GENE EXPRESSION ANALYSIS OF TRIGEMINAL GANGLIA AND PERIPHERAL BLOOD MONONUCLEAR CELLS IN A RAT OROFACIAL PAIN MODEL

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Objective: The origin and precise pathomechanism of migraine are still being debated. Trigeminal nociceptor sensitization is proposed to play a role by eliciting hyperalgesia and allodynia. We aimed to investigate gene expression changes in trigeminal ganglia (TRG), central trigeminal nucleus caudalis (TNC) and peripheral blood mononuclear cells (PBMC) evoked by Complete Freund's Adjuvant (CFA) induced peripheral inflammation.
Methods: Orofacial inflammation was induced by unilateral s.c. injection of 50 µl CFA into the whisker pad of male Wistar rats (n=8). Transcriptome analysis was performed using cDNA microarray on TRG tissue samples collected after 7 days. Five differentially expressed genes were selected for validation by quantitative PCR (qPCR) on days 1, 3 and 7 of a second experiment (n=12). Three genes were detected as markers of neuronal or glial activation. TRG and TNC tissue samples, and PBMCs comprising of monocytes and lymphocytes were taken. Saline-treated animals and contralateral sides of CFA-injected rats served as controls. Mechanical pain thresholds of the orofacial region were determined with a series of von Frey filaments.
Results: 253 differentially expressed genes were found between CFA treated and contralateral TRG samples 7 days after CFA injection. The mRNA expression changes of G-protein coupled receptor 39 (Gpr39), kisspeptin-1 receptor (Kiss1r), Lkaaear1 and Otoraplin were validated. They were most upregulated on day 3 in TRGs of the CFA-treated side. CFA induced significant orofacial mechanical allodynia in one day with a maximum on day 3. This correlated with patterns of neuronal (FosB), glial (Iba1), and astrocyte (GFAP) activation markers in both TRG and TNC, and surprisingly in PBMCs. In TNCs, gene expression changes similar to TRGs were observed but Kiss1r transcripts were not significantly altered while Neurod2 was observed only in TNC.
Conclusion: The genes revealed by our study may participate in the cascade of events resulting in the sensitization underlying migraine headache and the accompanying facial allodynia. Expression changes of Lkaaear1, Otoraplin and Neurod2 can be related to the modulation of synaptic plasticity. Gpr39 and Kiss1 receptors have emerging roles in pain but have not been implicated in migraine before thus may become potential targets in treatment. Corresponding mRNA changes in peripheral leukocytes is an intriguing result that might lead to the identification of biomarkers.

The present study was supported by National Brain Research Program B (KTIA_NAP_13-2014-0022), EFOP-3.6.1.-16-2016-0004, KA-2015-20, GINOP 2.3.2-15-2016-00050 „PEPSYS" and StayAlive GINOP (GINOP-2.3.2-15-2016-00048). J. K. was supported by a scholarship from the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TAMOP-4.2.4.A/2-11/1-2012-0001 'National Excellence Program Apaczai Scholarship'. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

THALAMIC DUAL-CONTROL MECHANISM FOR SLEEP AND WAKE

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Animals alternate between wakefulness and sleep during the circadian cycle which is a vital component of homeostatic control. During wakefulness, neocortical neurone show tonic activity whereas during non-rapid eye movement sleep (NREM) they burst fire with high synchrony across the whole neocortex. However, the circuit mechanism controlling this activity remains unclear. Excitatory drive from the midline thalamus has emerged as an essential hub for control of cortical excitability. Indeed, lesions of the midline thalamus may produce loss of consciousness as well as failure to consolidate NREM. Here we identify excitatory neurones in the centromedial thalamus (CMT) as being phase-advanced to the cortex and sensory thalamus in sleep slow-waves in freely moving mice. Channelrhodopsin-2 transfection of CMT neurones for anatomical mapping, optogenetic activation and optrode recording revealed that monosynaptic projection to the cingulate cortex controls both wakefulness and cortical slow-waves. Furthermore, we show that widespread synchrony of cortical slow-waves is dependent on a relay in the dorsal thalamus (AD) using combined optogenetic activation and inhibition recordings. Finally, intact function of both the CMT and AD are required for sleep recovery following a period of deprivation. Together these results demonstrate CMT dual control of neocortical activity and vigilance state which is dependent on AD and provides a circuit mechanism for cortical synchrony and sleep homeostasis.

NEUROSERPIN EXPRESSION IN DEVELOPING HUMAN CORTEX

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Neuroserpin is a serine-protease inhibitor that is mainly expressed in the cerebral cortex and has a role in the neuroprotection following hypoxia-ischaemia. Several studies have demonstrated its function in preserving vascular membrane integrity of blood-brain barrier and protecting neurons from ischaemia-induced cell death in the adult. These make neuroserpin a promising therapeutic agent in preventing neuronal death following hypoxia-ischaemia that could be particularly relevant in conditions such as neonatal hypoxia and stroke (Millar et al., 2017 Frontiers in Cellular Neuroscience).
We are currently investigating the role of neuroserpin in neonatal hypoxia in mouse models and therefore we wanted to compare the expression pattern in mouse and human during 'physiological' cortical development. We obtained human brain samples from the Oxford Brain Bank and King's College London (REC 07/H0707/139, gestational week 13, 14, 16, 18, 19, 21, 22, 25 and 40). Neuroserpin was expressed from the earliest age examined (13gw) and has been localized to migrating neurons particularly abundant i) in the germinal zone ii) in the interface of the marginal zone and cortical plate, iii) the deep cortical plate and iv) the subplate.
These results were corroborated by using different antibodies against neuroserpin (Abcam ab 55587 and ab 33077), PAS-Alciane Blue and Nissl-stainings. The earlier localizations of neuroserpin-immunoreactive migrating neurons were retained during the second trimester. By full term, neuroserpin-immunoreactive positive neurons formed four characteristic bands in the developing cortex situated i) between the marginal zone and the upper cortical plate, ii) in the upper cortical plate, iii) in the lower cortical plate and iv) in the still present but thinner layer of subplate. Morphological assessment of cortical plate neurons and colocalization experiments have shown that the majority of the neuroserpin-immunopositive neurons were SMI31.1-immunopositive pyramidal cells and to a lesser extent they were calretinin-immunopositive interneurons situated mainly in the upper cortical plate.
Our future goal is to give a detailed description of the neuroserpin-immunopositive migrating neurons throughout the first and third trimester as well as early childhood and compare their localization between diagnostic groups such as acute and chronic hypoxia.

Funded by the Medical Research Council Grant numbers: G00700311 and G00900901; Clarendon Fund, University of Oxford; The Academy of Medical Sciences Newton Advanced Fellowship in partnership with The Royal Society and The National Natural Science Foundation of China for collaborative projects (UK/China grant numbers: NA160314/816110158).

CALRETININ INTERNEURON DENSITY IN THE CAUDATE NUCLEUS IS LOWER IN AUTISM SPECTRUM DISORDER

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Autism spectrum disorder is a debilitating condition with possible neurodevelopmental origins but unknown neuroanatomical correlates. Whereas investigators have paid much attention to the cerebral cortex few studies have detailed the basal ganglia in autism. The caudate nucleus in particular is a nexus of converging circuits including a massive corticostriatal input and therefore it may serve as a node for the pathophysiology of autism spectrum disorder.
We used immunohistochemistry for calretinin and neuropeptide Y in 24 age and gender matched subjects with autism spectrum disorder and controls ranging in age from 13 to 69 years. Autism subjects had a 35% lower density of calretinin+ interneurons in the caudate that was driven by loss of small calretinin+ neurons. This was not caused by altered size of the caudate, as its cross-sectional surface areas were similar between diagnostic groups. Controls exhibited an age dependent increase in the density of medium and large calretinin+ neurons, whereas subjects with autism did not. Diagnostic groups did not differ statistically regarding Iba1 immunoreactivity for microglia, suggesting chronic inflammation did not cause the decreased calretinin+ density. There was no statistically significant difference in the density of neuropeptide Y+ neurons between subjects with autism and controls.
The decreased calretinin+ density may disrupt the excitation/inhibition balance in the caudate leading to dysfunctional corticostriatal circuits. The description of such changes in autism spectrum disorder may clarify pathomechanisms and thereby help identify targets for drug intervention and novel therapeutic strategies.

