pharmacological treatments had different effects on the correlated activity of RGCs. While, the loss of GABAergic inhibition did not significantly alter correlated activity, the GJ blockade diminished concerted RGC signaling. These data indicate that inner retinal inhibitory signaling serves individual coding, while GJs rather contribute to the RGC population code.

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HYPOTHALAMIC CNTF VOLUME TRANSMISSION SHAPES CORTICAL NORADRENERGIC EXCITABILITY UPON ACUTE STRESS

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Stress-induced cortical alertness is maintained by a heightened excitability of noradrenergic neurons innervating, notably, the prefrontal cortex. However, neither the signaling axis linking hypothalamic activation to delayed and lasting noradrenergic excitability nor the molecular cascade gating noradrenaline synthesis is defined. Here, we show that hypothalamic corticotropin-releasing hormone-releasing neurons innervate ependymal cells of the 3rd ventricle to induce ciliary neurotrophic factor (CNTF) release for transport through the brain's aqueductal system. CNTF binding to its cognate receptors on norepinephrinergic neurons in the locus coeruleus then initiates sequential phosphorylation of extracellular signal-regulated kinase 1 and tyrosine hydroxylase with the Ca2+-sensor secretagogin ensuring activity dependence in both rodent and human brains. Both CNTF and secretagogin ablation occlude stress-induced cortical norepinephrine synthesis, ensuing neuronal excitation and behavioral stereotypes. Cumulatively, we identify a multimodal pathway that is rate-limited by CNTF volume transmission and poised to directly convert hypothalamic activation into long-lasting cortical excitability following acute stress.

EXPRESSION OF KCC2 IN INJURED MOTONEURONS FOLLOWING VENTRAL ROOT AVULSION

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Down regulation of KCC2 is associated with developing spasticity and increased excitatory transmission in the acute spinal cord injury. Avulsion injury results in motoneuron death due to the excitatory action. In this study we examined the alterations of KCC2 expression of injured motoneurons with or without riluzole treatment.
The left lumbar 4 and 5 (L4-5) ventral roots of the spinal cord were avulsed. Animals were treated with riluzole for 2 weeks. Riluzole treatment started immediately on the day of surgery daily for 1 week and every second day for the next 1 week. In control animals the L4-5 ventral roots were avulsed without riluzole treatment. Expression of KCC2 in the injured motoneurons and in the affected side of the L4 and L5 spinal segments were detected 5, 7, 10, 16, and 21 and 63 days after the injury with immunohistochemistry followed by confocal microscopy and dSTORM imaging.
KCC2 immunoreactivity was significantly higher in the ventral horn of treated animals than in the controls 5, 10 and 16 days after the injury. The KCC2 labelling in the lateral and ventrolateral part of the L4 ventral horn was weaker compared to the medial gray matter of L4-5 ventral horn in both groups. The quantitative analysis of mean fluorescence cytoplasmatic signal in the injured motoneurons revealed that KCC2 staining was different between the groups.
Taking together, the present results indicate that pharmacological blockade of voltage activated Na^+ and Ca^{2+} channels influences the expression of KCC2 in injured motoneurons.

EXAMINATION OF THE PERISOMATIC INPUT TO PRINCIPAL NEURONS IN DYSGENETIC CORTICES OF HUMAN EPILEPTIC PATIENTS

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The previous investigations of our group were performed on hippocampal samples with temporal lobe epilepsy (TLE), and enhanced perisomatic inhibition was found, which may increase the seizure probability. Therefore, changes of perisomatic inhibitory inputs were examined in focal cortical dysplasia (FCD) cases to find whether mechanism of epileptogenesis is similar to TLE.

Surgically removed frontal cortical samples from patients with FCDIIb were compared to post-mortem perfusion fixed cortices of the same cortical regions with short-terminterval (4-4 subjects). We used antibodies against NeuN, and SMI32 (labelling pyramidal cells), PV (labelling perisomatic inhibitor elements), GFAP and IBA1 (labelling glial elements). The samples were examined with light, confocal, and electron-microscopy.

In severalepileptic samples, there were numerous giant, dysmorphic neurons, appear to have a particularly dense inhibitory input.In some cases, presumably balloon cells were found in the white matter. The quantitative parameters showed a tendency in the elevation of the number of perisomatic terminals in FCD cases. It was most prominent in case of the giant cells.

Our results suggest that perisomatic inhibition is preserved in FCD and cell size correlates with the abnormal perisomatic input, which may have a role in the seizure generation caused by dysgenesis.

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EARLY IMPAIRMENTS OF HIPPOCAMPAL NEUROGENESIS IN 5XFAD-MICE ARE ASSOCIATED WITH ALTERED EXPRESSION OF SOXB PROTEINS

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Alzheimer’s disease (AD) is a progressive neurodegenerative disorder with sex-related epidemiological profile, affecting two times more women than men.Hippocampus,crucial structure in learning and memory processing is important site of adult neurogenesiswhich can be impaired in AD. Members of SOXB transcription factors play critical roles in regulating neurogenesis in embryonic and adult nervous system, including maintaining the multipotency, renewal and cell fate decision of neural stem/progenitor cells. The aim of the present study was to evaluate the expression patterns of SOXB proteins in the subgranular zoneof 5xFAD mice (Tg)of both genders that rapidly develop severe amyloid pathology. Expression was analysed in 8-week-old animals, time point when the formation of amyloid-beta plaques in the abovementioned model begins.Immunohistochemical analysis showed a significant decrease in the number of cells expressing SOXB transcription factors throughout the SGZ of Tg mice in comparison to their non-transgenic counterparts. Despite observed changes in expressional pattern of examined SOXB proteins, the proliferative capacity evaluated by the number of Ki-67 immunoreactive cells remained unaffected. Finally, differences in SOXB protein expressioncoincidence with reduced number of doublecortin (DCX) immunoreactive immature neurons found in Tg males, but not in females. Based on our results we can conclude that1) SOXB proteins might be considered as new biomarkers in research for detection of early impairments in adult neurogenesis and 2) there aregender-specificities in DCX-immunoreactivity related to surviving period and differentiation of immature neuron.The cause of this sex difference has yet to be elucidated.

MOLECULAR AND PHARMACOLOGICAL INVESTIGATION OF ALPHA7 NACHRS IN HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED DENTATE GYRUS GRANULE CELLS

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Alpha 7 nicotinic acetylcholine receptors (alpha7 nAChRs) have received considerable interest in light oftheir selective localization in the central nervous system and their unique physiological and pharmacological properties. Intense pharmacological research focuses on this receptor population that could potentially be targeted for the development of precognitive medications. Alpha 7 nAChRs arealso present in human induced pluripotent stem cell (hIPSC) derived dentate gyrus granule cells, an in vitro model of hippocampal neurogenesis, therefore this model system could be used to study the properties and function of alpha7 nAChRs in human neurons. Here we demonstrate results about alpha7 nAChRs in hIPSC-derived granule cell using RNASeq and qPCR data and immunofluorescence and staining. By means of single neuron patch-clamp electrophysiology we characterizedbasic properties of these receptors. The selective agonist choline evoked an inward current in the presence of the positive allosteric modulator PNU-120596, while no current was evoked by PNU-120596 alone. The α7 nAChR antagonist methyllycaconitine (MLA), inhibited the current evoked by choline and PNU-120596. Finally, investigating the same neurons with fluorescent calcium imaging showed, that neuronal networks reacted both to choline and PNU-120596 with increases in calcium transients, which could be abolished with MLA. These results suggest that human induced pluripotent stem cell based granule cells are amenable to functional assays toinvestigate alpha7 nAChR function and could also be used for testing new molecules targeting this receptor.

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THE INFLUENCE OF HIPPOCAMPAL INTERICTAL EPILEPTIFORM DISCHARGES ON NEOCORTCAL SLEEP SPINDLES IN TEMPORAL LOBE EPILEPSY

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The hippocampo-neocortical information transfer is critical for the slow wave sleep (SWS) related memory consolidation; it is coordinated by the temporal coupling of the hippocampal sharp wave ripple (SPW-r) and neocortical sleep spindle activity. Hippocampal interictal epileptiform discharges (IEDs) are known to correlate with impaired memory consolidation. IEDs surpass the physiological SPW-r-spindle coupling, moreover they tend to couple with spindles and induce them in behavioral states that do not naturally express these oscillations.

The present study focused on characterizing the influence of the hippocampal IEDs on cortical spindles during SWS in 20 patients with focal pharmacoresistant epilepsy undergoing scalp-foramen ovale electroencephalography. We analyzed a period of 1500ms of the first NREM cycle performing visual spindle and automated IED detections; and compared the duration, frequency, and amplitude of cortical spindles that temporally coupled with hippocampal IEDs with spindles with no temporal hippocampal IEDs connection. Temporal coupling was defined as an IED occurring within a 500ms interval of the spindle.

We found that spindles connected to hippocampal IEDs lasted longer and had higher amplitude, and their maximum power tended to be in a lower frequency range. IEDs had more influence on the centro-parietal (fast) spindles, than on the frontal (slow) spindles and they effected more robustly the spindles of the ipsilateral side.

These findings support the hypothesis that IEDs could impair memory through the derailment of physiological mechanisms of the hippocampo-cortical coupling; epileptic spiking contributes more to memory disturbances in MTLE than we had expected earlier.

INVESTIGATION OF SYNAPSES IN THE NEOCOTRICAL WHITE MATTER IN HUMAN TEMPORAL LOBE EPILEPSY

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Neurons are present in the neocortical white matter (WM) of healthy adults. In previous research we revealed significantly higher numbers of WM neurons in temporal lobe epilepsy (TLE) patients than in controls. The aim of our present work was to investigate whether WM neurons are functionally active and are part of neuronal circuitry responsible for the development or maintenance of seizures. Therefore, we studied the distribution and density of synapses in the neocortical WM in pharmacotherapy-resistant TLE patients' surgically resected tissue samples. Neocortical WM of temporal lobe tissues from non-epileptic patients with intracranial tumor and from autopsy were used as controls.

Synapses and neurons were visualized by immunohistochemistry using antibodies against synaptophysin and NeuN, respectively, and were investigated under light microscope and quantification of WM neurons and synaptophysin immunoreactivity was performed. The presence of synaptophysin in presynaptic terminals was verified by electron microscopy.

In TLE group, synaptophysin density in the WM was significantly higher than in control samples.Analyzing density of synaptophysin immunoreactivity and clinical data, we observed that synaptophysin density was significantly higher in samples from TLE patients who had the epileptogenic lesion on the left side than in patients with lesion on the right side. No correlation was found between synaptophysin immunoreactivity and other clinical data.

Our results suggest that WM neurons found in TLE patients receive large numbers of synaptic inputs indicating that they may be integrated in epileptic neuronal networks.

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ANTAGONISM OF FP-RECEPTOR SIGNALING INHIBITS THE EVOLUTION OF SPREADING DEPOLARIZATION IN CEREBRAL ISCHEMIA

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Spontaneous, recurrent spreading depolarizations (SD) are increasingly more appreciated as the pathomechanism behind delayed ischemic brain injuries. The inhibition of the FP receptor of prostaglandin F2a was shown to limit secondary neuronal damage during brain ischemia. Thus, we test the hypothesis that the neuroprotection by FP receptor blockade is achieved by the inhibition of SD. Global forebrain ischemia was induced in isoflurane-anesthetized, young, adult, male Sprague-Dawley rats (n=16) by the bilateral occlusion of the common carotid arteries. Two open craniotomies on the right parietal bone served the elicitation of SD with 1M KCl (caudal), and the acquisition of local field potential (rostral). The entire dorsal cranium was thinned to track regional cerebral blood flow (CBF) variations by laser speckle flowmetry. The femoral artery was prepared for the monitoring of mean arterial pressure (MAP). The femoral vein was used for the infusion of an FP receptor antagonist (AL-8810; 1mg/bwkg) or its vehicle (0.1% DMSO). Physiological parameters were similar in the two groups (e.g. MAP:82.7±8 vs. 84.5±9.1mmHg; AL-8810 vs. control). However, AL-8810 markedly reduced the duration of evoked SDs (36±14 vs. 56±15s). In addition, total depolarization time was reduced by 50% in the AL-8810 group (1339 vs. 2589s). The CBF response to SD involved a more restricted cortical surface in the AL-8810-treated animals. In summary, the antagonism of FP receptors emerges as a promising approach to inhibit the evolution of injurious SDs in cerebral ischemia. Further studies should address, whether the volume of the ischemic infarct is reduced accordingly by this intervention.

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CYTOPLASMIC TRANSPORT DEFICIT IN ALS PATIENT-SPECIFIC HUMAN iPSC-DERIVED ASTROCYTE PROCESSES

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Synapse dysfunction and loss are early features in Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disease, which in part underlie its rapid course of muscle paralysis, and dementia. Both intrinsic neuronal and non-neuronal causes have been proposed, but the precise mechanisms are unclear. We mechanistically explored the changes in astrocyte processes in light of their emerging role in synapse modulation and ALS pathogenesis. To take an unbiased approach, we initially integrated ALS-related genome wide association data with published proteomic and transcriptomic datasets deriving from human astrocytes affected by an ALS-causing SOD1 mutation. We have found that the concordant defect in KIF5a transcript and protein levels in SOD1 astrocytes overlaps with disease-driving gene modules in a broad spectrum of mutations. Since the loss of KIF5a, a molecular motor for intracellular transport, has been shown to be responsible for collapses in neurites, we next examined its potential effect on astrocyte process morphology and function. We have found that KIF5a gene-silencing in mouse astrocytes leads to decreased astrocytic arborization and velocity of mitochondrial transport.Thishas been also observed in human induced pluripotent stem cell (iPSC)-derived SOD1 astrocytes in which KIF5a was found deficient. This highlights a potentially targetable common astrocytic pathway in ALS, which may be responsible for the early synapse dysfunction and therefore it is a subject of our current investigations.

COMPARATIVE ANALYSIS OF FUSARIUM MYCOTOXINS ON CELL VIABILITY OF PRIMARY NEURONAL AND ASTROGLIAL CELL CULTURES

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Fumonisin B1, Deoxynivalenol (DON) and Zearalenone (ZEA) are toxic secondary metabolites produced by Fusarium mold. These mycotoxins are common food and feed pollutants and represent a risk for human and animal health. Although most of their physiological effects are described, it is not yet clarified how they influence neural cell functions. We investigated cell viability effects of these toxins on specified neural cell types, including mouse primary neuronal, astroglial and mixed cell cultures after 24 or 48 hours of mycotoxin administration in 1 nM - 50 μM concentration range. MTT-assay revealed that DON decreased cell viability in a dose-dependent manner, independently from the cultures types. Fumonisin B1 increased cell viability significantly on astroglial and mixed cell cultures in lower doses, while in 50 μM, it exerted a highly toxic effect. ZEA had no significant effect on mixed cell cultures, however in 10 nM, it increased the cell viability of neurons and astrocytes, as well. Since ZEA is a mycoestrogen, we analyzed the effects of ZEA on the expression of mitochondrial membrane protein VDAC1 and estrogen receptor isotypes ER-alfa and ERbeta with qRT-PCR. In neuronal and mixed cultures, ZEA administration decreased ER-alfa expression, while in astroglial cultures, it induced the opposite effect. ER-beta expression was not altered by ZEA in either culture types. ZEA decreased the expression of VDAC1 only in neuronal cultures. Our results demonstrate that Fusarium mycotoxins are acting on a cell specific manner.

