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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Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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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Hungarian Academy of Sciences
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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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Hungarian Academy of Sciences
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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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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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Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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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16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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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16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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

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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

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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.

16th Annual Conference of the Hungarian Neuroscience Society
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January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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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16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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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Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
January 17-18, 2019, Debrecen, Hungary

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.

16th Annual Conference of the Hungarian Neuroscience Society
Hungarian Academy of Sciences
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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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Hungarian Academy of Sciences
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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.