THE ROLE OF ASTROCYTE-SPECIFIC ECTO-5'-NUCLEOTIDASE/CD73 IN CELL MIGRATION IN VITRO: POTENTIAL THERAPEUTIC TARGET IN NEUROINFLAMMATION

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It is known that ATP potentiates inflammation, while adenosine (Ado) acts as an anti-inflammatory and immunosuppressive factor, with role in cell survival, proliferation, and motility. Both ATP and Ado are signaling molecules and part of the purinergic signaling system, which additionally comprises of highly specific membrane receptors, and ectonucleotidase enzymes. Extracellular ATP is normally found in nanomolar concentration in the CNS. However, its level massively increases in response to danger signals, such as metabolic injury, hypoxia and inflammation. In these conditions, other components of the purinergic signaling network are dynamically changed as well, struggling to recover the impaired balance between pro- and anti-inflammatory effects. Ecto-5'-nucleotidase (eN/CD73), acting as a membrane-bound ectoenzyme, has one of the key roles in homeostasis maintenance of the system and immunoregulation, since its main function is the conversion of AMP to Ado. It has been shown that CD73 functions as the cellular adhesion molecule (CAM), and that it is engaged in direct interactions with ECMs. It is clear that CD73 has significant role in cell motility, by producing Ado, thus shifting the signaling pathways from P2 to P1 purinergic receptors, and by being directly involved in migratory capacity of the cell.
Significant upregulation of ecto-5'-nucleotidase expression and activity, mostly on activated astrocytes, has been observed in several animal models of human neuropathologies, such as MS and ALS, including the cortical stab wound injury. Astrocytes are one of the main cellular effectors of neuroinflammation, making them an important target in understanding of this prominent CD73 upregulation in the neuropathological conditions, in aspects of cellular migration. The large body of evidence in cancer biology (including glioma tumors) suggests that adenosine, produced by catalytic action of CD73, suppresses immune surveillance and promotes tumor growth. CD73, as highly expressed in tumorigenic tissue, is involved in the process of tumor cell migration. The CD73 inhibition has been identified as potential therapeutic target in cancer treatments, using specific anti-CD73 antibodies and different specific CD73 inhibitors. Thus, we have conducted several *in vitro* studies on primary rat cortical astrocyte cultures.
The aim of our present study was to estimate the impact of the anti-CD73 antibodies, the specific inhibitor of CD73 activity (α,β-metADP, APCP) and CD73-siRNA, on the CD73 potency in astrocyte migration in parallel with its mRNA expression and enzyme activity. Moreover, we wanted to compare the results of the direct CD73 inhibition with the effects of the extracellular ATP or Ado (via P2 and P1 receptors, respectively) on both CD73 functions. Our data suggest that CD73 is involved in the process of astrocyte migration, and represent potential target for neuroinflammation therapy, which should be further investigated.

ROLE OF S-NITROSGLUTATHIONE IN HYPERGLYCEMIA INDUCED DISRUPTION OF BLOOD BRAIN BARRIER MEDIATED BY ALTERATIONS IN TIGHT JUNCTION PROTEINS AND CELL ADHESION MOLECULES

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Diabetes is associated with increased blood brain barrier (BBB) permeability causing neurological deficits. The present study investigated the role of tight junction proteins [Zona occludens-1 (ZO-1), occludin, claudin-5] and cell adhesion molecules [intercellular cell adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1)] in aberrated BBB permeability and assessed the effect of S-nitrosoglutathione (GSNO) in diabetic model. Diabetes was induced by intraperitoneal injection of streptozotocin (40 mg/kg body weight) for 5 days in mice. GSNO was administered orally (100 µg/kg body weight) daily for 8 weeks after the induction of diabetes. A significant decline in learning and memory was observed in diabetic mice gauged by radial arm maze test. Relative mRNA and protein expression of ZO-1 and occludin were found to be significantly lowered in isolated microvessels obtained from diabetic cortex and hippocampus while claudin-5 remains unchanged. Furthermore, immunofluorescence of tight junction proteins suggested that the fluorescent intensity for both ZO-1 and occludin appeared to be reduced in diabetic brain. In addition, a significant upregulation was observed in mRNA and protein expression of ICAM-1 and VCAM-1 in diabetic animals. Also, ultrastructure of microvessels from diabetic brain was found to be aberrant suggesting BBB damage. However, GSNO administration to diabetic animals was able to ameliorate loss of ZO-1 and occludin as well as upregulate ICAM-1 and VCAM-1, restoring BBB integrity and improving cognition. Our findings clearly suggest that GSNO may present a therapeutic potential by protecting BBB, thus preventing neurological complications in diabetes.

MUTUAL INHIBITION BETWEEN DISTINCT NEURONAL CLUSTERS IN THE HABENULO-INTERPEDUNCULAR PATHWAY

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The habenulo-interpeduncular (Hb-IPN) pathway is an evolutionary conserved neural circuit recently implicated in fear, anxiety and neurobehavioral disorders such as substance abuse. The developing zebrafish is the ideal organism for the study of neuronal transmission dynamics in the Hb-IPN pathway due to its small size, genetic accessibility and optical transparency. The Hb consists of cholinergic and non-cholinergic neurons that are mainly localized to the right or left nuclei, which project to the ventral and dorsal IPN domains, respectively. The ventral and dorsal IPN neurons project to distinct brain regions, such as the raphe nucleus and griseum centrale. First, by analyzing calcium activity in larval zebrafish brain explants we have discovered that an increase in calcium activity in cholinergic Hb axon terminals at the ventral IPN is correlated with a decrease in calcium activity in non-cholinergic neurons at the dorsal IPN. This finding suggests intrinsic inhibitory mechanisms between the two distinct pathways. We next investigated whether this inhibitory interaction between the two circuits occurs *in vivo* upon external stimuli. In larval zebrafish, aversive stimuli such as mild electric shock, preferentially activates right Hb cholinergic neurons, while flash of light activates the left Hb non-cholinergic neurons. We found that neurons that respond to shock are inhibited upon light flash, and vice versa. Thus, we demonstrate that the two neuronal populations located in the left or right Hb nuclei are indeed part of distinct neuronal circuits that repress each other's activity. The habenula has been suggested to function as a switching board for behavior selection. Our finding of mutual inhibition of neuronal activity between the cholinergic and non-cholinergic circuits demonstrates an innate mechanism in place that could explain how selection of one behavior extinguishes the other.

OCULOCUTANEOUS ALBINISM: A FUNCTIONAL NEUROIMAGING CASE STUDY

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Background: Albinism is a well-studied, heterogeneous group of inherited melanin synthesis disorders with a worldwide prevalence of 1 in 17,000. In humans, albinism can be divided into Oculocutaneous albinism (OCA) and Ocular albinism. Apart from hypopigmentation, the most clinically significant symptoms are related to the visual system. Nystagmus, iris transillumination, macular hypoplasia, and optic nerve fiber misrouting together result in significantly decreased visual function. Of these, irregular optic nerve routing within the optic chiasm and the subsequent abnormal representation of the visual fields in the primary visual cortex can be studied with fMRI to further clarify the diagnosis. This study aimed to investigate the degree, if any, of optic nerve fiber misrouting in a child with a proposed diagnosis of OCA using electrophysiological and fMRI methods. We present our results together with ophthalmological and neurological findings.
Methods: Our study included one seven-year-old female albino patient and one nine-year-old healthy male subject with normal vision, who served as control. We obtained a complete medical history and performed a computer based stereovision test, with random-dot stereograms. Native MRI and fMRI were performed for both subjects. Two monocular (full-field and hemi-field), checkerboard pattern-onset stimuli, with check sizes of 60 and 120 arcmin, presented in a block design pattern were used for fMRI measurements. Steady state flash VEPs were evoked by LEDs. The VEPs were analyzed using independent component analysis in an attempt to blind-separate hemispheric sources.
Results: The clinically relevant data included the skin and hair hypopigmentation, a horizontal nystagmus, iris transillumination and decreased visual acuity (O.D 0.2, O.S 0.15) The results of the stereovision test revealed no measurable stereopsis. Native MRI demonstrated no structural abnormalities of the parenchyma. The fMRI results demonstrated an abnormal optic nerve routing at the level of the chiasm. Each stimulus generated an exclusively contralateral primary visual cortical activation. The VEP responses to monocular flashes were predominantly detectable in the contralateral hemisphere.
Conclusion: Through fMRI we were able to determine that either all of the patient's optic nerve fibers cross contralaterally at the level of the chiasm or the number of uncrossed fibers is so few that it is undetectable with our methods. Based on the patient's hypopigmented appearance, a typical nystagmus pattern and the results of our neuroimaging studies, we can conclude that the patient indeed has OCA.