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THE PROGNOSTICROLE OF INVASIONSPECTRAIN GLIOBLASTOMA

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Keywords: glioblastoma, invasion, extracellular matrix, MGMT, IDH1

Introduction: The glioblastoma is the most common primary malignant brain tumor. Median overall survival ranges between 16-24 months. There are only a few prognostic factors eg. age, *IDH1* mutation and *MGMT* methylation status. Their questionable clinical applicability calls forth the need of other markers.. A promising aspect of glioma research is the background of the invasion potential, and the relating ECM molecules.

Methods: The clinical factors of 41 GBM patients were examined, along with their *IDH1* mutational-, *MGMT* methylation status and the expression of 46 ECM molecules (invasion spectrum). The used techniques were qRT-PCR, IHC, MSP and pyrosequencing. The overall survival time were used to divide the patients into two prognostic groups (A, B).

Results: Significant differences were determined in the KPS-score and rate of reoperations. All of the tumor samples were *IDH1*-mutant. The rate of methylation status in each prognostic groups were the following: „A”: 28.6 %; „B”: 68.8 % (p= 0.03) by the MSP, which was further confirmed by the pyrosequencing. The statistical classifier algorithm could predict the prognostic groups based on the invasion spectrum. Identification rate: 83.3%, pos. pred. value (A):0.93. Significantly differing ECM molecules: cadherin-12, VEGFR-3, versican.

Discussion: Differences in the clinical parameters are in concordance with the scientific literature, along with the differences in methylation rate. The latter results underlines its significance as a useful marker. The high accuracy of the invasion spectrum in differentiating the prognostic groups proposed its role as a great prognostic tool, while the identified molecules could be the target of anti-invasive agents in the future.

EXAMINATION OF NON-CONVENTIONAL MARKERS IN POLYTRAUMA VICTIMS

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Severe trauma is the most frequent cause of death in people below the age of 40 years. Associated tissue injuries result in cellular necrosis with leukocyte activation and consequent swelling that leads to immediate immunoreactions and trigger further reactions and consequent organ failures in the host. New monitoring methods could be the leukocyte antisedimentation rate (LAR) or the changes in pituitary adenylate cyclise-activated polypeptide (PACAP) levels which is a neuropeptide with several antiapoptotic, antioxidant, regulatory and antiinflammatory effects proved by numerous in vitro and in vivo studies.

Our aim was to examine the characteristics of non-conventional markers (PACAP-38, LAR) in polytrauma victims and their correlation to conventional laboratory parameters (serum C-reactive protein(CRP), procalcitonin(PCT) used in the daily intensive care.

Patients were followed for 5 days (T1-T5) after admission to a critical care unit with severe polytrauma (Injury Severity Score $\geq$ 16). Serum PACAP-38 was measured with sandwich ELISA, while LAR, CRP and PCT levels were determined with conventional laboratory methods.

Thirteen patients were examined, their median age was 21 (27-55) years. Compared to the control group both LAR and PACAP-38 levels of polytraumatic patients were markedly elevated and reached their peak at T4. CRP levels showed an increasing tendency, on the other hand PCT failed to indicate any consistent kinetics. We found moderate positive correlations between LAR and CRP, as well as PACAP and CRP levels.

Based on the similarity in PACAP and LAR kinetics after polytrauma we suggest that they have a potential biomarker function in severe trauma patients.

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EFFECS OF DORSAL ROOT AVULSION INJURY ON THE SPINAL GANGLIA AND SPINAL CORD

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High impact vechicle accidents and sport injures often result in avulsion injuries when the dorsal and ventral roots of the spinal cord are torn out. The changes in the ventral horn after ventral root injury are well-known, however, there are only a few studies investigating the effects of the avulsed dorsal root. In this study, our goal was to examine the avulsion-induced changes in the cell population of the affected dorsal root ganglia and in the spinal cord. Lumbar 4 and 5 (L4-5) dorsal roots were avulsed. The animals were perfused 3 or 21 days after the surgery. The injured and contralateral dorsal root ganglia along with the L4-5 spinal segments were removed. Immunohistochemical analysis carried out on cryostat sections included neurofilament 200 kDa protein, Transient Receptor Potential cation channel subfamily V member 1 (TrpV1) receptor, Calcitonin gene-related peptide (CGRP) immunostainings and Griffonia Simplicifonia Isolectin-B4 (GSAB4) histochemistry. These aimed at the changes both in the ganglia and in the spinal cords following the injurys.
Our preliminary data suggest that there are no significant topological or morphometric changes in the TRPV-1 and CGRP expression levels even 21 days after the injury in the L4-5 ganglia. However, dorsal root avulsion injury severely affected the ipsilateral gracile tract of the spinal cord. Moreover, we could detect an impact of the injury on the contralateral side of the cord, too.
It can be concluded that this avulsion injury model represents well the subsequent changes in the spinal cord, while the ganglion cells remain preserved.

CHRONIC CEREBRAL HYPOPERFUSION INDUCED SYNAPTIC PROTEOMIC CHANGES IN RAT CENTRAL NERVOUS SYSTEM

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Chronic cerebral hypoperfusion (CCH) is an ischemic state where cerebral blood flow is gradually and permanently reduced. CCH leads to cognitive impairment and neurodegenerative diseases, such as vascular dementia and Alzheimer's disease. It can induce neuroplasticity damage, inflammatory response and hypoperfusion-related metabolic changes in different brain regions, such as various cortical areas and hippocampus. Experimental animal models are effective tools to study the mechanism of neurodegeneration to achieve potential therapeutic targets of CCH. The most widely used model of CCH is the permanent bilateral common carotid artery occlusion in rats. In our study, we have performed an unbiased survey of synaptosome proteome changes of hippocampus and two cortical areas (frontal and occipital cortex) by a high-resolution quantitative proteomic approach. Stepwise bilateral common carotid artery occlusion or sham operation were performed on rats. The occlusion was monitored by 3D TOF angiography. Eight weeks after the first occlusion, rats were sacrificed and synaptosome samples were prepared from the 3 brain areas of 12 animals. Then, the proteins were separated by 2D-DIGE and analyzed with DeCyder software. Finally,94, 32 and 15 proteins were identified from the altered spots by LC-MS from the occipital, frontal cortex and hippocampus, respectively. The interaction network of the proteins with significantly changed levels was analyzed, common regulator and common target analyses were performed.

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THE CENTRALLY PROJECTING EDINGER-WESTPHAL NUCLEUS IN THE ROTENONE MODEL OF PARKINSON'S DISEASE

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Introduction:The neuropathological diagnosis of Parkinson's disease (PD) is based on cell loss in the dopaminergic substantia nigra (SN) and the presence of Lewy bodies. Anxiety and depression are commonlyoccurringnon-motor symptoms preceding the occurrence of the motor deficit. Morphological changes were found in numerous other nuclei of the brainstem includingthe Edinger-Westphal nucleus (EW).In this nucleus, the centrally projecting cells (cpEW) expresses urocortin1 (Ucn1) thatcontributes to stress response and emotional reactions. Our aim was to showthe involvement of cpEW-Ucn1 in PD-associated mood disorders using the rotenone model of PD in the rat. We hypothesized that besides thewell-known neurodegenerative alterations in the SN,morphological changes in the urocortinergiccpEWwill occur, that contributes to depressed mood and increased anxiety.

Methods: To induce PD,Wistar rats received subcutaneous rotenone injections for 5 weeks vs. solvent treated controls. Open field (OFT) and sucrose preference tests (SPT)were conducted. Morphological changes were assessed by multiple label immunofluorescence.

Results:Rotenonetreated ratsshowed increased anhedonia level in SPT. In OFT, increased anxiety was found, besidesmotordysfunction. The model's validity was proven by the reduced dopaminergic cell countin the SN that correlated with the loss of theurocortinergic cells and the reduction of Ucn1 density.The drop of Ucn1 expression correlated with the behavioural changes. Occasionally, activated microglia cells were found performing phagocytosis on Ucn1 cells upon rotenone treatment.

Conclusion:The impairment of the Ucn1 neurons in the cpEWmay contribute to the non-motor symptomsofPD.

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ASTROCYTES ARE REMARKABLY VULNERABILE TO ISCHEMIC/ANOXIC INJURY

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Glutamate excitotoxicity is responsible for cell death and lesion progression in multiple cerebrovascular disorders, such as ischemic stroke. Anoxic depolarization leads to extensive glutamate-release but its contribution to excitotoxicity remains unclear. Further, while the impact of glutamate excitotoxicity on neurons has been much investigated, less is known about the vulnerability of astrocytes. We aimed to compare the susceptibility of astrocytes and neurons to ischemia/anoxia. Ischemia/anoxia was induced by the bilateral occlusion of the common carotid arteries in combination with O2-withdrawal from the anesthetic gas mixture (arterial blood pO2=35±10 mmHg in anesthetized, old (18 months) male Sprague-Dawley rats (n=7), followed by reoxygenization 5 min later. Brains were removed after transcardial perfusion with physiological saline and 4% paraformaldehyde, and sliced to 20µm coronal sections with a freezing microtome. Cleaved caspase-3 (CC3), an apoptosis marker was co-localized with astrocytes (GFAP) and neurons (NeuN) relying on immunocytochemsitry. Semiautomatic cell counting was performed with ImageJ software in cortical, hippocampal and striatal regions. Our preliminary results show that astrocytes are more sensitive to anoxic stress than neurons. In the cortex, less than 10% of the NeuN positive neurons were engaged in apoptosis, meanwhile half of the GFAP positive astrocytes expressed CC3. In addition, the presence of CC3 near the nucleus of astrocytes was clearly associated with GFAPdegradation. While astrocytes are thought to be resistant to ischemic stress more than neurons, our data suggest that ischemia in combination with severe anoxia predominantly injures astrocytes in the old rodent brain.

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DIFFERENTIAL EFFECTS OF GAP JUNCTION MODULATORS IN ABSENCE AND TEMPORAL LOBE EPILEPSYMODELS

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The influence of astrocytic cell networks on neuronal network activity is an emerging issue in epilepsy.Among the various mechanisms by which astrocytes modulate neuronal function, astrocytic gap junction coupling is widely considered to be acrucial mechanismin epileptic conditions, contributing to the synchronization of astrocytic and neuronal cell networks, possibly inducing recurrent epileptiform activity. Here weexplored whether modulation of astrocytic gap junctions could alter epileptic seizures in differenttypes of epilepsy. Openingof gap junctions bytrimethylamine intensifiesseizure-like events in the low-Mg^{2+}*in vitro*epilepsy model of temporal lobe epilepsy, while alleviating seizures in the*in vivo*WAG/Rij rat model of absence epilepsy. In contrast, application of the gap junction blocker carbenoxolone prevents the appearance of seizure-likeevents in the low-Mg^{2+} epilepsy model, butaggravates seizures in non-convulsive absence epilepsy, *in vivo*. We conclude that astrocytic gap junctions are key players in the formation of epileptiform activity and have different mode of action in the convulsive and non-convulsiveepilepsy types.

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INVASION POTENTIAL OF GLIOBLASTOMA SAMPLES IN THE ASPECT OF ECM MOLECULES – A CASE REPORT

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Keywords: glioblastoma, invasion potencial, prognosis, extracellular matrix molecules, survival

Introduction: The glioblastoma is the most common and most aggressive malignant primary brain tumor. Its outstanding invasion potential and remarkable intratumoral heterogeneity set back the successful treatment and creates significant interpatient prognostic differences. Molecules of the ECM are important part of this process, therefore they are promising prognostic marker candidates.

Methods: Two prognostically different GBM patients have been compared to each other from a study that consist of GBM patients with favorable and unfavorable prognoses. The expression of 19 ECM molecules was measured by qRT-PCR in fresh-frozen tumor samples. The mRNA expressional patterns were used to differentiate the prognostic groups.

Results: The overall survival time of the patients was 16 (A-patient) vs >71 months (B-patient, still alive). The clinical courses showed distinct aggressiveness based on the clinical and radiological progression, as well as treatment response. The different character of the tumors could be confirmed by the investigation of ECM molecules. The fold-changes between the two samples were more than 2-fold in case of ITGAV, ITGB1, EGFR, MMP2, PDGFA, VCAN, MKI67. The overall usability of the expressional pattern in the differentiation of the prognostic groups was satisfying (sensitivity: 0.71, pos. pred. value: 0.71).

Discussion: The expressional pattern of the ECM molecules correlates greatly with the prognosis of GBM patients, therefore, adopting it as a prognostic factor is encouraged. The distinct invasion potential was also confirmed on individual patient level, highlighting the significance on a clinically-relevant manner.

IN VIVO DIFFUSION TENSOR IMAGING OF THE BRAINS OF STRESSED RATS

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AIM:
Stress is the most important triggering factor for the development of various psychiatric disorders, but the underlying neurobiological events are not completely understood. Stress exposure can affect neuroplasticity and structural integrity of limbic brain areas. Here, we used diffusion tensor imaging (DTI) to study the temporal dynamics of stress induced structural changes in the brains of laboratory rats.
METHOD:
Young adult male Sprague-Dawley rats (control group: 16 animals, stress group: 16 animals) were subjected to restrain stress (6 hours/day) for 21 days. DTI were acquired with a 4.7T Bruker PharmaScan pre-clinical MR scanner. Baseline measurements were performed before stress and the protocol was repeated three times: one week (acute stress), three weeks (chronic stress) after stress initiation and two weeks after the end of the stress (recovery). A pre- and post-processing pipeline was built up by using FMRIB Software Library. Repeated-measures ANOVA was used to assess within-subject differences.
RESULTS:
Diffusion data were corrected for eddy currents and subject movements by the detection and the replacement of positive and negative outliers and then fractional anisotropy (FA), mean diffusivity (MD), eigenvalues ($L_{1,2,3}$) and eigenvectors ($V_{1,2,3}$) were calculated. After Bonferroni adjustment significant within-subject differences were found in FA and MDin the corpus callosum, external capsule and inferior colliculus of control rats, while no differences were observed in stressed rats.
CONCLUSION:
After the development of a modern image processing pipeline, stress appears to have negative impact on the development of rat brain.

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TRANSGENIC MICE EXPRESSING THE HUMAN SOMATOSTATIN RECEPTOR 4: A NOVEL HUMANIZED MODEL FOR TRANSLATIONAL RESEARCH

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We discovered that the somatostatin 4 receptor (sst_4) mediates analgesic, anti-depressant and anti-inflammatory functions of somatostatin without endocrine actions. This proposed new drug developmental perspectives and small molecule sst_4 agonists are currently tested. Sst_4 was shown to be present in pain and mood-related brain regions of the mouse, but its expression and function in humans is not known.
Therefore, we constructed a PiggyBac transposon vector containing human chromosomal fragment with the SSTR4 gene that also expresses the Luciferase-tdTomato reporter fusion protein. P2A self-cleaving site ensures that the human sst_4 is expressed separately from the reporter fusion protein not affecting the function.

We did transgenesis in SSTR4-deficient mice and one transgenic female was obtained which had offsprings. This first generation mother had several copies of the randomly inserted transgene. We bred mice carrying one copy of the transgene. With ligation-mediated PCR, we located 3 copies on chromosome 3, 10 and X, and there are 2 lines with yet unknown locations of their transgenes.

In vivo imaging showed Luciferase luminescence in the brain with the strongest signal in the bulbus olfactorius, but tdTomato was not detectable either in vivo or on histological sections.

In the elevated plus maze sst_4 mice spend less time in the open arms showing greater anxiety compared to wildtypes, but insertion of the human SSTR4 gene reversed this anxious phenotype providing evidence for its functionality.

This novel humanized model is very useful for detecting pathology-related expression changes and the effect of our novel sst_4 agonists.

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REPRESENTATIONAL UNTANGLING BY THE FIRING RATE NONLINEARITY IN V1 SIMPLE CELLS

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An important computational goal of the visual system is “representational untangling” (RU): representing increasingly complex features of visual scenes in an easily decodable format. RU is typically assumed to be achieved in high-level visual cortices via several stages of cortical processing. Here we show, using a canonical population coding model, that RU of low-level orientation information is already performed at the first cortical stage of visual processing by a fundamental cellular-level property: the thresholded firing rate nonlinearity of simple cells in the primary visual cortex (V1). We identified specific, experimentally measurable parameters that determined the optimal firing threshold for RU and found that the firing thresholds of V1 simple cells extracted from *in vivo* recordings in awake behaving mice were near optimal. These results suggest that information re-formatting, rather than maximisation, may already be a relevant computational goal for the early visual system.

INSULIN EXERTS DIFFERENTIAL NEURITE OUTGROWTH-PROMOTING EFFECTS ON SUBPOPULATIONS OF CULTURED DORSAL ROOT GANGLION NERONS

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Introduction: Neurite outgrowth-promoting effect is one of the salient features of insulin's action on cultured dorsal root ganglion (DRG) neurons. However, the significance of the insulin receptor(InsR) expression and the chemical phenotype of DRG neurons in relation to the neurite outgrowth-promoting effect of insulin have not been examined. The aim of the present study was to evaluate the effect of insulin on neurite outgrowth of DRG neurons of different chemical phenotypes which express or lack the InsR.

Materials and methods: Selected parameters of neurite outgrowth of cultured DRG neurons which expressed the InsR, transient receptor potential vanilloid type 1 receptor (TRPV1), calcitonin gene-related peptide (CGRP) and/or bound the *Bandeiraeasimplicifolia*isolectin B4 (IB4)by using immunohistochemical and quantitative stereological methods were assessed in the presence or absence of insulin.

Results: Insulin, at a concentration of 10 nM, significantly increased the quantified parameters of neurite outgrowth of cultured DRG neurons as compared to neurons cultured in control medium. ~43% of neurons displayed InsR-immunoreactivity. The proportions of TRPV1-, CGRP-immunoreactive(IR), and IB4-binding neurons amounted to ~61%,~57% and ~31% of DRG neurons IR for the InsR. Of the IB4-positive population only the InsR-IR neurons were responsive to insulin. In contrast, TRPV1- and CGRP-IR neurons showed increased tendency for neurite outgrowth.However, the responsiveness of DRG neurons expressing the InsR was superior to populations of DRG neurons which lack this receptor.

Conclusions: These findings suggest distinct regenerative propensity for differing populations of DRG neurons which is significantly affected through insulin receptor signaling.

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DETECTION AND NEUROCHEMICAL CHARACTERIZATIONOF SOMATOSTATIN 4 RECEPTOR EXPRESSION IN THE MOUSE BRAIN

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Somatostatin is expressed in brainregionsrelatedtopain- and moodregulation. Itsinhibitory G-protein coupled receptor subtype 4 (sst_4) mediatesanalgesic, anti-inflammatory and antidepressant effects without endocrine actions. Itwas suggested to be a novel drug target for chronic neuropathic pain. However, its central nervous system distribution and mechanism of the inhibitory actions are not known due to the lack of specific antibody.

We mapped sst_4 expression using β-galactosidase immunohistochemistry in the brain of *Sst*$_4$knockout (KO) mice where sst_4 was replaced by the l*acZ* gene. Since KO mice are not appropriate for determining functional changes and co-localization, we alsoperformed ultrasensitive RNAscope-based in situ hybridization and neurochemical characterization on formalin-fixed, paraffin-embedded coronal sections. *Sst*$_4$ mRNA was quantified by qPCRin thepositive regions.

Strongsst_4-related β-galactosidaseimmunopositivity was detected in the hippocampus, moderate in the medial septum, amygdala, habenula in both sexes. Remarkablymore intensive signalwas seenin the primary somatosensory cortex of male mice compared to females. *Sst*$_4$ mRNA wasabundant in the hippocampal CA1 region, amygdala, spinal cord, sensory and motor cortices (layer 5). It was co-localized with vesicular glutamate transporter 1 in most regions and choline-acetyl transferase in the habenula. Sst_4mRNA was significantly higher in the dorsal root ganglia compared the spinal cord.

These are the first data for sst_4 expression in glutamatergic and cholinergic excitatory neurons of the nociceptive pathway. This might explain its unique value to simultaneously inhibit chronic pain and depression.

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HETEROGENEOUS EXPRESSION OF PARVALBUMIN AND EXTRACELLULAR MATRIX MOLECULES IN THE RED NUCLEUS OF RAT

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Previously we described that distribution of extracellular matrix (ECM) molecules showedregional differences in the red nucleus (RN). We also observed that the expression pattern of a highly condensedECM, theperineuronal net (PNN)is related to the morphofunctional characteristics of neurons. Other studies revealed neurochemical differences between the neurons of red nucleus, e.g., the parvalbumin (PV) showed heterogeneous distribution within the nucleus. The aim of our study was to examine thedistribution and molecular composition of PNN around the PV positive neurons in the red nucleus of rat.

The experiments were performed on adult female Wistar rats. Hyaluronan (HA) was detected with biotinylated Hyaluronan Binding Protein. WFA histochemistry was performed using biotinylated *Wisteria floribunda* agglutinin as a general marker of PNN. Lecticans (aggrecan and brevican) and the PV were detected with antibodies.

Our results showed significant differences between the distribution of PV positive/PNN bearing neurons in the parvo- and magnocellular parts of the red nucleus. In the parvocellular part, the majority of small sized neurons showed intense PV staining, whereas the ECM reactions were negative or weak in the pericellular area.In contrast, the large–sized neurons of the magnocellular area were surrounded by robust PNN with each ECM reaction, but the PV immunostaining was faint.The HA or brevican positivedots along the axons may represent the nodes of Ranvier.According to our finding, both the PV positivity and expression of PNN is closely related to the morpholofunctional properties of rubral neurons.

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THE EFFECT OF INSULIN ON THE NOCICEPTIVE EFFERENT FUNCTION IN MENINGEAL TISSUES

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Stimulation of chemosensitive afferents results in the release of vasoactive neuropeptides e.g. calcitonin gene-related peptide (CGRP). A distinct part of chemosensitive neurons expressing the nociceptive receptor transient receptor potential vanilloid 1 (TRPV1) also express the insulin receptor (IR). Previous findings showed that IR can sensitize TRPV1 through intracellular signal transduction mechanisms. In this study, we aimed to investigate the functional interaction between IR and TRPV1 in dura mater encephalipreparations of rats.

Using ‘ex vivo’ dura mater preparations we tested the effect of insulin on the release of CGRP and also studied the potentiating effect of insulin on the TRPV1 agonist capsaicin-induced CGRPrelease. We measured the amount of CGRP with ELISA technique. In ‘in vivo' experiments we measured the effect of locally applied insulin on TRPV1-mediated meningeal vascular functions using laser Doppler flowmetry. Besides, using immunohistochemical technique we investigated the colocalization of IR and TRPV1 in the trigeminal ganglion supplying the dura mater.

Our results showed that insulin evoked CGRPrelease due to the activation of IR. Further, administration of insulin increased the amount of capsaicin-induced CGRPrelease. Preincubation with a TRPV1 antagonist capsazepine decreased the effect of insulin on CGRPrelease. According to ‘in vivo’ results, application of insulin enhanced the TRPV1-mediated meningeal blood flow changes. Immunohistochemical staining proved the colocalization of IR and TRPV1 in a high number of trigeminal neurons.

According to our findings, the presence of insulin may mediate meningeal nociceptive functions due to the activation and/or sensitization of the TRPV1 receptor.

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CORTEX-WIDE ACTIVATION OF VIP-EXPRESSING INHIBITORY NEURONS BY REWARD AND PUNISHMENT

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The balance between excitation and inhibition is crucial in cortical computations. Disinhibition appear to be a broadly implemented mechanisms for changing this balance and allows associative learning to take place. A subset of cortical inhibitory interneurons that express the vasoactive intestinal polypeptide (VIP) target other inhibitory interneurons and are therefore a suitable candidate to be involved in such processes. In order to understand whether the VIP interneurons contribute to associative learning, we engaged mice in an auditory as well as visual Go/NoGo task and used 3D random access two-photon imaging to measure the calcium responses of up to 120 VIP neurons in the mouse cortex simultaneously. Our data revealed that VIP neuronsare highly activated by reward and punishment throughout different cortical areas. More than 80% of all measured neurons responded to water delivery or air puff or both. Within a given measurement, about 50% of the measured VIP neuronpopulation was recruited after reinforcement onset during a trial. VIP-neurons in the visual cortex also responded to drifting gratings of different orientations. These visual responses were uncorrelated with their reinforcement-related activation.In addition, the amplitude of VIP-responses to the reinforcement was in some cases significantly modulated by the arousal of the animal (assessed by the pupil diameter). These data indicate that VIP neurons might be an important part of a general cortical circuit necessary for associating specific stimuli with positive and negative behavioral outcomes.

ELECTROPHYSIOLOGICAL EXAMINATION OF UNDERLYING NEURONALMECHANISMS OF TASTE REACTIVITY IN THE NUCLEUS ACCUMBENS OF BEHAVING RATS

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The nucleus accumbens (NAcc) is a key structure in the integration of chemical and other signals arising from the endogenous and exogenous environments. In our previous examinations, the existence of taste responsive NAcc neurons was proved in anesthetized rats. In our recentresearch project,in thissame brain region,we planned to provide complex characterization of the tastedetectionmechanism of neurons in behaving animals.
In this study, extracellular single neuron activity is recorded in the NAcc of male rats by means of tungsten wire microelectrodes when taste reactivity test is performed with 2 concentration series of the five primary taste qualities (NaCl, HCl, monosodium-L-glutamate [MSG], sucrose, QHCl). During the intraoral infusion period, all the ingestive and aversive mimics and postural-locomotor response patterns of rats are recorded by video camera.After the frame by frame analysis of these above records, the behavioral results are correlated to the electrophysiologically recorded neuronal firing patterns.
These new series of ourexperiments, i.e. when the analysis ofelectrophysiological examinations is combined with that of the simultaneous behavioral tests, are supposed to unravel so far unknowncorrelationsbetween distinct neuronal firing patternsandthe specific taste reactivity response phases.These genuinely new findings may further elucidate the distinguished role of limbic forebrain neurons in adaptive taste detection mechanisms ofthe central feeding control.

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INVESTIGATION OF PERISOMATIC INPUTS ON GIANT MOTOR NEURONS IN THE HUMAN PRIMARY MOTOR CORTEX

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One of the most special neuron population of the human central nervous system is the group of giant pyramidal cells in the primary motor cortex (Betz cells). Their axons constitute ca. 10% of the corticospinal tract, and they play an important role in fine motor movements. Our focus was to investigate and characterize their perisomatic input for better understanding their function since the literature is controversial in that field.

We have investigated the primary motor cortices of eightpost mortem perfusion-fixed subjects (PMI: 2-5 h) without any known neurological deficits. We used SMI32 and parvalbumin (PV) immunostaining to visualize Betz cells to optical-, fluorescent- and electron microscopic examination. SMI32 labels all of the giant motoneurons, and according toprimate data PV is also present in a subpopulation of them. PV-immunostained Betz cells were further investigated in the electron microscope.

In our human samples, various portion of SMI32-labeled Betz cells were PV-immunopositive, too. Betz cells are heavily covered by mostly inhibitory synapses. Asymmetric-like synapses were proved to be vGlut1-negative, originating presumably from subcortical sources. The projecting neurons of the ventral lateral nucleus of the thalamus, which are PV+ in the primata, are the most likely candidates.

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CARBOXAMIDO STEROIDS INHIBIT THE TRP ION CHANNEL ACTIVATION AND HAVE ANALGESIC EFFECT VIA LIPID RAFTS

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Transient Receptor Potential Vanilloid 1 and Ankyrin 1 channels (TRPV1 and TRPA1) are nocisensors playing important role to trigger pain. We provided evidence that the disruption of the plasma membrane microdomains of lipid rafts with sphingomyelinase and methyl β-cyclodextrin influenced the activation mechanisms of TRP channels. We described also that a carboxamido-steroid compound (C1) had an inhibitory effect on TRP ion channel activation through lipid raft disruption. The aim of this study is to examine the potential analgesic effect of C1 in *in vivo* mouse models beside the *in vitro* actions.

The effect of C1 was analysed on isolated trigeminal (TG) neurones by measuring agonists-induced Ca^{2+}-transients with ratiometric technique, and on TRPV1- or TRPA1-expressing CHO cells by measuring ^{45}Ca-uptake. We investigated the mechanonociceptive and thermonociceptive threshold of the animals in RTX-induced thermal, and mechanical hyperalgesia, and formaldehyde-evoked hyperalgesia model. The analgesic effect of C1 was also measured in capsaicin-evoked acute nocifensive („eye-wiping") test.

The results show, that C1 treatment diminished the percentage of responsive cells, and the magnitude of Ca^{2+} transients in TG neurones, and decreased the ^{45}Ca-uptake on receptor-expressing CHO cells. C1 treatment significantly reduced the RTX-induced thermal, and mechanical hyperalgesia the formaldehyde-evoked hyperalgesia and the number of capsaicin-evoked eye-wiping movements in *in vivo* models.

On the basis of *in vitro* and *in vivo* results we suggest that the hydrophobic interactions between the TRP channel and lipid raft interfaces modulate the opening properties of these channels. Therefore, targeting this interaction might be a promising tool for drug developmental purposes.

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PUTATIVE POSTSYNAPTIC TARGETS AND FUNCTION OF LOCAL AXON COLLATERALS OF SPINAL DORSAL HORN PROJECTION NEURONS

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Approximately ten years ago our research group was the first to report that the main axon of projection neurons (PNs) in the superficial spinal dorsal horn (SDH) – involved among other tasks in pain transmission – give rise to distinct types of local axon collaterals before leaving the spinal grey matter (Szucs et al., 2010). The course and distribution of the collaterals suggestthat they establish local and propriospinal synaptic connections. However, the target neuronal elements and the function of these synaptic contacts remain to be elucidated.

To achieve the above goal we used retrograde tracing methods to identify and selectively manipulate PNs in the SDH. 1) DiI was injected in the parabrachial complex to allow identification of PNsomata during in vitro recordings. 2)AAV-pgk-Cre was injected in the parabrachial complex of tdTomato reporter mice to allow visualization of PN collateral axons without prior biocytin staining. 3) The same retrograde vector was injected into ChR2 reporter mice to allow selective activation of PNs or their axon collateral terminals during in vitro recordings.

We found that axon terminals of PNs contact mostly local interneurons within the SDH. Contacts are present on somata and proximal dendrites of SDH neurons. The activation of PNs (or their axons) evoked different types of responses in the recorded non-PN neurons including slowly developing tonic depolarization and fast transient inhibitory events.