Aknowledgements: Hungarian Brain Research Program KTIA_NAP_13120130001; OTKA K108747 EFOP-3.6.1.-16-2016-00004
Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs
The project has been supported by the European Union, co-financed by the European Social Fund
The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

A STATIONARY NEURONAL CONTINGENT RELEASES MATRIX METALLOPROTEASE-2 TO REGULATE FORWARD NEUROBLAST MIGRATION IN THE ROSTRAL MIGRATORY STREAM

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The rostral migratory stream (RMS) is viewed as a glia-enriched conduit of forward-migrating neuroblasts in which chemorepulsive signals control the pace of forward migration. Here we demonstrate the existence of a scaffold of neurons that receive synaptic inputs within the rat, mouse, and human fetal RMS equivalents. These neurons express secretagogin, a Ca2+-sensor protein, to execute an annexin V-dependent externalization of matrix metalloprotease-2 (MMP-2) for reconfiguring the extracellular matrix locally. Mouse genetics combined with pharmacological probing in vivo and in vitro demonstrate that MMP-2 externalization occurs on demand and that its loss slows neuroblast migration. Loss of function is particularly remarkable upon injury to the olfactory bulb. Cumulatively, we identify a signaling cascade that provokes structural remodeling of the RMS through recruitment of MMP-2 by a previously unrecognized neuronal constituent. Given the life-long presence of secretagogin-containing neurons in human, this mechanism might be exploited for therapeutic benefit in rescue strategies.

EVALUATION OF COGNITIVE AND MEMORY DYSFUNCTION FOLLOWING MILD TRAUMATIC BRAIN INJURY IN RATS

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Mild traumatic brain injury (mTBI) is a common form of head trauma, and causes minor symptoms that usually resolve on their own. Previous studies have shown that TBI causes cognitive impairment, and acts as a risk factor for subsequent neurocognitive disorders in old age. Similarly, repetitive mild TBI, such as successive concussions in footballers, has been shown to affect cognitive abilities, and in some cases can lead to chronic traumatic encephalopathy. Rats with repetitive mild TBI hold potential as a functional model to study neurocognitive disorders such as Alzheimer's. In the present study, our aim was to develop a mild TBI treatment model in adult rats, which would otherwise not have any prolonged effect on cognition and memory.
TBI was caused under general anaesthesia using the Marmarou weight-drop injury model. In this, a weight of 450 grams is dropped from a pre-determined height onto a metallic disc fixed to the intact skull of the animal. Forty-eight male Wistar rats were divided into 4 groups: Sham (no injury), Mild (weight is dropped from a height of 15 cm), Moderate (25 cm), and Severe (150 cm). We applied behavioural tasks such as Novel Object Recognition (NOR) and Morris Water Maze (MWM) to assess learning. In NOR (delay time: 30 minutes), animals have to discriminate familiar and novel objects. In the MWM task, the animals have to find a hidden platform in the maze, and gradually learn the position of the platform. The acquisition phase lasted 4 days, and probe trial was performed on day 5. In the pre-injury control NOR test, all groups showed normal memory performance, as all groups were able to recognize the novel object. In post-injury 1-week and 1-month tests, all groups showed marked decrease in memory performance, as no group recognized the novel object. In the post-injury 2-month test, Sham and Mild groups performed better than at the 1-month test. Compared to Sham, both Moderate and Severe groups still displayed deficits. In the MWM, in the post-injury 3-week test, all groups took significantly less time to find the platform on the fourth day compared to the first day. In the probe trial, the Severe group performed the worst, while Sham, Mild and Moderate performed similarly (marginally significant between-subject effect was found).
From the results obtained, we can infer that the Mild group rats did not sustain any persistent cognitive dysfunction, except mild impairment in one session of NOR. These observations make it suitable to utilise the Mild injury protocol as a base for further assessing behavioural effects of repetitive mTBI in the future, and to quantify the extent of cognitive impairment caused by repetitive mTBI.

MRI AND IMMUNOHYSTOCHEMICALLY DETECTED DIFFERENCE IN TOXIC-INDUCED (CUPRIZONE) AND CONGENITAL (B4GALT1-NULL) DEMYELINATION

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Mice with a disrupted *B4galnt1* gene lack the enzyme UDP-GalNAc:GM3/GD3 N-acetylgalactosaminyltransferase or simply GM2/GD2 synthase. Therefore, they lack complex brain gangliosides such as GD1a and GT1b, which are found mostly on axons. The total amount of myelin production in *B4galnt1*-null mice is similar compared to wild types. However, with the lack of GD1a and GT1b, myelin-associated glycoprotein (MAG), a constituent of myelin, cannot make proper connections with these complex gangliosides on axonal surfaces. With age, these mice exhibit axonal degeneration and demyelination resulting in motor and behavioral deficits. Cuprizone-induced demyelination is a well characterized model for central nervous system demyelination. Its administration leads to specific cell death of oligodendrocytes. To compare these two mouse models, we performed brain magnetic resonance imaging and immunohystochemistry. We imaged ex-vivo brains of 3 groups of mice: *B4galnt1*-null, wild-type (C57BL/6) and cuprizone-fed. Each group was consisted of 3 subjects. Each subject was 3 months old at the time of brain removal. Upon removal they were perfused with 4% PFA, kept in the PBS, and underwent imaging. Diffusion tensor data were acquired on 7 T Bruker BioSpec 70/20 USR using the spin-echo diffusion weighted imaging sequence in 64 directions, and isotropic voxels with resolution of 150 μm. Using anatomical markings, regions of interest (ROI) were placed in the rostral part of corpus callosum and analyzed according to their scalar radial diffusivity maps. Radial diffusivity is well defined diffusion tensor imaging measure for evaluating differences in white matter. It is calculated as an average of 2 shortest eigenvalues, λ_2 and λ_3. Immunohistochemical comparison of both models was performed using typical markers for demyelination (central myelin markers MAG, MBP), and non phosphorylated or phosphorylated fibers (SMI 311, SMI312). Results were analyzed using non-parametric Kruskal-Wallis test and showed significant statistical differences across the groups ($p=0.004$). The difference between *B4galnt1*-null and cuprizone-fed group, as well as *B4galnt1*-null and wild-type group were $p=0.002$ and $p=0.026$ respectively. Results showed that radial diffusivity values are highest in the cuprizone-fed group, which can be explained solely due to process of demyelination. On the other hand, lowest values of radial diffusivity were in the *B4galnt1*-null group, which may be explained due to unwinding of myelin around axons causing less diffusion of water perpendicularly to axon, which then present as lower values. Myelin markers proved demyelination in *B4galnt1* mice, as well as different demyelination pattern in two mice models. Fiber markers proved accompanied dephosphorylation of neurofilaments, suggesting axonal pathology.

Acknowledgements: The MRI scans were performed at the Laboratory for Regenerative Neuroscience - GlowLab, University of Zagreb, Croatia.

GENETIC INTERACTIONS IN MEDULLOBLASTOMA AND HYDROCEPHALUS

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Background Medulloblastoma (MB), a tumor of the cerebellum is the most common malignant brain tumor of childhood. The origin of MB has been a subject of controversy for many years but currently it is believed that granule neuron precursors (GNPs) are a site of origin. Deregulation in Sonic hedgehog (Shh) signaling pathway has been shown to cause a subtype of MB and Patched (*Ptch1+/-*), an antagonist of the Shh pathway, mutant mice are one of the most studied models of MB. More recently, *Chd7 (*Chromodomain-helicase-DNA-binding protein 7) has been identified as a tumour suppressor for MBs and mutations in Chd7 have been hypothesised to cooperate with *Ptch1* to induce MBs. One of the main features of MBs is the development of hydrocephalus which is believed to responsible for high lethality of this condition.
Aims This project aims to explore the incidence of MB and hydrocephalus in single gene mutant mice (*Ptch1*+/-) compared with double heterozygous mice (P*tch1*+/;*Chd7*+/-) whilst also exploring some of the hypothesised underlying mechanisms leading to the formation of hydrocephalus namely, expansion of choroid plexuses responsible for the over production of cerebrospinal fluid (CSF).
Materials and set up Breeding model were set up to generate Ptch1+/- (n=8) mice and Ptch1+/- ;Chd7+/- (n=5) mice. This study looked at two developmental stages at E12.5 and P0 stages.
Results This study confirmed that roughly 40% of *Ptch1*+/- mice develop tumors in keeping with previous experiments. In contrast, we observed the development of severe hydrocephalus in *Ptch1*+/-;*Chd7*+/- mice in the absence of any tumor formation. This might be partly due to early embryonic lethality as a consequence of the severe hydrocephalus. Furthermore, in both of these genotypes the abnormal wide midline cleft was observed pointing towards midline developmental defects. These defects were exacerbated in *Ptch1*+/-;*Chd7* +/ - embryos. Finally, the expression of Transthyretin, a molecular marker specifically expressed in the choroid plexus at E12.5, revealed no structural disorganisation or ectopic expression of the choroid plexus either genotypes.
Conclusion: This study confirms the formation of MBs in Ptch1+/- mutant mice. The double heterozygous mutant mice however, develop severe hydrocephalus with no obvious tumour formation which we hypothesis is due to early lethality before tumours growth is visibly detect under microscope. To overcome this problem, we aim to repeat our mice set up using Math1Cre mice instead as Math1 expression is specific to GNPs in the postnatal cerebellum.Furthermore, although both genotypes of mutant mice showed signs of incomplete midline development there were no structural disorganisation or ectopic expression of the choroid plexuses, in either genotype as previously hypothesised. This study serves as an impetus for a subsequent study to further understand the link between Chd7 and Ptch1 genes.

EFFECTS OF PACAP-38 TREATMENT ON DIABETIC TESTICULAR ATROPHY

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Introduction: Pituitary adenylate cyclase-activating polypeptide (PACAP) occurs at highest concentration after the central nervous system in the testis. In the male reproductive system, effects of PACAP have been reported on spermatogenesis, sperm motility, and testosterone synthesis of Leydig cells. The protective effects of PACAP have been proven in diabetic retinopathy and nephropathy. It is not known, however, how PACAP affects the outcome of diabetic testicular atrophy.
Methods: To induce diabetes, 65 mg/kg intravenous streptozotocin was administered to adult male Wistar rats. For 8 weeks, 20µg PACAP-38 was injected intraperitoneally to control+PACAP and diabetes+PACAP groups. We measured blood glucose level and body weight on a weekly basis. On the eighth week testes were dissected, measured and processed for light microscopical histological analysis on routine sections.
Results: The diabetic groups had a significant rise in the blood glucose level after the seventh day of the experiment, and there was a significant weight loss compared to the control groups. These parameters were not altered by the applied PACAP treatment. Testis weight was decreased as well as the gonadosomatic index, the macroscopic length and diameter of the testes in the diabetic group. All these parameters were significantly less pronounced in the PACAP-38-treated group. Microscopic examination revealed that the seminiferous tubule diameter of the diabetic+PACAP group was reduced significantly less than in the diabetic group. Moreover, the PACAP-38 treatment prevented the severe reduction in the epithelium area, observed in the diabetic group. In the PACAP-38-treated group, the Johnsen score was significantly higher than in the diabetic group.
Conclusions: Our study confirmed the protective effects of PACAP in the testis: the organ weight, the gonadosomatic index, the length and diameter of the testes, and the microscopic changes, including the seminiferous tubule diameter, the epithelial area and the Johnsen score.

Support: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, GINOP-2.3.2-15-2016-00050 "PEPSYS", MTA-TKI, the National Research, Development and Innovation Fund K119759. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

EXOGENOUS KETONE SUPPLEMENTATION IMPROVED MOTOR FUNCTION IN RODENT MODELS

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Nutritional ketosis has beneficial therapeutic effect on epileptic seizures and on other neurological disorders, but many patients find the ketogenic diet (KD) unpalatable and difficult to sustain. In order to circumvent the need for severe dietary restrictions, exogenous ketone supplementation has been developed to increase blood ketone level to therapeutic levels. The focus of this study was to investigate the effects of ketone supplementation on motor function in healthy and pathologic animal models.
Accelerating rotarod test were performed and recorded along with the circulating blood beta-hydroxybutyrate (βHB) level measurements in Sprague-Dawley (SPD), Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats and Glucose Transporter Type-1 Deficiency Syndrome (G1D) mice strain and hanging wire test in G1D mice. We performed acute and sub-chronic experiments on gavage fed rats one day or seven consecutive days and we performed chronic experiment feeding G1D mice with special diet for 10 weeks.
The effect of ketogenic diet (KD), butanediol (BD), ketone-ester (KE), ketone-salt (KS) and combinations (KE+KS) or mixtures with medium chain triglyceride (MCT) (KE+MCT, KS+MCT) were compared with the control group and/or with the baseline.
The motor performance of healthy SPD rats was enhanced by the single administration of KE+MCT supplement. KE and KS alone improved the motor function of the sub-chronic experiment in SPD rats, while KD and KE in the chronic experiment in G1D mice. The motor function of the WAG/Rij rats showed improvement in KE+KS and KE+MCT groups.
Blood ketone levels were elevated by the KD and exogenous ketone supplementation effectively in all rodent models. We conclude that exogenous ketone supplementation improved motor function without dietary restrictions in rodent models with and without pathology.

"Project no. FIEK_16-1-2016-0005 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the FIEK_16 funding scheme."

PROTEOME ANALYSIS OF TEARS FROM WILD TYPE AND PACAP-DEFICIENT MICE

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a pleiotropic and multifunctional neuropeptide distributed throughout the body. PACAP has been shown to have diverse effects in the eye, including regulation of tear production. The diverse biological actions provide the background for the variety of deficits observed in mice lacking endogenous PACAP. PACAP-deficient mice display several abnormalities, such as completely lost pupillary light reflex, and increased sensitivity to in vitro hypoxia, oxidative stress and excitotoxic insults. However, the proteomic background of these differences observed in PACAP-deficient mice is still not clear.
Our aim was to investigate the proteome alterations in the tears of PACAP-deficient mice compared to wild type mice (WT) between healthy and 12mg/kg i.p. LPS-treated conditions. Tear fluid was collected by non-invasive method 3 and 6 hours before and after treatment. Pooled mice tear samples were separated with sodium dodecyl sulfate polyacrylamide gel electrophoresis. The peptide samples were subjected to liquid chromatography-mass spectrometry (LC/MS/MS) on AmaZon SL Ion Trap mass spectrometer.
The ratios of proteins did not show any significant differences in healthy conditions. Six hours after LPS treatment, we found some significant changes between the two groups. Serum albumin precursor, aldehide dehydrogenase 3 and lipase were upregulated, while lacrein protein was downregulated in the PACAP-deficient mice compared to the WT. Gluthatione S-transferase alpha and omega levels were decreased during the acute inflammation in both treated groups, however, the downregulation of the peptides was significantly stronger in the LPS-treated wild type mice compared to LPS-treated PACAP KO mice. These results clearly show that endogenous PACAP plays a protective role in acute inflammation in the eye.

This research was supported by EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, GINOP-2.3.2-15-2016-00050 "PEPSYS", PTE AOK Research Grant (KA-2017-15), Supported by the ÚNKP-16-4 new national excellence program of the ministry of human capacities, Bolyai Scholarship, MTA-TKI PACAP Research Group. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

IS AGE-RELATED DEGENERATION OF THE CORPUS CALLOSUM ASSOCIATED WITH ALTERATIONS IN HOMOTOPIC FUNCTIONAL CONNECTIVITY OF THE DORSOLATERAL PREFRONTAL CORTEX?

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Resting-state fMRI has been used to identify large-scale networks in the brain. These networks show strong within-network connectivity and have a particular topological signature (e.g., visual, frontal, and default mode network). Previous research supports the notion that functional connectivity reflects the underlying structural connectivity of the brain, as these measures are inter-related. However this statement does not always hold. Moreover, it remains unclear whether decline in structural connectivity, measured by integrity of white-matter pathways, accounts for alterations in functional connectivity in aging. Here, we will look at longitudinal changes in the corpus callosum, the largest white-matter tract, measured by Tract-Based Spatial Statistics (TBSS), in a group of 190 subjects, with ages between 25 and 80. Independent component analysis (ICA) will be used to extract relevant resting-state networks. We will investigate specific parts of the corpus callosum that project into each hemisphere (i.e., genu, splenium) to explore whether larger changes in these tracts are associated with lower (or higher) functional connectivity in the dorsolateral prefrontal cortex (DLPFC). Possibly, individuals with lower white-matter integrity will also show lower connectivity in these regions. The study will provide new insight into the relationship between structural and functional connectivity, and would help clarify whether age-related changes in white-matter are, at least partly, responsible for decreases/increases in functional connectivity in the DLPFC.