Our preliminary findings support our earlier hypothesis that PNs are not simple output elements of the SDH circuitry but active participants of local information processing.

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COMPLETE SYNAPTIC COVERAGE OF ONE DENDRITE OF A CALBINDIN-D_{28K} IMMUNO-POSITIVE INTERNEURON IN MOUSE V1

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The functional implication of the remarkable diversity of GABAergic inhibitory interneurons (INs) from the circuitry point of view has gained recently attention because different subtypes can be implicated in specific functional roles. Quantitative data on INs regarding their synaptic connectivity with other members of the neural network are quite rudimentary. Our major goal is to generate a quantitative electron microscopic (EM) database of the complete synaptic coverage of major subtypes of INs in the mouse primary somatosensory (S1) and visual (V1) cortices.

60 μm thick coronal vibratome sections were collected from tissue blocks containing S1 and V1. Adjoining sections were reserved for EM analyses and stained for a particular GABAergic subtype marker, respectively. Thereafter, we utilized the “mirror” technique which rests on the precise identification of “mirror” cells which are cut in half by the sectioning plane of adjoining sections.

At first a Calbindin-D_{28K} immuno-positive IN was chosen from layer 5 in V1. 50 nm ultrathin serial sections (~1200) were collected and processed for TEM analysis. The first 30 μm segment of the selected dendrite has been traced (photographed) and reconstructed in 3D until now. We were able to determine the main synaptic parameters: distance from soma location, surface area and volume of the presynaptic boutons; vesicle content, surface extent of the active zones.

Our immunohistochemistry-correlated EM method proved successful in allowing to tracing long dendrite segments originating from the parent soma in specimens free of ultrastructural deficits caused by the immunohistochemical procedure.

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EXCITATORY DYNORPHINERGIC INTERNEURONS ARE INVOLVED IN NOXIOUS HEAT-ASSOCIATED NOCICEPTION MEDIATED BY P-S10H3 IN MICE

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Phosphorylation of serine 10 (S10) in histone 3 (H3; p-S10H3) has been recently demonstrated to participate in processing nociception at the spinal level following noxious stimuliation. However, the distribution and types of superficial dorsal horn (SDH) neurons involved in p-S10H3-mediated nociception remains to be elucidated. Thus, in the present work we performed immunohistochemistry to determine dorsal horn neuronal populations responding with increased p-S10H3 levels to acute application of noxious heat (60 °C), noxious cold (4 °C) and ultraviolet B (UVB) irradiation.

We found that around half of the SDH neurons with p-S10H3 belonged to the inhibitory SDH neuronal population when assessed in VGAT/tdTomato mice. We observed that phosphorylation of S10H3 induced by noxious heat was restricted to SDH neurons exhibiting dynorphin-precursor protein, preprodynorphin- (more than 60%) and calretinin-immunostaining (18%) in spinal cord of mice, while co-expression with other tested inhibitory neuronal markers (neuropeptid Y, nNOS, parvalbumin) was negligible. We also reveal that the majority of p-S10H3-expressing dynorpihenrgic neurons lack Pax2 and thus are excitatory. We also found that this class of excitatory dynorphinergic neurons showed p-S10H3 expression only in response to noxious heat but not to acute appliaction of noxious cold or exposition to UVB.

We provided evidence that p-S10H3 level is elevated in a dedicated subset of excitatory dynorphinergic SDH neurons in the mouse following noxious thermal stimulus.

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COMPARISON OF ENDOCRINE DISRUPTOR INDUCED CHANGES OF INFLUENCED RECEPTOR MRNA EXPRESSION IN DIFFERENT RODENT MODELS

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The term “endocrine disruptor” (ED) refers to a group of substances, which – even in small doses – alter the physiological regulatory pathways of endogenous hormones, and thus, disorganize the normal neuroendocrine functions of the body. The hormonal imbalance caused by these foreign substances is a result of dysregulated feedback loops and/or disturbed cellular signaling pathways, manifesting in the change of hormone-receptor transcription and translation in the target cells. Altering the balance of the neuroendocrine regulation will lead to serious developmental, medical and even agricultural consequences, therefore ED effects are widely researched in the European Union. However, the effects of EDs are mainly tested on rodent models and in some cases conclusions are drawn from only one model.

We treated primary cerebellar cell cultures (originated from postnatal 7 days old rat and mouse pups) with estrogen, thyroid hormones, bisphenol-A, zearalenone and arsenic and combinations of the substances. The change of thyroid receptor α, β and estrogen receptor α, β mRNA expression were measured by qPCR. Results were compared to non-treated controls, and the difference inthe change of transcription were examined between rat and mouse samples.

Our results show the difference in ED affected receptor expression of cerebellar granule cells cultured from mouse and rat – not just in the level of mRNA transcription but even in the direction of change – thus proving the need of a wider test model for experiments in the field of endocrine toxicology.

THE ROLE OF NEUROKININ SIGNALLING RECEPTOR IN THE DEVELOPMENT OF ENDOTOXIN FEVER

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Introduction: The neurokinin-1 (NK1) receptor and its ligand (Substance P) were shown to contribute to the development of lipopolysaccharide (LPS)-induced fever, but the exact mechanism is unknown.
Methods: We used adult NK1 receptor knockout (*Tacr1*$^{-/-}$) and wild type (*Tacr1*$^{+/+}$) mice of both sexes. After intraperitoneal administration of LPS (120 µg/kg), thermoregulatory responses and changes in inflammatory biomarkers (serum cytokine levels, tissue cyclooxygenase-2 [COX-2] expression, prostaglandin E_2 [PGE_2] concentration) were studied in the mice.
Results:LPS caused fever, as expected. At 40 minutes after LPS administration, the increase in deep body temperature and oxygen consumption was attenuated in *Tacr1*$^{-/-}$ mice compared to wild type mice (38.1 ± 0.2 vs. 38.5 ± 0.2°C and 173 ± 9 vs. 189 ± 6 ml/kg/min; $p < 0.05$). The fever response to intracerebroventricular administration of $PGE_2$was unchanged in *Tacr*$^{-/-}$ mice. After LPS administration, COX-2 mRNS expression increased in the lungs,liver, and brain in both genotypes. The LPS-induced increase in COX-2 protein expression was attenuated in the lungs and it tended to be diminished in the liver of*Tacr1*$^{-/-}$ mice. After injection of LPS, PGE_2concentrationsignificantly increased in the lungs of*Tacr1*$^{+/+}$ (but not *Tacr1*$^{-/-}$) mice.
Conclusion: Our results suggest that NK1 receptors play a role in fever development. The NK1 receptor contributes to the early phase of LPS-induced fever through enhancementof peripheral COX-2 protein expression. Our findings further advance our understanding about the connection between NK1 receptor pathway and the “cytokine-COX-2-PGE_2” axis in fever.

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FUNCTIONAL ASYMMETRY OF THE HYPOTHALAMUS IN MALE RATS

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Like many other areas of the brain, hypothalamic nuclei are symmetrically located in the right and left side of the III. ventricle. Earlier results of our research group proved that the hypothalamic hemispheres do not work parallel and do not take part in particular regulatory processes with the same intensity. This kind of functional asymmetry has been described concerning the control of reproductive function in female rats. In the present experiment, we hypothesised that hypothalamic functional asymmetry can be observed also in male animals, and this sort of task-sharing is manifested in the control of food intake and reproduction, two intensively regulated and energy-requiring of the hypothalamic functions. To test our hypothesis, we performed metabolic examinations through the analysis of mitochondrial respiration. In order to obtain viable mitochondrial fractions from the isolated left and right hypothalamus of male rats for this purpose, we applied differential and Percoll-based gradient fractionation procedure. Experimental animals were examined in different reproductive (bilateral orchiectomy, testosterone treatment) and satiety states (food deprivation, scheduled feeding, etc.). Results on reproductive states did not confirm our assumption of lateralized operation of the hypothalamus in males. On the other hand, with regard to satiety state, it has been proved that starvation and fasting increase the metabolic activity of the left hypothalamus by suppressing the otherwise characteristic right sided dominance. Our results strongly suggest that regulation of satiety state and energy expenditure is controlled in a lateralized manner on the hypothalamic level at least in male rodents.

RAPID EFFECT OF 17β-ESTRADIOL ON THE ELECTRICAL ACTIVITY OF STRIATAL CHOLINERGIC INTERNEURONS

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The cholinergic interneurons (ChIs) in the striatum play a pivotal role in the basal ganglia circuitry, and dysfunction of striatal acethylcholine neurotransmission has been implicated in the pathogenesis Parkinson'sand Alzheimer's disease (PD, AD). Among many different factors controlling the functions of cholinergic neurons 17β-estradiol (E2) is an essential factor. Besides its genomic effects E2 exerts rapid non-classical actions on the electrical activity and signaling of neurons.The aim of our study was to examine the rapid non-classicaleffect of E2 on the function of striatal ChIs by the means of the patch clamp technique in adult cholineacetyltransferase(ChAT)-tdTomato transgenic mice. Our immunofluorescence experiments showed that over 99% of tdTomato-expressing striatal neurons are ChAT-positive i.e. they are cholinergic interneurons. These neurons lack parvalbumin and $K_v2.1$ but express membrane estrogen receptor (GPER1). Furthermore, our results showed classical estrogen receptor (ERα) expression in a subpopulation of ChIs. Cell-attached/loose patch-clamping experiments revealed that 23% and 50% of examined neurons were tonically active in adult female and male mice, respectively. Physiological dose of E2 (100 pM) or pharmacological dose (100 nM) changed firing variability but not the frequency of patched ChIs with firing rate > 0.33Hz in 5 minutes. These results indicate that E2rapidly tunes the activity of ChIs. Further studies are needed to elucidate the detailed mechanism of this rapid non-classical effect of E2 on the electrical activity of ChIs.

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AGE DEPENDENT NEURONAL ACTIVATION OF STRESS CENTERS IN ACUTE STRESS MODEL IN THE RAT

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Introduction:The hypothalamus-pituitary-adrenal axis (HPA) is the chief regulator of the stress-response. The key of the HPA is the parvocellular paraventricular nucleus of the hypothalamus (pPVN) controlled by higher-order limbic stress centres. The HPA axis reactivity is considered to be a function of age, but to date, little is known about the background of this age-dependency. Sporadic literature data suggest that the stress sensitivity as assessed by semi-quantitation of the neuronal activity marker c-Fos may also be influenced by age.

Methods: We investigated the HPA activity and c-Fos immunoreactivity 2h after the beginning of a single 60-mins acute restraint stress in eight age groups of male Wistar rats. We hypothesized that the function of the HPA axis (i.e., pPVN c-Fos and blood corticosterone (CORT) level), the neuronal activity of nine stress-related limbic areas (i.e., magnocellular PVN (mPVN), medial (MeA), central (CeA), basolateral nuclei of the amygdala, the oval (ovBNST), dorsolateral (dlBNST), dorsomedial (dmBNST), ventral and fusiform (fuBNST) divisions of the bed nucleus of the stria terminalis (BNST)), and two brainstem stress centres such as the centrally projecting Edinger-Westphal nucleus (cpEW) and dorsal raphe nucleus (DR) show age dependency in their c-Fos response.

Results indicate that the stress-induced rise in blood CORT-titer was lower in young age reflecting relatively low HPA activity. All 12 stress-related brain areas showed c-Fos response that peaked at 2 months of age.

Conclusions: Stress centres show strong age-dependent basal- and stress-induced c-Fos expressions, which indicate the importance of further examinations in age- and stress-associated mood disorders.

DIFFERENTIALLY EXPRESSED GENES IN THE PREOPTIC AREA OF MOTHER RATS - AN RNA-SEQ STUDY

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Postpartum behavioural and physiological changes are important parts of reproduction. The central regulation includes the preoptic area whose lesionin rodents abolishes maternal behaviours while its stimulation enhances maternal behaviours. Our objective was to identify genes involved in maternal adaptation of the preoptic area. Therefore, we compared gene expression on 10th postpartum day in the preoptic area of lactating rat mothers and mothers whose pups were taken away immediately after delivery. The pup-deprived controls did not take care of the pups on the 10th postpartum day when the preoptic area was dissected forRNA sequencing. After false discovery rate correction, we found 7 differentially expressed genes betweennormal and pup-deprived mothers. Subsequently, we validated the changes in Nwd1 (NACHT And WD Repeat Domain Containing 1), Rbm3 (RNA-binding protein 3) and Ndufs5 (NADH:Ubiquinone Oxidoreductase Subunit S5). Nwd1, a regulator of androgen receptor levels, and Ndufs5, an accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase showed significantly higher while Rbm3, which modulates translation, was reduced in maternally behaving animals suggesting that these genes are involved in the maternal adaptation of the preoptic area.

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THE EFFECTS OF ENERGY SUBSTITUTION DURING SLEEP DEPRIVATION ON THE FOLLOWING REBOUND SLEEP

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Cholinergic basal forebrain (BF) neurons are implicated in cortical activation and the induction of recovery sleep (RS). Wake (W) associated increase in the activity of these cells results in a subsequent decrease in activity which contributes to the induction of RS. It was speculated that the suppression of energy reserves in BF neurons due to the increased activity during W may be an important factor in this mechanism. To test this hypothesis, we studied how local BF administration of energy sources, glucose (GLU), lactate (LAC) and pyruvate (PYR), during sleep deprivation (SD) influences the subsequent RS.

In 8 Han-Wistar rats with implanted EEG/EMG electrodes and guide cannulae for microdialysis probes, the probe targeted into the BF was perfused (1µl/min) with artificial cerebrospinal fluid (CSF) on the baseline day. Then, on the subsequent SD days, the rats were sleep deprived for 3 h, and during SD, the microdialysis probe was perfused with CSF or with a solution containing 20 mM GLU and 10 mM LAC or 20 mM GLU, 10 mM LAC and 20 mM PYR. Sleep was recorded for 24 h on each day.

The GLU-LAC-PYR solution suppressed non-REM sleep (NREMS; SD-CSF: 121.6±2.7%, SD-GLU-LAC-PYR: 102.6±5.3% of the baseline day value) and resulted in a tendency to increase REM sleep (REMS) during RS. The GLU-LAC solution resulted only in a tendency to decrease NREMS and increase REMS.

Suppression of energy reserves in BF neurons during SD may contribute to the induction of the subsequent NREMS rebound.

PUP-INDUCED BRAIN ACTIVATION IN MOTHER MICE BRAIN IN THE ABSENCE OF PROLACTIN

Szilvia Olah

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Prolactin as well as direct neuronal input from the pups can mediate behavioral and endocrine changes in mothers. Both types of stimuli can activate the nervous system. Immunohistochemical detectation of pSTAT5 is an accepted marker of prolactin-induced signaling in the rodent forebrain. We found an elevated number of pSTAT5-ir neurons in the maternal mouse brain 2-h after reuniting the dams with their litter following a 22-h separation. Bromocriptine, a dopamine 2 receptor agonist, eliminates prolactin secretion from the pituitary. Bromocriptine treatment indeed resulted in the disappearance of pSTAT5 labeling in lactating mice. Suckling can also directly activate specific neuronal pathways, which reach different brain centers to maintain maternal behavioural and endocrine alterations. In the lactating period, neuronal populations activated by pup exposure can be visualized by Fos immunohistochemistry. Indeed, pup exposure and suckling induces the expression of Fos in several different brain areas. Fos induction following suckling remained largely undisturbed in bromocriptine-treated suckled mother mice. Double labeling of pSTAT5 and Fos was performed with and without pup-exposure, and the colocalization was quantitatively analyzed in different brain regions. We found that most neurons responding to suckling in mothers are driven either by prolactin or direct neuronal input from the pups while some neurons are affected by both types of inputs. In addition, the ratio of neurons directly influenced by both routes varies in different brain region. These data suggest that the 2 major forms of inputs from the pups towards the mothers, prolactin and direct neuronal sensory inputs affect brain networks, which partially overlap depending on the brain region, but are generally largely separated from each other, and can be independently activated.