EFFECT OF PACAP ON DIABETIC NEUROPATHY: EXAMINATION OF THE FUNCTIONAL AND ULTRASTRUCTURAL ALTERATIONS OF THE PERIPHERAL NERVES

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Introduction: The protective effect of PACAP in diabetic retinopathy and nephropathy has been reported in several studies previously. However, the potential protective effect of PACAP in diabetic neuropathy is not known.
Methods: To induce diabetes, 65 mg/kg intravenous streptozotocin was injected to adult male Wistar rats. We measured blood glucose level and body weight on a weekly basis. For 8 weeks, 20µg PACAP-38 was injected in 100 µl saline intraperitoneally in the control+PACAP and diabetes+PACAP groups. We examined the mechanical nociceptive thresholds weekly, using Randall-Selitto test. The structure of the sciatic nerve was investigated with light-, and electron microscope.
Results: Diabetic groups had a significant rise in the blood sugar level after the seventh day of the experiment, and there was a significant weight loss, which was not affected by PACAP treatment. After the sixth week, we observed the enhancement of mechanical hyperalgesia in the diabetic groups, which was significantly less increased in the diabetic+PACAP group on the seventh and eighth weeks. Ultrastructural examination of myelinated axons revealed that axon-myelin separation of the diabetic+PACAP group was reduced significantly less than in the diabetic group. We did not observe the rise of the mitochondrial number in myelinated axons of the diabetic+PACAP group, in contrast to untreated diabetic group, where a marked rise was found. Moreover, we observed unmyelinated fiber atrophy in the diabetic groups, and it was less severe in the diabetic+PACAP group. Examining the endoneurial capillaries, PACAP-38 has significantly inhibited the thickening of the basement membrane compared to the untreated diabetic group.
Conclusions: The Randall-Selitto test confirmed the presence of neuropathy and the protective effect of PACAP. The electron microscopic analysis verified the protective effect of the neuropeptide regarding axon-myelin separation, mitochondrial number increase, thickening of the basement membrane and unmyelinated fiber atrophy. The protective effect of PACAP could be functionally and morphologically confirmed.

Support: Supported BY the ÚNKP-16-2 New National Excellence Program of the Ministry of Human Capacities; MTA-TKI, GINOP-2.3.2-15-2016-00050 "PEPSYS", Hungarian Brain Research Program, Lendület Program. EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

TRPA1 AND TRPV1, KEY PLAYERS IN LIGHT-INDUCED PAIN

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Transient Receptor Potential (TRP) comprise a superfamily of ion channels, some of which are polymodal, expressed in primary afferent neurons and involved in sensory function. While certain TRP channels are known to be thermo-sensitive (TRPV1, TRPM8, possibly TRPA1), mechano-sensitive (TRPV1, TRPM3, TRPA1) or chemo-sensitive (most of them), until recently there was limited information concerning photo-sensitivity among these channels. Two TRP channels involved in pain signalling, TRPA1 and, to a lesser extent, TRPV1, are now known to be activated by UV or even visible light, and this activity can be substantially enhanced by endogenous photosensitizer molecules (e.g. porphyrins) or exogenous agents. Experiments carried out on heterologous expression systems, cultured primary afferent neurons from wild type and null mutant mice and intact tissue converge to demonstrate a crucial role of these two channels in mediating light-evoked activation and sensitization of nociceptors. These effects are likely to become relevant in pathophysiological states such as cutaneous porphyrias and may also be involved in triggering the pain experienced by patients treated with photodynamic therapy. The putative involvement of these channels in light-evoked pain in several genetic diseases will be discussed at the meeting, as well as the roles of reactive oxygen species in mediating TRPA1 and TRPV1 photosensitivity.

DIAGNOSTIC POTENTIAL OF CEREBROSPINAL FLUID BIOMARKERS IN ALZHEIMER'S DISEASE COMBINED WITH TAU GENOTYPES

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Alzheimer's disease (AD) is the most common sporadic tauopathy. Although it is not caused by mutation in the microtubule-associated protein tau (MAPT) gene, previous studies showed that biomarkers of AD differ between patients with different MAPT genotypes. In this study, we tried to reveal whether the diagnostic potential of cerebrospinal fluid (CSF) biomarkers amyloid β1-42 (Aβ1-42), total tau (t-tau), tau phosphorylated at epitope 181 (p-tau181), epitope 199 (p-tau199), epitope 231 (p-tau231) and visinin-like protein 1 (VILIP-1) could be improved by MAPT haplotype-tagging polymorphisms (rs1467967, rs242557, rs3785883, rs2471738, del-In9, rs7521). First, we compared if levels of Aβ1-42, t-tau, p-tau181, p-tau199, p-tau231, and VILIP-1 differed between patients with different MAPT genotypes (rs1467967, rs242557, rs3785883, rs2471738, del-In9, rs7521). Second, we analyzed the distribution of MAPT haplotypes (H1 and H2 haplotypes and their sub-haplotypes) in Croatian population. Of the analyzed polymorphisms the del-In9 insertion-deletion in MAPT intron 9 defines the H1/H2 division caused by the inversion, while other single nucleotide polymorphisms (SNPs) define sub-haplotypes. The study was conducted on 113 AD and 53 mild cognitive impairment (MCI) patients, 9 healthy controls and 54 patients with other causes of dementia (14 with vascular dementia (VaD), 22 with frontotemporal dementia (FTD), 7 with dementia with Lewy bodies, 3 with AD + VaD, 1 with corticobasal syndrome (CBS), 2 with Parkinson disease (PD), 1 with epilepsy and 4 with unspecified dementia). Levels of t-tau were significantly higher in subjects with AG in comparison to GG rs1467967 tau genotype (when all patients were analyzed together). This observation was also confirmed in the combined group of AD, MCI patients and healthy controls. Differences in levels of CSF biomarkers between other MAPT genotypes (rs242557, rs3785883, rs2471738, del-In9, rs7521) were lost after Bonferroni correction for multiple comparisons. Additionally, we detected 23 H1 sub-haplotypes and 5 H2 sub-haplotypes in the Croatian population.

This work was supported by Croatian Science Foundation grant IP-2014-09-9730.

OSCILLATING FIELD STIMULATION REDUCES INFLAMMATION AND PROMOTES HEALING

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Spinal cord injury is one of the most devastating causing temporary or permanent changes in spinal motor, sensory and autonomic functions. In order to improve the neurologic conditions of patients after spinal cord injury, several potential therapeutic approaches have been identified. One of the promising approaches is the mobilization of endogenous regeneration mechanisms in damaged nervous tissue by means of application of a weak exogenous electric current in the site of injury. In our experiments we were explored whether oscillating field stimulation could efficiently promote motor function recovery in the rat model of spinal cord injury. The spinal cord injury was performed under the general anesthesia by the compression device at the level of Th9 segments with weight of 40g for 15 minutes. For the application of weak electrical current, the miniature oscillating field (OSF) stimulator was used, which was designed in our laboratory. OSF stimulator reverses polarity of electric field every 15 minutes during 6 weeks to support axonal outgrowth in both directions. Implanted stimulator delivered current through the site of injury by means of two Ir/Pt electrodes that were inserted into the epidural space two segments cranial and caudal from the Th9 segment. Experimental (Wistar albino rats) animals were divided into three groups: 1) intact animals with implanted OSF stimulator, 2) the animals with spinal cord injury - SCI, 3) spinal cord injured animals with implanted stimulator - SCI+OSF. After 28 days, animals were perfused and the spinal tissue was used for immunohistological and histological analysis. In control group of intact animals we observed, that OSF stimulator implantation does not induce an inflammatory reaction or necrosis of the surrounding tissue, and does not present a risk or any restriction for the animals. Our results also shows that the experimental SCI+OSF group of rats had better motor activity of hind limbs (using BBB scale and CatWalk) 4 weeks after injury. OSF stimulation decreased the number of activated astrocytes and microglial cells and promoted wound healing after surgery compared with the SCI group of animals. Based on preliminary results, our findings suggest that the implantation of OSF stimulator represents suitable, safe and stable methods that, in the context of combinational therapy, could effectively help in the regeneration and functional restoration after nervous tissue damage.

Supported by APVV grant No. 15/0766 and VEGA grant 0168/17

THE EFFECTS OF SELECTIVE CRF RECEPTOR ANTAGONISTS IN RATS EXPOSED TO CHRONIC NICOTINE TREATMENT AND CONSEQUENT ACUTE WITHDRAWAL

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The aim of our study was to investigate the participation of the CRF receptors (CRF1 and CRF2) in the behavioral and biochemical changes observed following chronic nicotine treatment and consequent acute withdrawal. In this purpose male Wistar rats were exposed to repeated intraperitoneal (ip) injection with 1.4 mg/kg nicotine or saline solution for 7 days and consequently to acute withdrawal for 1 day, and then to a single intracerebroventricular (icv) injection with 0.1 µg/2µl of selective CRF1 receptor antagonist antalarmin or 1 µg/2µl of selective CRF2 receptor antagonist astressin 2B or saline solution. After 30 minutes the changes of horizontal and vertical activity were monitored with an *in vivo* conducta system and then the changes of dorsal and ventral striatal dopamine release were measured with an *in vitro* superfusion system. Half of the rats were treated icv and tested on the 8th day, following 7 days of chronic treatment and half of them on the 9th day, following 1 day of withdrawal. On the 8th day the horizontal and the vertical activities increased in parallel with the dorsal and the ventral dopamine releases in nicotine-treated rats, compared to the control. On the 9th day the horizontal activity and the dorsal dopamine release were increased, but the vertical activity and the ventral dopamine release were decreased in nicotine-treated rats, compared to the control. A single icv administration of antalarmin, but not astressin 2B, reversed all changes of the locomotor activity and the striatal dopamine release observed on the 8th or the 9th day. Our study suggests that CRF1, but not CRF2 receptor, mediates the behavioral and biochemical changes described following chronic nicotine treatment and consequent acute withdrawal.

The present study was supported by the Hungarian Brain Research Program (KTIA_13_NAP-A-III/8)

INVESTIGATION OF THE EFFECT OF AGING ON BLOOD-BRAIN BARRIER MORPHOLOGY AND FUNCTION IN WISTAR RATS

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Introduction: Several research articles reported increased permeability of blood-brain barrier (BBB) in advanced age. A close connection has also been described between the Alzheimer pathology (especially the impaired beta-amyloid clearance) and the BBB dysfunction. The aim of our study was to compare the efflux transporter function, the microvascular morphology and the magnetic resonance image of the brain in young adult and aged Wistar rats.
Methods: The efflux transporter interactions were studied using a well-known P-glycoprotein (P-gp) substrate, quinidine (QND) and a specific P-gp inhibitor PSC-833 (valspodar) by in vivo microdialysis technique. The cerebral microvascular morphology was analyzed by electronmicroscopy (EM). The macroscopic differences between the brain structure of young and aged animals were studied by MR imaging.
Results: The brain penetration of QND increased significantly after pretreatment with PSC-833 in young rats. With similar chemical knocking out, the brain exposure to QND was less increased compared to the baseline in the aged rats determined by dual –probe microdialysis. The post-mortem studies by EM revealed decreased tight junction protein expression between the endothelial cells in the brain capillaries of old animals compared to the young ones. The size of astrocyte endfeet surrounding endothelial layer enlarged with aging and also the thickness of the basal membrane increased in old rats. The MRI investigations showed a remarkable increase in the volume of all cerebral ventricles with advanced age. The total size of the brain changed minimally, but in the bodyweight of young and aged animals tested, a more than 3 fold difference was determined.
Conclusion: Our results indicate that the BBB permeability changes during aging. This can be the consequence of the increased paracellular transport between the endothelial cells (decreased number of tight junctions). But other factors, like enlarged thickness of the basal lamina and the better protective function of P-gp against QND entrance into the brain suggest that the protective function of some elements of the BBB is preserved or even improved with aging.

ANALYSIS OF EEG CORRELATES DURING N-BACK WORKING MEMORY TASK IN EPILEPSY PATIENTS INVESTIGATED WITH SURFACE AND INVASIVE ELECTRODES

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Introduction: Working memory (WM) is a neuro-cognitive system of limited capacity, where information from perception is temporally maintained and manipulated (Baddeley, 2003). Available evidences indicate that mainly fronto-parietal regions are involved in complex WM tasks, but there are some data supporting the participation of the temporal lobes too (Tudesco et al, 2010). N-back task (NBT) is a typical task conceptualized to induce WM processing-load, also used in electrophysiological researches (Scharinger et al, 2017). Earlier electrophysiological studies examined mainly the alpha (8-13 Hz) and beta (14-24 Hz) frequency band power (Gevins and Smith, 2000), but gamma or higher frequencies were not studied. In the present study we used scalp and invasive EEG to detect event related wide band frequency changes in different locations associated with different WM storage load of NBT
Methods: We involved 6 therapy resistant epilepsy patients undergoing video-EEG with scalp and invasive intracranial electrodes for visual and verbal NBT. Stimuli were displayed on a black background in the center of a computer screen. Random sequences of five nouns were used as verbal, and five non-verbalizable geometric shapes as visual stimuli. All stimuli were presented for 500ms, inter stimulus interval was set to 1500 ms. We used three WM storage load of NBT (0, 1, and 2-back conditions).
EEG montage included 32 standard scalp leads positioned according to the 10-20 system. Sampling frequency was 512 Hz. iEEG montage included SEEG electrodes and strips sampled at 2048 Hz. The EEG graphs were filtered and averaged, and time-frequency analysis was performed using EEGLAB.
Results: All subjects completed the six conditions of the NBT. The proportions of good replies were 83% (±12%), 86% (±12%), 64% (±19%), 63% (±20%), for conditions verbal 1-back, visual 1-back verbal 2-back, visual 2-back respectively. We saw a tendency, that gamma frequency band power was increased in 1 and 2-back conditions, that was not observed in baseline condition.
Conclusions: Video-EEG examination during presurgical evaluation of the patients is suitable for complex neuropsychological testing including detailed working memory tasks. Gamma frequency band changes may be psychophysical correlates for working memory processes.

AGE AND NUTRITIONAL STATE MODIFY ACUTE CENTRAL LEPTIN EFFECTS REGARDING ENERGY BALANCE

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Middle-aged people tend to grow obese, while old people develop anorexia leading to weight loss. As these trends are also observed in other mammals, regulatory alterations may also be assumed in the background. Leptin is a catabolic adiposity signal produced in white adipose tissue acting mainly in the hypothalamus. It suppresses food intake (FI) and enhances energy expenditure leading to weight loss. Both aging and obesity have been associated with leptin resistance.
The present study aimed to analyze age-related changes in the anorexigenic and hypermetabolic responsiveness to acute intracerebroventricular leptin administration in different age-groups of normally fed male Wistar rats (adult and old rats from 3 to 24 months of age, NF3 to NF24, respectively). The expressions of the long form of the leptin receptor (Ob-Rb) and inhibitory SOCS3 genes were also assessed by quantitative RT-PCR in the arcuate nucleus (ARC). The influence of high-fat diet-induced obesity (HF) was also tested on anorexigenic and hypermetabolic leptin effects in younger and older middle-aged groups (HF6 and HF12).
Leptin-induced anorexia varied with age: leptin suppressed fasting-induced re-feeding FI strongly in young adult (NF3), but not in younger or older middle-aged (NF6 or NF12) or in aging (NF18) rats. However, leptin-induced anorexia became significant again in old NF24 rats. Leptin-induced hypermetabolism, on the other hand, showed monotonous age-related decline and disappeared by old age. Ob-Rb expression declined until 12 months of age followed by a partial recovery in NF18 and NF24 groups. On the other hand, SOCS3 expression was high in NF6 and NF18 and to some extent in NF24 rats. Age-related alterations of Ob-Rb and SOCS3 expression in the ARC may partly contribute to the explanation of age-related variations in anorexigenic but not hypermetabolic leptin effects. High-fat diet-induced obesity was associated with resistance to leptin-induced anorexia in HF6, similar to that seen in NF6. However, instead of the expected leptin-resistance in HF12, a strong leptin-induced suppression of re-feeding was detected in these obese middle-aged rats.
Our results suggest that acute central effects of leptin on anorexia and hypermetabolism change in disparate ways during aging, implying separate mechanisms (e.g. signal transduction pathways) of different leptin actions. The age-related pattern of leptin-induced anorexia may contribute to the explanation of middle-aged obesity, and partly to that of aging anorexia. Our findings concerning obese rats are in accord with previous observations on anorexigenic effects of peripherally administered cholecystokinin: diet-induced obesity appeared to accelerate the development of age-related regulatory alterations. Similarly, our present data also raise the possibility that chronic diet-induced obesity promotes responsiveness to centrally applied leptin at least concerning anorexigenic effects. (ÁOK-KA 2017/12, ÁOK-KA 2017/13)

DEVELOPMENT OF PUTATIVE INHIBITORY NEURONS IN THE EMBRYONIC AND POSTNATAL MOUSE SUPERFICIAL SPINAL DORSAL HORN

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The superficial spinal dorsal horn is the first relay station of pain processing. It is also widely accepted that spinal synaptic processing to control the modality and intensity of pain signals transmitted to higher brain centers is primarily defined by inhibitory neurons in the superficial spinal dorsal horn. Earlier studies suggest that the construction of pain processing spinal neural circuits including the GABAergic components should be completed by birth, although major chemical refinements may occur postnatally. Because of their utmost importance in pain processing, we intended to provide a detailed knowledge concerning the development of GABAergic neurons in the superficial spinal dorsal horn, which is now missing from the literature. Thus, we studied the developmental changes in the distribution of neurons expressing GABAergic markers like Pax2, GAD65 and GAD67 in the superficial spinal dorsal horn of wild type as well as GAD65-GFP and GAD67-GFP transgenic mice from embryonic day 11.5 (E11.5) till postnatal day 14 (P14). We found that GABAergic neurons populate the superficial spinal dorsal horn from the beginning of its delineation at E14.5. We also showed that the numbers of GABAergic neurons in the superficial spinal dorsal horn continuously increase till E17.5, but there is a prominent decline in their numbers during the first two postnatal weeks. Our results indicate that the developmental process leading to the delineation of the inhibitory and excitatory cellular assemblies of pain processing neural circuits in the superficial spinal dorsal horn of mice is not completed by birth, but it continues postnatally.