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PROTEOMIC ANALYSIS OF SALIVA IN PACAP KO AND WILD TYPEMICE

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PACAP (Pituitary adenylate cyclase-activating polipeptide) is an endogenous neuropeptide with widespread occurrence. PACAP mainly acts via its PAC1, VPAC1 and VPAC2 receptors, stimulating cAMP/PKA and several other downstream pathways. PACAP has diverse physiological effects and plays various roles under pathological circumstances.

PACAP also affects the secretion of exocrine serous glands such as the lacrimal and salivary glands as well as the pancreas. Immunohistochemical studies have also shown the presence of PAC1 receptors in the salivary glands. In animal experiments exogenously administered PACAP stimulates the amount of secretion of the above mentioned serous glands and the excretion of several factors. Therefore, we hypothesized that PACAP may also affect the protein composition of saliva.

To confirm our hypothesis, we analysed saliva of PACAP knockout (KO) and wild mice with liquid chromatography mass spectrometry (LC-MS). This method issuitably sensitive for detecting small protein concentrations and for qualitative and quantitative comparative studies.

Hundreds of proteins were identified from our samples. Between samples from wild type and KO mice we found several differences in protein concentrations that can be divided into the following groups: antibacterial enzymes and immune response proteins (lactotransferrin, S100A8, cathelicidin antimicrobial peptide), stress response proteins (myeloperoxidase, Annexin-A2), metabolic enzymes (alpha-enolase, glyceraldehyde 3-phosphate dehydrogenase) and other proteins.

Based on our findings, we assume that PACAP affects the salivary composition and may also have immune functionsand effects on the bacterial flora in the oral cavity due to the proteins like lactotransferrin, S100A8 and cathelicidin antimicrobial peptide.

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ASTROCYTIC NETWORK SYNCHRONIZATION PROMOTES NEURONAL SLOW-WAVE ACTIVITY IN THE RAT NEOCORTEX IN VIVO

Zsolt Szabó
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Slow-wave activity (SWA)recording is characterized by barrages of action potentials interspersed by silent phases at low frequency. The generation of this prototypical neuronal activity requires synchronization at large spatial scales, but the underlying cellular mechanism remains largely unexplored.Here, we asked what roles gap-junction interconnected astrocyte networks play during SWA in vivo. Transgenic rat line expressing the fluorescent calcium sensor GCaMP2 in astrocytes and interneurons allowed us to monitor astroglial activity during ketamine-induced SWA. Astrocytic activation displayed similar temporal dynamics to the neuronal activation pattern, but the astrocytic component dominated the early phase of SWA. Moreover, spatial correlation revealed that astrocytes*per se* might trigger the synchronization of neuronal firing.Further supporting this notion, we demonstrate that blockade of astrocytic gap junctional communication reduces the ratio of both astrocytes and neurons involved in SWA. During the late phase of SWA, the astrocytic synchronization gets disintegrated, while the neuronal activity increasingly predominates. These in vivo findings conclusively suggest a causal role of the astrocytic syncytium in large-scale SWA generation.

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PROTEOMIC ANALYSIS OF THE MATERNAL PREOPTIC AREA IN RATS

Edina Brigitta Udvari

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The behavior of female rats changes profoundly as they become mothers. The preoptic area has a central role in the regulation, furthermore, its lesion eliminates maternal behavior. Because the molecular background is poorly understood, we performed proteomic analysis to compare protein level changes associated with motherhood. We used 2-dimensional differential fluorescence gel electrophoresis followed by identification of altered proteins with mass spectrometry. We found 12 proteins with significantly increased and 6 proteins with significantly reduced level in mothers. These results show some similarities with previous genomics approaches in the preoptic area. Functional analysis suggested that most of the altered proteins are involved in glucose metabolism and neuroplasticity. These proteins may support the maintenance of increased neuronal activity and morphological changes in preoptic neuronal circuits known to take place in mothers. Increase in the level of *alpha-crystallin B chain*(Cryab) was confirmed with Western blotting, too. This small heat shock protein may also contribute to maintaining the increased activity of preoptic neurons by stabilizing protein structures and protecting from stressful events. Common regulator and common target analysis of the altered proteins suggested a role of prolactin in the molecular changes in the preoptic area. The results first identified the protein level changes in the maternal preoptic area. The altered proteins contribute to the maintenance of maternal behaviors and may also be relevant to *postpartum* depression, which can occur as a molecular level maladaptation to motherhood.

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CELL TYPE-SPECIFIC CORTICAL INNERVATION OF THE MESOLIMBIC SYSTEM

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Cortical control over the mesolimbic system is important in reward processes. Prefrontal cortex (PFc) provides a major glutamatergic innervation in the ventral tegmental area (VTA) and the nucleus accumbens (NAc). However, exact details of these corticofugal pathways are unknown. Therefore, using single retrograde tracings, first, we mapped the region and layer-specificity of these connections. We found that VTA projecting cells were located in deep, while NAc innervating ones distributed in all cortical (pyramidal) layers. We also analysed the FoxP2 content of the retrogradely labelled cells and observed that large proportion of VTA-projecting population originate from deep L6. Then, with double retrograde approach, we investigated the proportion of PFc cells innervating both regions. Finally, to further dissect the layer-specificity of the cortical innervation of the mesolimbic system, we injected adeno-associated viral constructs into the PFc of cortical layer-specific strains of mice [Rbp4- (L5), Thy1- (L5), NTSR1-Cre (L6)]. The projection pattern and axon-density of these cell-types were different in the VTA and the NAc. NTSR1-positive cells did not project to either the VTA or NAc, while Rbp4- and Thy1-positive neurons intensively innervated both. Furthermore, our data showed that, despite the strict layer-specificity of these genes in the primary cortices, the infected neurons in PFc were intermingled with FoxP2 population and each other. Thus, it suggests that the anatomical layer-specificity of these mouse strains is not entirely valid in the PFc. Our data together opens new opportunities to investigate the cortical control of mesolimbic reward system in a cell-type specific manner.

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AUTOMATED MONITORING OF FEAR-RELATED PARAMETERS AND CLOSED-LOOP STIMULATION OF THE THALAMO-AMYGDALAR NETWORK IN AFFECTIVE BEHAVIOR

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Exploring neuronal networks underlying affective behavior is a heavily investigated field in neuroscience; however, exploiting advantages of modern techniques (optogenetics, multisite electrophysiological data, video and ultrasound recording) synchronously to collect automated and correlated readouts with behavioral relevance is still considered a challenge. We have started to configure a modular and versatile system based on the Bonsai visual programming language in order to investigate the thalamo-amygdalar pathway in associative fear behavior. This system enables the delivery of various (acoustic, visual, aversive somatosensory and optogenetic) stimuli, as well as the parallel monitoring of locomotion, electrical brain signals and vocalizations. As a starting point, we devised an automated method for the offline analysis of freezing behavior and locomotion-based fear responses with optional manual verification. The results of freezing level and open/closed arm relation in elevated plus maze test obtained with offline automated analysis were compared to manual scoring, and showed no significant differences. We also realized a behavior-coordinated, closed-loop optogenetic stimulation of midline thalamic cells which evoked short-term place aversion of a neutral place. As ultrasonic vocalizations (USVs) also imply the emotional state of mice, we have begun to record USVs during affective situations (e.g., isolation, restraint, courtship). We aim to employ these vocalizations as behavioral parameters in fear behavior analysis as well as innate emotional signals, so we can explore their behavioral effects and the underlying thalamic neuronal activity in their processing.

DICHOTOMY IN THE FRONTAL THALAMOCORTICAL SYSTEM

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The medial thalamic influence over frontal cortex (FC) plays important role in many cognitive functions. This network is built-up by parallel thalamocortical routes providing complex computation leading to cognition. Recently, by classifying the calretinin-expressing and nonexpressing midline thalamic population (CR+/CR- MT) with distinct arousal-related activity (Matyas, Komlosi et al, 2018), we have proposed a view of dichotomy in this network. Here, we performed cell-type specific anatomical and electrophysiological approaches to dissect the CR+ and CR- thalamocortical (TC) systems.By injecting a Cre-dependent AAVs together with a novel viral construct, which selectively infect Cre-positive and -negative thalamic cells in CR-cre mice, we mapped the topography of these CR+ and CR-TC axons (respectively) with regional-selectivity. The two population formed a rather non-overlapping cortical innervation. CR+ cells preferentially targeted prelimbic, infralmibic, orbital and insular cortices while CRones, cingulate and secondary cortical areas. In addition, their subcortical projections were also distinct in the field of the nucleus accumbens, amygdala, lateral septum, hypothalamus, dorsal striatum, bed nucleus of stria terminalis. Multisite in vivo recordings from frontal and parietal cortices along with thalamic optogenetic activation are designed to compare the cellular (multi-unit activity) as well as network (local field potential) – local and global – effects of CR+ and CR- TC cells. Our preliminary data shows that the two thalamic population provide qualitatively and quantitatively distinct cortical excitation, propagating differently to the parietal cortical regions. These findings indicate the dual nature of the frontal thalamocortical system which may fulfil different role in cognition.

CELL-TYPE-SPECIFIC INTERROGATION OF THE MOUSE THALAMUS IN AVERSIVE CUE PROCESSING

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Evolutionarily conserved and fast transmission of sensory-related information to the amygdala is provided by direct thalamic projections which are essential in fear behavior. We investigate the network elements and function of this thalamo-amygdala route.

The major, calretinin-positive (CR+) thalamic inputs to the amygdala carry essential information for auditory fear learning. We hereby examined the midbrain connectivity of posterior CR+ thalamic cells (PIL - posterior intralaminar and SG - suprageniculate nuclei) and their spontaneous, unimodal and multimodal sensory-elicited firing characteristics.

The collicular innervation of these neurons is cell-type-specific and strikingly different from those of the neighboring CR- cells.PIL/SG CR+ thalamic neurons are targeted by smaller boutons than their CR- neighboring cells which receive the large driver-type terminals from the inferior colliculus. Moreover, PIL/SG CR+ neurons are exclusively innervated by the superior colliculus which also suggests that these cells have non-primary, multimodal function in fear behavior.

In anesthetised animals, sound(CS)- and shock(US)-evoked as well as paired cue driven firing patterns were examined in PIL/SG CR+ and CR- auditory-related thalamic neurons. In freely behaving tetrode-implanted animals, CR+ and CR- thalamic cued responses were tested in fear conditioning and extinction. In both acute and chronic conditions, CR+ cells were more likely responsive to multimodal or associated cues.Short-latency sensory and optogenetically evoked amygdalar responses can be derived from PIL/SG CR+ neuronal firing and effectively inhibited by their optogenetic suppression as well.

According to our data, CR+ thalamic cellsprovide thekey components of associative learning directly conveying aversive sensory-related information to the amygdala.

ESSENTIAL ROLE OF THE LATERAL THALAMOAMYGDALA PATHWAY IN FEAR LEARNING

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The basis of associative learning is the association of a neutral stimulus with a valencebaring signal and then, stored them as an emotional memory. At a later time point, the presence of the same neutral stimulus on its own (conditioned stimulus) evokes a behavior linked the valence. This process consists of series of events with distinct time scale, the rapid perception, the temporally longer consolidation/memory storage, and the eventual retrieval. Here, using loss-of-function approaches, we selected temporally matching, genetic-based silencing methods to investigate how the calretinin-positive (CR+) thalamic population innervating the lateral amygdala, involved in fear associative learning. Timing the chemogenetic inhibition of CR+ TA cells – via hM4Di providing inhibition for several hours – to the conditioning as well as the consolidation phase suppresses fear learning as well as fear expression on the following day. Temporally precise timing of inhibition with optogenetic stimulation of NpHR pumps during perception erased fear behavior in all phases of associative learning. The NpHR animals were indistinguishable from non-shocked animals in many aspects. To investigate, how essential the presence of CR+ TA population in these fear processes, we lesioned them with the cell-type selective diphtheria toxin (DT)-mediated apoptosis. Our preliminary data show that DT-treated animals expressed elevated rather than suppressed fear in every phases of fear paradigm suggesting a generalized fear behavior. Altogether, these data highlights the pivotal role of CR+ TA cells in forming normal fear behavior.

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CELL-TYPE SPECIFIC THALAMIC MODULATION OF THE AMYGDALAR OSCILLATORY AND UNIT ACTIVITY

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The calretinin-positive (CR+) midline thalamic (MT) nuclei convey arousal-related signal, while the posterior intralaminar/suprageniculate cells (PIL/SG) transfer sensory information to the amygdala, which are both necessary in emotional memory formation. Moreover, the amygdalar gamma oscillations are known to play an important role in these processes, which raises the question how these thalamic inputs impact the amygdalar activity and oscillations. Here, we examined the modulation of the local field potential (LFP) and multiunit-activity (MUA) within the amygdala with subnucleus precision in response to optogenetic activation of these two thalamic inputs using high-density, multisite recordings. Furthermore, we investigated the underlying intra-amygdalar connectivity patterns with anterograde and retrograde tracings. Activation of MT inputs had excitatory effect in the amygdalostriatal transitional area (AStr) and intercalated cells of the amygdala (ITC), which then induced strong inhibition in the basal amygdala (BA). Similar activation of PIL/SG axonal arbors elicited excitation in the AStr, ITC and basomedial amygdala (BMA), but had an inhibitory effect on the lateral amygdala (LA). The multiunit-activity (MUA) reflected the corresponding changes in the LFP and well matched the input patterns of the two thalamic regions as well as the intra-amygdalar connectivity. The peripheral shock stimuli triggered identical areas and effects as the PIL/SG stimulation. The strength of the amygdalar gamma oscillation increased in response to both thalamic excitation and aversive stimulus but was strongest in case of PIL/SG inputs. Altogether, these data indicate complex but distinct nuclei-specific thalamic effects on segregated amygdalar microcircuits which could drive gamma oscillation-mediated emotional behaviors.

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EFFECTS OF CHRONIC D-AMINO ACID CONSUMPTION ON LEARNING,BEHAVIOURAL PLASTICITY AND NMDA RECEPTOR SUBUNIT COMPOSITION

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D-Serine (D-Ser) and D-Aspartate (D-Asp) function in the mammalian brain as co-agonist of the NMDA receptors (NMDAR). We investigated how the chronic consumption of these D-amino acids affects spatial learning in mice. To confirm the role of NMDAR underlying behavioral changes, we assessed the expression of the NR1, NR2A and NR2B subunits of NMDAR using quantitative western blot analysis. Mice consumed D-amino acids in drinking water for 6 weeks. Spatial memory was measured using the Morris watermaze test. We separated the effects of D-amino acids on short-, intermediate- and long-term memory. By detailed evaluation of the behavioral results we found that in the intermediateterm (hours) D-Asp treated animals remembered better the place of the hidden platform than did the control and the D-Ser treated groups. However, both D-amino acid consuming groups showed reduced behavioral plasticity during the reversal period of the test. Thus, D-Asp proved to be a memory stimulant at the 3-4 hours interval, which corresponds to the first wave of de novo protein synthesis. The expression of NMDARs in the hippocampus increased in the D-Asp treated group and decreased in D-Ser treated group. There was a shift in NR2 subunit composition toward the NR2A type in the D-Asp treated group. It is expected that D-Asp is primarily involved in cognition through mechanisms relevant to the translational processes that take place hours after behavioral training. Our results suggest a critical role of D-amino acids in NMDAR dependent plasticity via changes in receptor expression and subunit composition.