A UNIFYING KINETIC MODEL FOR VOLTAGE-GATED IONIC CHANNELS

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Modelling ionic channels represents a fundamental step to develop realistic neural models. Until recently, the voltage-gated ion-channels have been mainly modelled according to the formalism introduced by the seminal works of Hodgkin and Huxley (HH). However, following the continuing achievements in the biophysical and molecular comprehension of these pore-forming transmembrane proteins, the HH formalism turned out to carry limitations and inconsistencies in reproducing the ion-channels electrophysiological behaviour. On the contrary, Markov-type kinetic models have been increasingly proven to successfully replicate both the electrophysiological and biophysical features of different ion-channels. However, in order to model even the finest non-conducting molecular transition, such Markov models are often equipped with a considerable number of states and related transitions, which make them computationally heavy and not suitable to be implemented in multi-compartmental conductance-based biologically realistic neuron models and large networks of those.
We developed a Markov-type kinetic model for all human voltage-gated sodium channels (VGSCs), which is detailed, global (i.e., it accounts for all ion-channel isoforms) and computationally efficient (i.e. with a minimal set of states and transitions).
The real electrophysiological data to be modelled were gathered from previously published studies on whole-cell patch-clamp experiments in mammalian cell lines heterologously expressing the human VGSC subtypes (from Nav1.1 to Nav1.9).
As a result, the developed model faithfully replicates the following electrophysiological macroscopic features of every human VGSC subtype: intensity-voltage curves and intensity-voltage relationship, normalized peak conductance-voltage relationship, steady-state current-voltage relationship during fast inactivation, recovery from fast inactivation. In addition, the model consistently reproduces the first and second time constants of the decay from activation, as well as the deactivation kinetics.
By adopting a minimum sequence of states, and a unique state diagram for all the distinct isoforms, the model ensures the lightest computational load, and the most efficient use in neuron models and neural networks.
The transitions between the states are described by single original ordinary differential equations, which govern the rate of the transitions in function of time and voltage. By simply changing the ‘constant’ parameters of the differential equations the model is able to consistently approximate the functional properties of the distinct channel isoforms.
In summary, the kinetic model appears to be the simplest and most parsimonious way for a detailed phenomenological description of the human VGSCs electrophysiological behaviour.
The present study reports on the suitability of the unified model also for voltage-gated potassium and calcium channels.

ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR AGONIST PHA-543613 IMPROVES NOVEL OBJECT RECOGNITION MEMORY AND REVERSES AGE-RELATED IMPAIRMENTS IN RATS

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Preclinical screening of potential drug-candidate compounds has to be performed in animal models, which mimic certain pathological states with good validity. Since neurocognitive disorders are almost exclusively occur in the elderly population, it is reasonable to investigate cognitive deficits and possible therapies in aged animals. However, aging is not a uniform process, and aged animals often show large variability in cognitive tasks. Our aim was to find a suitable behavioral test paradigm for the comparison of memory performance in middle-aged adult and old aged rats, and to validate the model for preclinical pharmacological screening.
Novel object recognition (NOR) experiments were carried out on 25 middle-aged adult (11 to 17 month old, further referred to as Adult) and 19 aged (20 to 23 month old, Aged) male Long Evans rats. Recognition memory performance of the animals was measured as a function of retention time by performing NOR tests with different retention intervals between the acquisition and the recognition trials. Later, pharmacological relevance of the model was assessed using the α7 nicotinic acetylcholine receptor (nAChR) agonist PHA-543613 in 1 mg/kg and 3 mg/kg doses. Behavioral pharmacological experiments in the NOR paradigm were performed in both groups using the 24-hour retention interval. Furthermore, the Adult group were also tested after 48-hour retention interval. When using short retention intervals (0.5 or 6 hours) between the acquisition and recognition phase in the NOR test, Adult and Aged animals showed similarly good memory performance. However, Aged animals were more sensitive to the increase of the retention interval: while Adult animals performed well, Aged animals were not able to discriminate the novel object after 24-hour retention interval. Memory deficit of the Aged animals was completely reversed by 3 mg/kg dose of PHA-543613, significantly improving the performance compared to vehicle treatment. The initially good performance of Adult rats in the 24-hour retention NOR test was not further improved by the α7 nAChR agonist. However, after 48-hour retention interval, Adult rats also showed poor discrimination performance, which was partially recovered by the administration of PHA-543613. However, the improvement of the discrimination index of Adult animals due to the drug treatment was not significant compared to the vehicle treatment. The weak effect of the cognitive enhancer in the Adult animals can be explained by the lack of pathological processes.
To sum up, our results suggest that Aged rats show memory impairment in the NOR paradigm when using long retention intervals, and their deficit can be successfully reversed by pharmacological manipulations, making the model suitable for preclinical drug-screening in dementia research. In the future, we plan to improve our age-related cognitive impairment model with initial sorting of aged animals in unimpaired and impaired groups according to MRI biomarkers.

Acknowledgements: EFOP-3.6.1.-16-2016-00004
Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs
The project has been supported by the European Union, co-financed by the European Social Fund
The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

EXPLORATION OF LIGAND BINDING MECHANISMS TO HUMAN ESTROGEN RECEPTOR ALPHA

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We use computational methods for investigation of the binding mechanism of various ligands to human estrogen receptor alpha (hERα). Computational strategies complement experimental structure determination, and provide structural information regarding the binding mode at atomic level. They also predict the differences between binding mechanisms of various ligands. Blind docking and molecular dynamics simulations allow an unbiased exploration of the whole surface of hERα which is essential for finding multiple binding sites. Molecular dynamics simulations treat the ligands and hERα with full flexibility. Protein flexibility is particularly important in following the complete ligand binding pathway as induced fit happens on the protein side. Apart from a fully flexible hERα, molecular dynamics allow using explicit water molecules witch are considered to be highly important in the ligand binding process. We present an elucidation of the binding mechanism of various ligands to hERα for an exploitation of their therapeutic potential.

Acknowledgement: Hungarian Brain Research Program (KTIA_NAP_13-2014-0001), OTKA (112807), EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

NANOSCALE ARCHITECTURE OF CB1 CANNABINOID RECEPTOR DISTRIBUTION ON HIPPOCAMPAL EXCITATORY AXON TERMINALS

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CB_1 cannabinoid receptors play a fundamental role in the regulation of neurotransmitter release throughout the central nervous system. Cell-type-specific deletion of CB_1 receptors from excitatory neurons revealed that endocannabinoid-mediated control of glutamate release is critically important in epilepsy, anxiety, and in memory formation. However, somewhat paradoxically, forebrain GABAergic interneuron terminals contain at least one magnitude of order more CB_1 receptors compared to the axon terminals of the glutamatergic principal cells. How can very low CB_1 receptor levels control efficiently glutamate release remains elusive. In addition, the low copy number of CB_1 receptors makes it extremely difficult to investigate the putative molecular changes associated with physiological and pathophysiological processes. To overcome this limitation and to elucidate the nanoscale distribution of CB_1receptors on excitatory axon terminals, we used a combined approach by generating CB_1 antibodies with superior sensitivity and employed STORM super-resolution imaging, a single-molecule localization microscopy with exceptional detection sensitivity. We first applied a new immunological approach based on neonatal Fc receptor (FcRn)-overexpressing transgenic mice and rabbits to produce monoclonal and polyclonal antibodies, respectively against CB_1 receptors. Systematic analysis revealed that the transgenic animals produced better antibodies compared to their wild-type littermates with affinity values reaching the subnanomolar range. Immunohistochemistry with the affinity-purified transgenic antibodies resulted in unprecedently dense CB_1-immunostaining throughout the forebrain, which was validated in CB_1 knockout mice. By using the selected best antibody and a quantitative electron microscopic analysis, we could demonstrate for the first time that the vast majority of excitatory terminals contain presynaptic CB_1 receptors throughout the hippocampus. Additional STORM super-resolution imaging confirmed this result at the single cell level by visualizing CB_1 in most recurrent and Schaffer collaterals of biocytin-filled CA3 pyramidal neurons. Surprisingly, and in contrast to the general homogeneous distribution of CB_1 receptors on GABAergic terminals, the nanoscale analysis uncovered a highly clustered accumulation of CB_1 around the bassoon-positive active zones of excitatory terminals. This observation raises the possibility that the high efficiency of endocannabinoid signaling at glutamatergic synapses is due to the precise nanoscale targeting of the CB_1 receptors to the vesicle release sites. Taken together, these findings demonstrate that FcRn-overexpressing transgenic animals are ideal tools to generate highly sensitive and specific antibodies, and are instrumental to delineate the cell-type-specific nanoarchitecture of one of the most important retrograde signaling pathway in the central nervous system.