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VISUAL SHORT-TERM MEMORY FOR OBJECT-LOCATION ASSOCIATIONS IN MACAQUES AND HUMANS

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Visual short-term memory (VSTM) allows to remain aware of transient visual information, even in the face of intervening events competing forattention. VSTM for object-location associations has special diagnostic value for age-related neurocognitive disorders. The CANTAB Paired Associates Learning (PAL) task is an object-location VSTM paradigm, wherein the locations of 2-12 sequentially presented, putatively nonfigurative visual objects must be held in VSTM during a delay period, after which all of them are probed sequentially.Good performance in this taskrequiresskillful coordination and sequencing of executive and memory processes. PAL is used in clinical practice and translational research; however, individual and inter-species variability between PAL task performance levels and mnemonic strategies in humans and macaques has not been systematically investigated yet.Here we collected data from 12 rhesus macaques across a one-year training period, and from college students in three experiments. With a memory set size of 4, well-trained macaques performed comparably to humans (cf. V. Pál et al., this conference). We show thatserial position effects are compatible in macaques and humans, and compare the prevalence and strength of these patterns in the two species. In contrast, most human subjects efficiently incorporated information from preceding decisions within a trial, while for macaques this skill emerged only after a prolonged training period. We conclude that macaques can learn the PAL task to a degree that their performance constitutes a good model for human VSTM in translational research ifindividual and inter-species differences in cognitive skills are taken into account.

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REEVALUATING THE TRANSLATIONAL VALIDITY OF THE PAIRED ASSOCIATES LEARNING MEMORY TEST

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The Paired Associates Learning (PAL) task is a widely used memory paradigm in cognitive neuroscience. Starting with a presentation of four or six stimuli, which after a short delay are then followed by a phase, where the location of each previously presented stimulus has to be recalled individually. This location-stimulus binding seems to be sensitive to cognitive decline, therefore the PAL task may be a relevant clinical screening tool in neurocognitive disorders. As the PAL task involves only putatively non-verbal stimuli, it is also used in non-human primate (NHP) studies due to its high translational validity.

The human volunteers tend to perform in the PAL task with the help of verbal cues. To test this notion, we developed a variant of the PAL task which includes articulatory suppression (word repetition) to prevent using verbal cues in memory storage. Besides that, we used a different set of stimuli, which lacked familiar shapes and color cues. We found that replacing the original PAL stimuli with non-figurative ones did not prevent the volunteers from using verbal associations. However, with the application of articulatory suppression the volunteers' performance significantly dropped. It is crucial to clarify that the observed differences in human and NHP visual short-term memory performance could be signs of capacity differences, or, as our current study suggests, there might be differences in the usage of different short term memory storage mechanisms in the two species.

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INHIBITION OF DOPAMINE D2 RECEPTORS CAN ALTER THE POSITIVE REINFORCING AND ANXIOLYTIC EFFECTS OF OXYTOCIN

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The central nucleus of amygdala (CeA) plays important role in learning, memory, anxiety and reinforcing mechanisms. Our previous findings indicated that in the rat CeA, oxytocin (OT) had dose dependent positive reinforcing and anxiolytic effect. The aim of our present study was to examine in the CeA the possible effects of OT and dopamine (DA) D2 receptor antagonist sulpiride on reinforcement in place preference test and on anxiety in elevated plus maze test.

Bilateral microinjection of 10 ng OT (Sigma: O6379) was delievered into the CeA of male Wistar rats. In separate groups of animals, 4 µg DA D2 receptor antagonist (sulpiride: S7771), and the D2 receptor antagonist 15 min before the 10 ng OT treatment, or the vehicle solution per se were administered into the CeA.

Rats receiving OT spent significantly longer time in the treatment quadrant in conditioned place preference test. Preceding treatment with DA D2 receptor antagonist blocked the rewarding effects of OT. Antagonist itself did not influence the time that rats spent in the treatment quadrant. In elevated plus maze test, rats receiving OT spent significantly longer time on the open arms. Preceding treatment with DA D2 receptor antagonist blocked the effects of OT.

Our results show that in the rat CeA OT has positive reinforcing and anxiolytic effects. The DA system appars to control these positive reinforcing and anxiolytic effects of OT since DA D2 receptor antagonist can block these actions.

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MONETARY INCENTIVES ACTIVATE BRAIN REWARD SYSTEM BUT FAIL TO IMPROVE WORKING MEMORY IN OLDER ADULTS

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Aging is associated with declines in dopaminergic neurotransmission and functional integrity of the brain reward system which has a negative impact on the human cognitive functions, including working memory (WM). However, the neural processes underlying the modulation of WM performance by reward-related, motivational factors and their impairment with aging remains unexplored. Here we addressed this question by measuring the behavioural and neural effect of monetary incentives on visual WM performance in young and older adults using functional MRI. At the beginning of each trial, a cue indicated whether small or large monetary reward could be earned. The results revealed significantly higher WM performance in trials where high monetary reward was anticipated than in the low reward condition only in younger, but not in older adults. Furthermore, it was also shown that in young adults reward-triggered WM improvement is absent in the early phase of the measurement session and needs about 30 minutes to evolve. Importantly, in both young and elderly groups we found that the cue indicating high monetary reward evoked significantly higher fMRI responses in the brain reward system than the low reward cue. These findings reveal that modulation of WM performance by monetary incentives is not under volitional control but instead it is mediated by learning processes that translate incentive information into cognitive effort deployment and WM performance improvements. In older adults, monetary incentives keep activating the brain reward system but due to the impairments of the reward learning processes fail to trigger working memory improvement.

READING EXPERTISE-RELATED HEMISPHERIC SPECIALIZATION OFORTHOGRAPHIC PROCESSING IS IMPAIRED IN DEVELOPMENTAL DYSLEXIA

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Fluent reading comes as a result of extensive practice. Reading skills develop gradually from childhood to adolescence, and acquiring reading expertise has an overall effect on visual information processing. However, whether or how developmental dyslexia affects neural processes which underlie the evolvement of reading expertise remains unexplored. Here we addressed this question by investigating the hemispheric lateralization of fixation-related EEG components previously shown to be markers of reading expertise, that might correspond to the well-known word reading-related N1 ERP component exhibiting left hemispheric lateralization. Dyslexic and control young adults read isolated sentences in a natural way at their own pace, their eye movements and EEG activity were recorded simultaneously. Considering the early stage of the fixation-related N1 EEG component, significant leftward lateralization of occipito-temporal activity was obtained from 80 to 140 ms in control subjects only, while in the latter stage significant right hemispheric lateralization of occipito-temporal activity was found from 170 to 205 ms and from 160 to 210 ms in control and dyslexic subjects, respectively. Between-group differences of lateralization were revealed from 95 to 125 ms in occipito-temporal regions indicating stronger lateralization in control participants. Our results reveal that in dyslexics the early N1 component of the fixation-related EEG activity is evenly distributed over the two hemispheres as opposed to its strong left hemispheric lateralization observed in the control readers. These findings provide the first experimental evidence for disturbed reading expertise-related hemispheric specialization of orthographic processing in dyslexia.

INFLUENCE OF SCHEMA FAMILIARITY AND INCENTIVESON VISUAL EXPLORATION IN A NATURAL SETTING

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The presence of associative information networks of prior knowledge (schemas) has been shown to enhance encoding and retrieval of new information in both animal and human studies. However, little is known about their influence on decision making and on the sensitivity to different reinforcement schedules, especially that of different levels of risk.

Our aim was to design an ecologically valid paradigm incorporating both learning and decision-making in the same schema-based task. During the experiments, participants freely explored items belonging to different schemas, i.e. paintings of various painters (each painter and prior knowledge on their visual style constitutes one schema). Each exploration phase was followed by two decision phases (with each schema represented once), where they were instructed to choose four items belonging to the same schema and received appropriate rewards.

The paradigm was established with 112 participants (age=23±0.3). Exploratory patterns of schema items were negatively correlated with familiarity of schemas in a natural setting. Furthermore, feedback, concerning the actual reward value associated with each schema, provided after each decision phase clearly shaped further exploratory behaviour with a preference for higher rewarded schemas ($p<0.0001$).

Different risk levels, introduced as numbers of items selected from one schema leading to a bonus, were influential with higher risk-aversive behaviour leading to reduced learning ($p=0.002$).

The present study is an important first step towards understanding and modelling human decision making in a natural setting with the goal of developing measures (e.g.: reward sensitivity, impulsivity) that may appear different in mental disorders.

DOES THE ACTIVATION OF GAP JUNCTIONS INFLUENCE MEMORY CONSOLIDATION?

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There is a growing body of evidence for the involvement of astrocytes in oscillatory brain activity, both in physiological and pathophysiological processes. Our goal is to investigate various molecular interactions between neurons and astrocytes, with a specific focus on the role of the astrocytic syncytium and pave the way for the identification of new potential drug targets. We have previously shown that blocking astrocytic gap junctions suppresses slow wave activity in rats, suggestinga possible causal relationship between astrocytic and neuronal synchronization (Szabó et al. 2017).Since slow wave sleep is associated with memory consolidation, perturbation of the astrocytic syncytium during this process may impact the working memory of rats.In this study we tested this hypothesis in further detail. We applied trimethylamine, a known activator of astroglial gap junctions and determined its effect on slow-wave sleep and memory consolidation usinglocal field potential measurements andnovel object recognition tests, respectively.

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IMPORTANT REGULATORY FUNCTION OF TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 IN AGE-RELATED MEMORY LOSS OF MICE

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Gradual memory loss is a very common symptom of the aging population occuring during physiological aging or neurodegenerative diseases. Expression of the Transient Receptor Potential Ankyrin1 (TRPA1) receptors has been demonstrated in nociceptive neurons and in the brain, however their role in neurodegenerative diseases and neuro-immune interactions is still unclear. We studied the role of TRPA1 receptor in age-related memory loss.

For the investigation of memory loss we used male young (3-4-month old) and old (18-month-old) wild type ($TRPA1^{+/+}$) and TRPA1 receptor-gene deleted ($TRPA1^{-/-}$) mice. Novel object recognition test (NOR) as well as Y maze (YM), radial arm maze (RAM) and Morris water maze (MWM) tests were used to assess the decline of memory and learning skills.

In the behavioral studies significant memory loss was detected in aged $TRPA1^{+/+}$ mice with the NOR and RAM, but there was no difference measured in YM and MWM. $TRPA1^{-/-}$ showed significantly milder memory loss, which could be seen as higher discrimination index in the NOR and less exploration time in the RAM. Furthermore, young $TRPA1^{-/-}$ animals showed significantly less reference memory error in the RAM and slightly but significantly higher mobility both in NOR and YM compared to the young WTs.

Our present work has provided the first evidence that TRPA1 receptors play an important deteriorating role in the memory loss induced by aging. Understanding the underlying mechanisms can open new perspectives for the pharmacological treatment of dementia.

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THE SEPTUM CONTAINS PARENTING-ACTIVATED NEURONS, WHICH ARE INNERVATED BY TIP39 FIBERS ARISING FROM THE THALAMUS

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Tuberoinfundibular peptide of 39 residues (TIP39) is a neuromodulator that is involved in the central control of maternal adaptations.TIP39 is induced in the maternal brain during the early postpartum period. TIP39 expression isconfined to three brain areas, of which the posterior intralaminar complex of the thalamus (PIL) shows the most significant maternal induction.We aimed to determine if TIP39-containing fibersproject from the PIL to the septum and whether they are involved in the maternal activation of septal neurons. Following the injection ofthe anterograde pathway tracerbiotinylated dextran amine into the TIP39-expressing area of the PILin suckling rats,a large density of labelledfiberswas located in the ventral part of the lateral septum ipsilateral to the injection site suggesting that TIP39 fibers present in this part of the lateral septum originate in the PIL.Based on light microscopic observations,TIP39-containing fiber terminalsclosely apposed septal neurons. Moreover, wealso demonstrated by using electron microscopy thatTIP39-positivefibers innervate these neurons,as they receive multiple synapses from TIP39-positive axon terminals. Since the ventral subdivision of the lateral septal nucleus contains a number of activated neurons following suckling, we also addressedifTIP39fiberssurrounded parenting-induced c-Fosexpressingneurons. Indeed, a large ratio of TIP39fiber-apposedcells were c-Fos-positive.Theresults suggest that the TIP39-containingneurons in the PIL project to the lateral septum and innervate some neurons there, which may contribute to maternal activation of the area.

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THE BASAL FOREBRAIN MAY PROVIDE A LINK BETWEEN LOCOMOTION AND LEARNING

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Keywords: basal forebrain, medial septum, theta activity, locomotion, pavlovian conditioning

Brain states are controlled by neuromodulatory centers. Among these the basal forebrain has widespread projections thought to mediate multiple cognitive functions. From these, the GABAergic projection has been implicated in controlling the locomotion-related theta oscillation and the glutamatergic projection can directly control animal speed. At the same time, cholinergic cells respond rapidly and reliably to reinforcement, important for learning. Therefore we hypothesize a relationship between basal forebrain neuronal activity, locomotion and learning.

To test this, head-fixed mice were placed on a wheel and trained on an auditory cued outcome task. This allowed mice to move or stay still voluntarily during the task. We monitored neuronal activity in the medial septum using tetrodes. Thus it was possible to examine whether there were correlated changes in neuronal activity and behavioural performance across the two states.

We found that mice initially trained on a fixed wheel learned faster. When allowed to move freely, mice tended to run after reinforcement delivery, which could reflect approach or escape responses. Neurons displayed a diversity of responses to behaviourally relevant events with dominant subpopulations showing activation or suppression after air puff delivery. These medial septal cell types may convey locomotion dependent learning signals via the septo-hippocampal pathway.

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SOCIAL DEFICITS AND DISRUPTED NETWORK ORGANIZATION IN THE PREFRONTAL CORTEX FOLLOWING POST-WEANING SOCIAL ISOLATION

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Early-life adversity is a risk factor for the emergence ofpsychiatric disorders associated with social deficits, particularly with pathological aggression. The prefrontal cortex (PFC), which plays a crucial role in regulating social behaviour, undergoesrobust network reorganizationduring childhood. We usedpost-weaning social isolation, a rodent model of early-life social neglect,to investigate the behavioural and prefrontal corticalconsequences of early-life adversities.Mice reared in isolation showed decreased sniffing and increased defensive behaviours in the social interaction test. During aggressive encounters, socially isolated mice exhibited increased attack counts and showed abnormal attack patterns characterized by attacks on vulnerable body targets (head, throat, belly). Fighting evokedsubregion-specific neural hyperactivation within the PFCofisolation-reared mice compared to socially-reared mice. To investigate the functional network activity of the PFC,we generated matrices from correlation coefficients of c-Fos activation patterns of PFC subregions. Quadratic assignment procedure correlations revealed that social experience exerts differential c-Fos activation patterns in isolated animals. Both parvalbumin (PV)-positive interneurons and perineuronal nets (PNN) are implicated in network organization and closure of critical periods of plasticity but little is known about their activity during social encounters. We found that in the infralimbic cortex social interaction significantly increased the number of PNN-c-Fos-positive cells in both social and isolated mice but decreased the activity of PV-PNN neurons in socially-reared mice only, suggesting social isolation-induced impaired PV-PNN activity during social interaction. Our results contribute to understanding how disruption of neuronal network organization during development translates into social abnormalities in adulthood.

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SOCIAL RECOGNITION OF WISKET RATS

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Social recognition is a major problem underlying deficiencies in interpersonal relationships in schizophrenic patients. Selectively bred rats after peri-adolescence isolation rearing and subchronic ketamine treatment (WISKET) exhibit phenotypes related to schizophrenia. To further validate our WISKET rat line we examined their social recognition.

The sociability cage was used to test Wistar control and WISKET male rats. It is a three-chambered cage in which the middle chamber can be used as a neutral starting point and to measure basic activities during the habituation phase. The wired cages in each of the other chambers may hold a conspecific. To study the subject's interest in a social stimulus, one chamber presents a conspecific while the other remains empty. In a preference test for social novelty, the two wired cages contain a familiar and an unfamiliar conspecific. The interest is measured by assessing the time spent in the same chamber or in close proximity to the familiar or unfamiliar other rat.

The social interest of Wisket males significantly decreased, however, they showed similar social preference for social novelty compared to Wistar controls. If these parameters were related to the time spent in the appropriate chamber, Wisket rats spent significantly less time with social behavior. Furthermore, Wisket rats showed higher anogenital sniffing and lower nose-nose contacttoward the conspecifics, indicating social avoidance and increased aggression.

These results indicate significantly decreased social interest that increase the validity of our Wisket model regarding the negative symptoms of schizophrenia.

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ROLE OF DIFFERENT CELL-TYPES OF THE MEDIAN RAPHE REGION IN SOCIAL BEHAVIOR

BibiánaTörök

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MTA KOKI

Role of different cell-types of the median raphe region in social behavior Disfunctions of median raphe region (MRR) are associated with many psychiatric disorders due to its role in anxiety and social behavior. Optogenetic stimulation of MRR acutely decreased aggressive behavior in mice, but the contributing cell-types were unknown. MRR has heterogeneous composition: serotoninergic, GABA-ergic, glutamatergic and yet unknown cell populations have been described. Our aim was to detect further neuron-types in MRR and reveal their function in social behavior. Control, stimulatory or inhibitory DREADD sequence was injected into MRR of VGAT-Cre (vesicular GABA transporter), VGluT3-Cre (vesicular glutamate transporter 3), CRH-Cre (corticotropin releasing hormone) and DAT-Cre (dopamine transporter) mice using AAV vectors. Thirty minutes after intraperitoneal injection of clozapine-N-oxide (ligand of DREADD) social interaction (SI) and residentintruder (RI) tests were conducted. We confirmed the presence of all studied cell-types in MRR. Inhibition of VGAT-Cre in MRR increased friendly SI and diminished aggression. VGluT3-Cre inhibition increased social contacts without any sign of aggression. In contrast, inhibition of CRH and stimulation of dopaminergic neurons decreased friendly SI and increased aggressive behavior in SI test with similar tendencies in RI. MRR GABA-ergic, glutamatergic and dopaminergic neurons may contribute to aggression, while CRHergic neurons has opposite effect, more resembling the whole MRR stimulation. As SI reflects anxiety as well, it can be assumed that MRR glutamatergic neurons are important in social interaction among anxiogen conditions. Thus, each neuron-type of MRR may contribute to the fine regulation of social behavior.

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EARLY FIXATION-RELATED EEG ACTIVITY IS REDUCED IN AMBLYOPIA DURING FREEVIEWING OF HUMAN FACES

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Amblyopia is the most common cause of vision impairment in a single eye among children and young adults. However, how amblyopia affects brain activity in natural viewing conditions still remains to be explored. To address this shortcoming, here we assessed how amblyopia modulates the early fixation-related EEG response during active scanning of human faces. Twenty young amblyopic adults participated in this study. Subjects' eye movements and EEG wererecorded simultaneously. Although the duration of fixations did not differ significantly between the amblyopic and fellow eyes, and only a marginal difference was found in the case of saccade amplitude, EEG data was analyzed using hierarchical linear modeling to regress out the potential effects of eye movement covariates. Effects of amblyopia on EEG were evaluated separately for within- and between-face saccades. For within-face saccades significantly weaker fixation-related EEG response was found for the amblyopic eye from 35 to 105 ms in occipital and from 65 to 110 ms in occipito-temporal channels. Considering between-face saccades, the same trend was observed, but from 25 ms to 95 ms in occipital and from 65 ms to 105 ms in occipito-temporal electrodes. Moreover, in the case of within-face saccades, the significant effect of amblyopia on fixation-related EEG in right occipito-temporal channels significantly correlated with the visual acuity difference between the two eyes. Our results provide the first evidence for reduction of early fixation-related EEG activity during natural viewing in amblyopia.

A POSSIBLE ANIMAL MODEL OF AUTISM: THE SOCIAL BEHAVIOUR OF THE YOUNG DOMESTIC CHICK

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Social impairment accompanies all forms of the autism spectrum disorder (ASD). A widely used pharmacological model for ASD is the embryonic treatment of rodents with valproic acid (VPA), which causes social defects postnatally in the adults. Newly hatched chicks show several types of preference and predispositions toward social stimuli and they are capable of complex cognition and social behaviour immediately after hatching, therefore chicks can be a good model to study the effect of embryonic VPA treatment on acquired and innate behaviours. We compared the behaviour of control and VPA treated chicks using several behavioural tests. There were no cognitive or motor differences between the two groups. Despite of the VPA treatment, the chicks retained their innate social preferences: they preferred a larger group of conspecifics over a smaller one, and an unmodified video recording of chicks over one with blurred head features. The two groups did not differ in their choice between a socially raised and an isolated chick, however, the control animals showed more intensive social exploration. At the age of three weeks, the VPA treated chicks failed to recognize their cagemates. Thus, VPA impaired the acquired social behaviours such as social memory in young chicks, but it failed to cause any defect in innate predispositions. Since the individual recognition of conspecifics develops at the end of the third week after hatching, further research on the differences in early social exploration might contribute new knowledge relevant to the early diagnosis of ASD.

RAPHE-HABENULAR CONNECTIONS CAN SHAPE FEAR BEHAVIOR

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Median raphe region (MRR) has an important role in fear and anxiety, however the network connections of their known cell types do not explain this role. We found a new glutamatergic cell population in MRR that is vesicular glutamate transporter 2 (vGluT2) positive. Our stereological and viral tracing experiments showed that these neurons give rise to the most abundant ascending projections from MRR. We found that MRR vGluT2 neurons send a strong input to the lateral habenula and medial ventral tegmental area that are responsible for aversive behavior, and to the medial septum and hippocampus that are responsible for memory formation. Electron microscopy confirmed that their asymmetric synapses contain NMDA-receptors, typical for glutamatergic excitatory transmission. Using cell-type specific retrograde rabies virus experiments we found that MRR vGluT2 neurons receive monosynaptic contacts from forebrain and brainstem nuclei responsible for fear, motivation and memory formation. Finally, optogenetic stimulation of MRR vGluT2 neurons in contextual place aversion test caused fear and aversive behavior in mice. Our results suggest that these novel vGluT2 positive neurons are primarily responsible for the fear and anxiety-like actions of the MRR, and they may play a direct role in several types of mood-disorders.

SIGNAL PROCESSING IN NEOCORTICAL PYRAMIDAL NEURONS OF A MOUSE MODEL OF HUMAN TAUOPATHY

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Hyperphosphorilated tau protein destabilizes cytoskeletal microtubules that leads to cell death in and malfunction of neural networks, processes associated with Alzheimer's disease.

Effects of such tau protein were investigated on dendritic morphology, neuronal membrane, subthreshold dendritic impulse propagation and on synaptic integration in principal neurons of rTg4510 mice expressing high levels of human mutant tau. Spatial reconstructions of 20 transgenic (TG) and 9 wild-type (WT) labelled pyramidal neurons from layer 3 of the frontal lobe of 9 month old transgenic and control mice were selected for this study.

TG neurons were segregated into 9 atrophic (TGA) and 11 non-atrophic (TGNA) neurons based on size and on number of bifurcations of apical dendritic arbours.

Morphologically faithful passive segmental cable models of these neurons were created by the NEURON simulator. Membrane resistances and capacitances were estimated by fitting neuron input resistances and membrane time constants of model neurons to electrophysiological measurements.

Somatopetal dendritic impulse propagation was studied by analysing current transfers, steady-state and sinusoid voltage transfers, and delays of locally generated dendritic PSPs.

We concluded that mutant human tau protein affects morphology and subthreshold dendritic impulse propagation differentially in TG neurons. We detected more changes in dendritic signalling in TGA than in TGNA cells relative to control, WT neurons. Our modelling suggests virtually no alteration in passive membrane properties and in synaptic input pattern recognition of pyramidal neurons is associated with the presence of mutant tau. All these findings are independent of the somato-dendritic distribution of membrane conductances within physiological ranges.

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CONTRASTING INDIVIDUAL AND POPULATIONAL NEURAL CODE IN NATURAL VISUAL STIMULI SUGGESTS CORRELATIONS FACILITATE STIMULUS DISCRIMINATION

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Complex stimuli are represented by the activations of populations of neurons in the visual cortex. A key question regarding the neural code is whether the information carried by a population is simply the sum of the individual neural contributions or joint activation patterns provide further information. Recent studies demonstrated that a linear decoder, which does not take into account joint activations, is sufficient to decode information about the orientation of grating images. Representation of more complex images, however, is expected to exploit knowledge about statistical regularities in the presence of constituent features and therefore such statistical regularities can shape the activation patterns of neurons representing those features. We constructed decoders that capture different statistical structure in multiunit responses recorded from the primary visual cortex of macaques. We used natural and synthetic images to investigate how the presence of statistical structure in responses affects decoding performance. Using logistic regression we demonstrated that quadratic components, which capture joint activations of pairs of neurons, enhance decoding performance. Using correlation-specific mixture decoders we demonstrated that stimulus-dependent spike count correlation structure contributes to nonlinear decoding capabilities. Finally, comparing the coding of complex natural image patches and that of limited-complexity synthetic images we showed that a nonlinear decoding strategy is more advantageous for complex images than for images with simpler structure. Taken together, our results highlight that structure in stimuli introduces intricate joint statistics in V1 responses, which has the consequence that stimulus identity can be most efficiently established with nonlinear decoders.

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CONSISTENT, CELL TYPE-SPECIFIC FIRING RESPONSES OF BIOPHYSICALLY DIFFERENT MODEL NEURONS DRIVEN BY SYNAPTIC INPUT

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One of the central goals of today's neuroscience is to achieve the conceivably most accurate classification of neuron types in the mammalian brain. As part of this research effort, electrophysiologists commonly perform current clamp experiments to gain detailed characterization of the neurons' physiological properties. Yet, it is not well understood whether neurons that share physiological properties of a particular phenotype would operate in a consistent manner under the action of intense synaptic inputs such as those in active brain circuits. Hence, the question is whether classification of physiological phenotypes as obtained in current step experiments can be extended to conditions when the same neurons are integrating complex synaptic inputs. We approached this problem by simulating a biophysically diverse population of model neurons based on 3 generic phenotypes. First, we stimulated the model neurons using the standard current step protocol and then exposed them to simulated synaptic bombardment. We extracted physiological parameters from the current step responses and spike event reliability vectors descriptive of the model's responses under synaptic inputs. Next, we applied a variety of supervised and unsupervised classification methods to identify the underlying biophysical phenotypes. Our results suggest that alternative classification schemes of a biophysically diverse neuron population can be achieved under different stimulus conditions and operational regimes. Still, accurate identification and classification of biophysically different neuronal phenotypes is possible using only spike arrival times and using low-dimensional vector representations of their synaptic responses.

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REPRESENTATION OF UNCERTAINTY DURING HIPPOCAMPAL THETA SEQUENCES

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Behavioural studies suggest that both humans and other animals are able to perform probabilistic computations. Such computations imply that the nervous system of biological agents is capable of the representation and manipulation of probability distributions. However, the way encoded distributions are related to population activity of neurons remains hotly debated because measures that could dissociate alternative models based on experimental data are remarkably lacking.

Here, we focus on hippocampal activity in the context of exploratory behavior to derive contrasting predictions for the alternative models. Place cells, selective for specific locations in the environment, become sequentially activated during each theta cycle, thus neurons encoding past, present, and future locations outline the trajectory of the animal. We interpret this activity pattern as the result of repeatedly performing probabilistic inference about possible trajectories in a dynamical generative model. Critically, during a single theta sequence the uncertainty is expected to change systematically, thus providing a chance to identify how it is encoded in the population activity. Specifically, we consider four alternative encoding models: (a) encoding the most likely trajectory; (b) sampling from the posterior distribution; (c) standard probabilistic population coding and (d) convolutional encoding.

We create a synthetic dataset in which place cells are driven by trajectories encoded using one of the four alternative encoding models, all consistent with many important features of experimental data. Then we derive three novel measures that can be extracted from the data and can be used to distinguish the neuronal activity patterns characteristic for the competing models. These results are directly applicable to experimental data to identify if and how uncertainty of spatial trajectories are represented in the hippocampus. Our analysis is an important step towards elucidating the strategies used by the brain to encode probability distributions and to understand the computational role of neuronal variability.

INVESTIGATION OF LIGANDGATED ION CHANNEL MODULATORSUSING A MICROFLUIDICS-BASED AUTOMATED PATCH-CLAMP INSTRUMENT

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Fluxion Biosciences is currently the sole provider of commercially available microfluidics-based automated patch-clamp systems, the Ion Fluxfamily of instruments. The IonFlux Mercury is equipped with an improved pressure control system, which enables continuous flow, precise timing and unique flexibility for complex compound application protocols. In this study the system was tested using one of the most demanding tasks possible: positive modulators of alpha7 nicotinic acetylcholine receptors (nAChRs). This was a challenge for two reasons:

First, the alpha7 nAChR is the LGIC (ligand gated ion channel) that is probably most difficult to study in electrophysiology experiments. At a rapid agonist pulse no more than ~3% of the receptor population is activated, all the rest is desensitized without conducting current. In addition, the receptor is extremely sensitive to low agonist concentrations, upon which they also desensitize without detectable opening. For this reason any leakage, diffusion, cross-contamination, or insufficiently fast solution exchange can suppress activation.

Second, some of the positive allosteric modulators of alpha7 nAChRs are notorious for their incomplete wash-out, due to their exceptionally high adsorption to silicone and plastic surfaces and fast partitioning into cellular membranes.

Assay protocols had to be optimized to meet the two-fold demand of fast solution exchange and prevention of cross-contamination.

We optimized pressure settings, pre-incubation duration, wash protocols, and priming protocols. Our improved protocols allow the pharmacological study of even the most problematic LGIC types using the IonFlux Mercury instrument.

LONGTERM CALCIUM IMAGING WITH 3D-ACUSTO-OPTIC MICROSCOPY AT MORE THAN 1 MM CORTICAL DEPTH DURING LEARNING

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Functionaltwo-photonimaging of large neuronal assemblies is an essential tool in understanding connectivity and operation of functional networks in the brain. To achieve this goal, we need to gain access to deeper layers in the cortex, ideally, functional cortical imaging should gather data from all cortical layers in the area of interest. Understandably, recording from such a large population of neurons results in hundreds of thousands of traces and calls for automated analysis tools for data extraction.

Here we report a novel imaging method for fast deep imaging in the primary visual area of transgenic adult mice, encompassing all six layers of the cortex. The newest generation of our 3D acusto-optic microscope allows measurements up to 1000 cells from an 800 x 800 µm field of view 1000 µm deep under the pia, while maintaining 20-40 Hz temporal resolution for functional imaging of neurons. Experiments were performed for multiple numbers of repetitions over the span of several days, yielding large amount of data per animal. We have developed a complex, semi-automatic workflow for 3D acusto-optical measurements and analysis, involving preparation of the animals, repeated measurements for multi-day imaging sessions of the same set of neurons, data analysis, and visualization. Our analysis software enables automatic cell detection, background and ΔF over F calculation, sorting and graphical display of the large data sets, while having multiple checkpoints for human interaction minimizing the possibility of errors.

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GENERATION OF A FRET-BASED BIOSENSOR FOR THE MEASUREMENT OF NEURONAL THYROID HORMONE LEVELS

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Thyroid hormone (TH) is a crucial regulator of brain function. In the brain, TH activation occurs in the glial compartment and the generated T3 evokes a TH-dependent neuronal expression profile. Hypophysiotropic TRH neurons project to the hypothalamic median eminenceand this axonal pathway is hypothesized to transport T3 signal to the somas located n the PVN. Therefore, we aimed to develop a recombinant biosensor that could be used for future in vivo studies on this phenomenon. The sensor is based on T3-evoked conformational changes manifested in increased energy transfer that can be measured by fluorescent resonance energy transfer (FRET) in live cells. The sensor is consisted of the human TRβ ligand binding domain (LBD) inserted between well characterized FRET pair, mTurquoise2 and YPet. Bait-peptides (KAT5 and SRC2) were applied between the T3-sensing core domain and YPet FRET acceptor combined with a set of flexible linkers incorporated into the N- and C-terminal of TRβ LBD that increased the efficiency of T3 induction. The sensor was characterized in HEK293 cells and in solution followed by expression in E. coli and His-tag affinity purification. Using a time-lapse live cell imaging screen, the sensor showed superior responsiveness to T3 over T4 (200% vs. 10%) and insertion of flexible linkers could decrease the relatively high basal signal. These improved candidates showed faster and ~2.2-fold higher T3 responsiveness. The sensor undergoes testing in cultured Dorsal Root Ganglia neurons. We conclude that the developed FRET-based T3-biosensor can assess T3 availability in live cells and will be especially useful for studies in polarized cells.

A NOVELMEASURE OF THERAPEUTIC WHOLE-BODY HYPOTHERMIA IN SEVERE TRAUMATIC BRAIN INJURY: THE COOLING INDEX

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Introduction: The role of therapeutic hypothermia in the treatment of severe traumatic brain injury (TBI)is controversial. We aimed to determine the effectiveness of therapeutic whole-body hypothermia on the mortality of adult patients with severe TBI by using meta-analysis.
Methods: We performed an extensive literature searchusingPubMed, EMBASE, and Cochrane Library databasesfrom inception to February 2017. The identified human studies were assessed regarding statistical, clinical, and methodological designs to ensure interstudy homogeneity. From the reported cooling parameters, we calculated the cooling index, a measure of therapeutic hypothermia.
Results: With forest plot analysiswe found no difference in the outcome of TBIbetween cooled and not cooled patients, but interstudy heterogeneity was high. On the contrary, by meta-analysis of randomized clinical trials that were homogenous with regard to statistical, clinical designs, we showed decreased odds ratio for death in therapeutic hypothermia compared with no cooling. As influencing factors, milder and longer cooling, and rewarming at <0.25°C/h were associated with better outcome. The therapeutic whole body hypothermia showed beneficial effect only if the cooling index was sufficiently high.
Conclusions: The high methodological and statistical interstudy heterogeneity could influence the contradictory outcomes obtained in earlier studies. By analyzing methodologically homogenous studies, we demonstrate that cooling improves the outcome of severe TBI, and this beneficial effect depends on certain cooling parameters and on their integrated measure, the cooling index.

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DIFFERENTIAL EFFECTS OF NANOSTRUCTURING ON PRIMARY NEURONS AND ASTROCYTES

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The long-term application of central nervous system implants is currently limited by the negative response of the brain tissue, affecting both the performance of the device and the integrity of the neural network. A possible solution is the topographical modification of implant surfaces mimicking the structure and dimensions of the extracellular matrix, which has been shown to affect the attachment and behavior of neurons and astrocytes. In our study, primary mouse astrocytes and hippocampal neurons were seeded ontonanostructured and smoothsilicon or platinum surfaces without additional biological coatings. Fluorescent wide-field and confocal microscopy and scanning electron microscopy were used to characterize the attachment, spreading and proliferation of cells.

We found that both astrocyte cell number and average cell spreading was significantly larger on platinum, compared to silicon surfaces, while silicon surfaces impeded glial proliferation. Nanostructuring did not have a significant differential effect on either parameter in astrocytes. Neuronal attachment was impaired on metal surfaces, but nanostructuring had a differential effect on neuronal growth cone morphology, regardless of surface material. Our results indicate that nanostructuring in itself can to promote neurite regeneration but does not induce reactive astrocytic responses.

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DIRECTED CAUSAL INTERACTIONS FROM fMRI DATA

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We applied cross convergent mapping (CCM) to assess directed causal interactions from fMRI time series data. Interactions were investigated between eight brain regions associated with both visuo-motor responding and working memory tasks. CCM is wholly appropriate for discovering directed, possibly delayed and circular interactions between weakly coupled dynamic systems, of which the functioning brain is an excellent example.

During the visuo-motor task, only three significant causal interactions were revealed: V1 → M1, and a bidirectional, circular connection [(V1 ←→ superior parietal cortex (SPC)]. The working memory task induced much richer functional structure, revealing a more extended cortical network of significant uni- and bi-directional causal interactions between regions including the dorsolateral-prefrontal cortex, SPC, supplementary motor area and dorsal anterior cingulate cortex (DACC), while the strong unidirectional interactions were observed from the DACC to the M1. Moreover, significant time delayed interactions (over lags of up to 6 seconds) between brain regions were observed during working memory but not visuo-motor responding. CCM recovered functional structure where conventional bi-variate correlation analyses applied to the same time series data did not.

These analysis are the first to apply CCM to the process of functional discovery from fMRI data and indicate that the method is well suited for the discovery of rich functional structure in a complex system like the brain, and even from relatively imperfect signals such as fMRI.

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THE RELIABILITY OF TABLET VISUAL ACUITY TEST USING DIFFERENT LUMINANCE AND CONTRAST CONDITIONS

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The classical form of visual acuity (visus) testing may involve a number of errors. In our present study we aimed to eliminate some of these possible mistakes, such as improper light conditions, possible learning of responses and a low retest rate using EuvisonTab screening tool. Our investigation was based on the current ISO standard (Nr. 8596:2017), which determines the parameters of optotype and the settings for the measurement of the distance visual acuity. The visus examination was performed with a Landolt C optotype at a distance of 4.66 meters. As a test tool, a 10" Samsung Galaxy tablet was used in a darkened room. The monocular visus values of subjects were compared for 6 different luminance/contrast conditions (320, 200 and 80 cd/m2 luminance, 100% and 15% contrast, and combinations thereof). The monocular visus was estimated using 24-letters fitted in size on an adaptive manner. The order of the individual conditions was randomized within a test session. In our current research phase, we processed the test-retest and inter condition variance results of 20 adult subjects. Using one sample t-test for the test-retest reliability and one-way ANOVA (in Matlab) to compare the visus values estimated using the 6 conditions we did not find any significant difference. Based on these the visus testing using EuvisionTab is reliable.

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CAMKIIα-GFP MOUSE LINE PROVIDES A NEW TOOL FOR MICROSCOPIC AND ELECTROPHYSIOLOGICAL ANALYSIS OF HIPPOCAMPAL NEURONS

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CaMKIIalpha-GFP mouse line expresses GFP in a cell-specific manner under the control of CamKIIalpha promoter (Wang et al., 2013). In this work, we analyzed the expression of the endogenous CamKIIalpha gene as well as the CaMKIIalpha-GFP transgene in developing embryonic mouse brain and checked whether the cellular morphology and electrophysiological properties of neurons were affected by the long-term expression of GFP within hippocampal organotypic slice cultures and dissociated neuronal cultures.

Our results show that GFP expression begins early in embryonic brain development and reaches a plateau at the third week after birth. Strong GFP expression is detected in the developing cortex and hippocampal formation, especially in the dentate gyrus and CA1 region. Detailed in vitro analyses showed that GFP expression selectively visualized pyramidal neurons. The lack of glutamic acide decarboxylase (GAD65/67) immunopositivity indicated that GFP positive cells are not GABAergic neurons. Using pre- and postsynaptic markers, we did not experience any difference in the maturation of these cultures compared to the control (CD1) ones. Analysis of the passive and active membrane properties also confirmed that expression of GFP did not affect the electrophysiological properties of the neurons. Thus, our results indicate that the CaMKIIalpha-GFP transgenic mice could serve as an ideal tool for further electrophysiological or anatomical studies and labeling of pyramidal neurons.

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EFFICIENT DEVELOPMENTAL NEUROTOXICITY METHOD USING A HUMAN IPSC DERIVED 3D-NEUROSPHERE ASSAY

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Neuronal Stem Cells (NSCs) can be differentiated from pluripotent stem cells, provide an attractive *in vitro* tool for studying CNS disorders or for drug development purposes. Here, we present a 3D human induced pluripotent stem cell (hiPSC)-based *in vitro* toxicology assay that can be used to test developmental neurotoxicity. Human iPSCs derived neurospheres grown in 3D culture were characterised timewise to monitor their complexity and homogeneity over a 7-weeks-long period using immunocytochemistry and electron microscopy. 3D neurospheres were exposed to 10 different toxicants (e.g. Paraquat, VPA, acrylamide, mercury chloride) activating different toxicity pathways. Samples were examined at different developmental time points (21, 28 and 42 days after plating), representing different developmental stages and maturity, with an ATP-based cell viability assay, optimised for 3D-tissues in 96-well plate format. Concentration-responses were investigated after acute (72 hours) exposure and the effect of toxicants were determined by histology as well. In addition, Transcriptional activity of major developmental, structural and cell type specific markers were investigated at weekly intervals. The results demonstrated that the acute exposure to different classes of toxicants resulted in distinct cell susceptibility profiles in different developmental stages, indicating that hiPSC-based *in vitro* neurodevelopmental models might be used effectively to evaluate developmental neurotoxicity. This will open new avenues for 3Rs replacement of animal models with *in vitro* assays in various academic and pharma-, chemical- and cosmetics industry applications.This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002.

TRANSFER LEARNING IMPROVES BRAIN AGE PREDICTION BASED ON RESTING-STATE FMRI CONNECTIVITY PATTERN ANALYSIS USING CNNS

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Using deep neural networks to predict brain age is attracting significant attention due to its potential as a biomarker of individual brain health. However, collecting the amount of data this method requires is not feasible in typical neuroimaging experiments. Here we investigated how transfer learning—repurposing already-trained deep networks by fine-tuning them to target datasets with fewer exemplars—can aid age category classification and regression based on brain functional connectivity patterns derived from resting-state functional MRI. We trained a convolutional neural network (CNN) on a larger public dataset and then examined how the knowledge learned can be used effectively to perform age category classification and regression on smaller target datasets collected with a different type of scanner and/or imaging protocol and pre-processing pipeline. Age classification improved when the convolutional layers' weights were initialized based on the values learned on the public dataset and then fine-tuned to the target datasets. Transfer learning also improved prediction of chronological age based on fMRI functional connectivity. Transfer learning is a plausible solution to improve brain age prediction by adapting CNNs to neuroimaging data with few exemplars and different data acquisition and pre-processing protocols.

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CENTRAL ROLE OF DIET CONSISTENCY COMPARED TO COMPOSITION IN OVERCONSUMPTION OF LEAN AND DIO MICE

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Introduction: Diet induced obesity (DIO) test is one of the best known animal models of obesity, however, application of healthier nutrition regime during drug treatment phase is rarely applied in mice. In order to study the potential pitfalls of diet switching, we investigated diet choice characteristics of obese and lean mice.

Methods: Male C57BL6OlaHsd mice fattened by D12492 high fat diet were used as obese, lab chow fed mice as lean subjects. After habituation, diet choice and diet switch tests were conducted using three different pelleted and grounded diets (standard lab chow diet - SD, low fat control - LF, high fat diet - HF) along with body weight measurements.

Results: Offering pelleted SD or LF diets to obese mice had no influence on intake pattern or body weight, but removal of HF resulted in dramatic decrease of consumption and weight loss. In our second experiment, lean mice preferred pelleted HF over SD, but after only six days, switching to their former chow resulted in significant decrease of their caloric intake and body weight, while offering pelleted LF diet had no effect on lean mice. Finally, access to grounded SD or LF diets induced food intake pattern changes similar to high fat diet in both lean and obese mice.

Conclusion: Regarding overconsumption, food consistency seems to be more important than diet composition. Furthermore, even a few days access to palatable diet seems to decrease the subjective value of less preferred diets, even causing starvation and body weight loss.

RESISTANCE OF PREVIOULSY ACQUIRED COGNITIVE CAPABILITIES TO IMPAIRMENT INDUCED BY CHRONIC UNPREDICTABLE STRESS IN RATS

Bence Tamás Varga

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We have developed a cognitive test system where rats are simultaneously trained in different cognitive tasks and then subjected to an impairment method with the aim to test cognitive enhancer agents. In our present experiment, we examined the effects of chronic unpredictable stress as impairing agent.

Twenty-one month old male Long-Evans rats were regularly trained in the five-choice serial reaction time test (model of attention and impulsivity), in the Morris water-maze (spatial memory), in a co-operation-test in skinner box (social cognition), and in a pot-jumping task (motor-learning). The latter two methods were internally developed. During the stress period animals had to learn a new task (T-maze alternation), too. The animals were randomized to stressed (N=24) and non-stressed (N=10) groups based on their test-performances. The unpredictable stress procedure was applied for 4 weeks and consisted of various combinations of restrain, electric foot-shock, exhaust swimming, frequent cage-moving, wet litter, cage tilting, water deprivation, randomly played dog barking, altered light-dark cycle. The cognitive performance was measured both during and after the stress procedure.

Stress caused a significant, 13% decrease in bodyweight and a 250% increase in corticosterone level. However, with the exception of a transient impairment in the five-choice reaction time test on the 10th stress-day, significant changes were not detected in any of the cognitive assays.

Our results show that well-trained tasks are resistant to even massive chronic stress. As in human disorders established knowledge deteriorates this resistance should be taken into account in designing models for testing putative cognitive enhancers.