SIGNALING EFFECTS OF UROCORTIN2 IN RAT PHEOCHROMOCYTOMA (PC12) CELL LINE

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Urocortins (Ucn) are ligands of corticotropin-releasing factor (CRF) receptors and have 3 different types called urocortin 1, 2 and 3. CRF receptors exist as two main isoforms: CRF-R1 and CRF-R2. Ucn1 can bind to both receptors but Ucn2 and Ucn3 have higher affinity towards the type 2 receptor. CRF-R1 is expressed in the brain and in the pituitary gland while CRF-R2 predominates in the central nervous system, the heart, peripheral organs, the brain, epididymis and in the intestinal tract. Urocortins have important roles in the control of stress response, anxiety, alcohol consumption, hemodynamic and neuro humoral regulation, in the physiology of the heart and circulation and also in pathological alterations of these systems. Urocortin can regulate the hypophysis pituitary adrenal axis, the immune system, behavior and general well-being.
Urocortin2 is produced in the brain and can pass through the blood-brain barrier. It is also expressed together with its receptor in the adrenal medulla from where the rat pheochromocytoma cell line (PC12) has been derived. PC12 cells are used as model system for the investigation of neuronal differentiation. In response to nerve growth factor (NGF) treatment the cells start to grow neurites and after many biochemical changes these cells will be similar to sympathetic neurons. In this cell line system Ucn2 can increase the level of cyclic AMP (cAMP) that leads to the secretion of noradrenalin. It also causes the activation of proteinkinaseA and the phosphorylation of ERK proteins that in turn control the biosynthesis of catecholamines.
Upon treatment of PC12 cells with urocortin2 or NGF the phosphorylation of ERK becomes elevated. However, if both agents are used simultaneously in these cells the activation of ERK is weaker. To find out the mechanism of these antagonistic effects we used different mutant cell lines. The activation of ERK was measured by Western blotting and immunocytochemistry. Densitometry of the Western signals was followed by statistical analysis.

This work was supported by EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

SHAPING OF THALAMIC NETWORK ACTIVITY BY LAYER 6 CORTICOTHALAMIC FEEDBACK

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The thalamus plays a central role in relaying information from the periphery, providing strong feedforward projection to the neocortex. Nevertheless, it acts not just as a simple relay station, but has a more complex role in sensation that is due to the robust feedback projection originating from layer 6 (L6) of the neocortex. This projection outnumbers thalamocortical (TC) projections by a magnitude and may play various roles in the TC network: sharpening the receptive fields of TC cells; influencing the generation of sleep rhythms; acting as classical modulatory pathway in selective attention, yet its actual effect has not been investigated in vivo.
In our research, we use electrophysiological and optogenetic methods to selectively manipulate L6 corticothalamic (CT) neurons in vivo, while simultaneously recording thalamic and cortical activity. We use NTSR1-ChR transgenic mouse line in which L6 CT neurons are exclusively expressing the photosensitive channel-rhodopsin.
Firstly, we investigate the spontaneous firing behaviour of L6 CT neurons under urethane anaesthesia with juxtacellular recordings. The spontaneous activity correlated more with the cortical UP-states, than with the sleep spindles.
Secondly, we investigate the effect of L6 feedback on single thalamic cells and also on thalamic network oscillations. L6 directly excites TC neurons, while it gives collaterals to the nucleus reticularis thalami (nRT), so it also indirectly inhibits them. The net effect of this excitation/inhibition pattern is not clear and may depend on several factors. We show that the projection provides different activation patterns to TC and nRT neurons and the net effect depends on network state and the strength of L6 activation.
Finally, we show that pulse-like activation of L6 can induce sleep spindles depending on the network state both in natural sleep and urethane anaesthesia. Our further goal is to elucidate the spontaneous firing of L6 CT cells in natural sleep.

STRUCTURAL BASIS FOR THE BASAL FOREBRAIN CONTROL OF THE MEDIAL PREFRONTAL CORTEX AND BASAL AMYGDALA

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The basal amygdala (BA) and the medial prefrontal cortex (mPFC), including both prelimbic (PL) and infralimbic (IL) regions, are two fundamental parts of a circuitry involved in fear memory learning. Previous studies have showed that the BA-PL connections are active during states of high fear, while the BA-IL pathway is recruited during fear extinction. Whether these two pathways can be synchronized by subcortical afferents conveying salient information has yet to be investigated. As the basal forebrain (BF) delivers profound innervation to several cortical regions and plays important role in attention and learning, we asked the question of whether this brain area can have an influence on the BA-mPFC fear learning circuits.
To elucidate the structural basis of this hypothesis, we investigated the localization and neurochemical identity of BF cells projecting to the mPFC and/or BA. To this end, we combined retrograde tracing with immunohistochemical staining. We observed that the PL and IL projecting BA neurons show a spatial segregation at the BA level. From the PL, cells could be labeled preferentially in the anterior part of the BA (BAa), whereas from the IL, retrogradely labelled cells could be detected mainly in the posterior part (BAp). Therefore, we investigated these two BA parts separately in regards of the BF projections. To label BF neurons projecting to mPFC and/or BA, we used FluoroGold, which was stereotaxically injected into the PL or IL, and FastBlue to the BAa or BAp. We identified the localization of the retrogradely labeled cells in the BF, prepared a map of projection cells in several cortical planes, and quantified the ratio of double projecting cells. In addition, we investigated the ratio of cholinergic and GABAergic projection neurons using immunohistochemistry to visualize cholinergic cells and a vGAT-Cre x ZsGreen reporter strain to detect GABAergic neurons. Our results show that PL and IL projecting BF neurons have a highly overlapping localization in the nucleus of the horizontal limb of the diagonal band, substantia innominata, and magnocellular preoptic nucleus. The BAa and BAp projecting cells could not be separated spatially either, they located dorsolaterally compared to IL/PL projecting neurons, mainly in the ventral pallidal area/substantia innominata. We found that 24-27 % of mPFC projecting and 61 % of BA projecting BF neurons were cholinergic, while the majority of projecting cells (80-85 %) was GABAergic giving rise to afferents to both cortical areas. Importantly, almost all cholinergic neurons co-expressed vGAT. We found 5 % of neurons that innervated both the mPFC and the BA.
These results indicate that, although a small portion of BF neurons has dual projection, both cholinergic/GABAergic and solely GABAergic BF neurons may provide an independent control for circuit operation in both the mPFC and BA.

NEURAL CREST CELLS MODIFY THEIR EXTRACELLULAR ENVIRONMENT DURING ENTERIC NERVOUS SYSTEM DEVELOPMENT BY HEPARAN SULFATE PROTEOGLYCAN PROTEINS

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The enteric nervous system (ENS), or the "gut-brain" is a network of neurons and glial cells within the wall of the gastrointestinal tract that is critically important in regulating motility and other fundamental aspects of gut function. Abnormalities of the ENS include a wide spectrum of functional gastrointestinal disorders, including Hirschsprung disease (HSCR), which is caused by the failure of enteric neural crest-derived cells to complete their migration along the intestine.
The ENS is derived from neural crest cells (NCC) that migrate, proliferate, and differentiate into enteric neurons and glia within the intestinal wall. ENS development relies on interactions between migrating ENCCs and their extracellular environment. Many extracellular matrix (ECM) components are present in the embryonic gut, but how they promote or inhibit enteric neural crest cells (ENCC) migration is largely unknown. Here, we identified heparan sulfate proteoglycan (HSPG) proteins, including collagen XVIII and agrin as important regulators of ENS development. Collagen XVIII is dynamically expressed during ENS development in the chick gut. This member of the HSPG family is expressed in preganglionic hindgut mesenchyme and its expression becomes limited to the region surrounding enteric ganglia in addition to the basement membranes of the blood vessels and gut epithelium. Aganglionic hindgut leads to the loss of HSPG expression in the submucosal and myenteric area, but remains strongly expressed in the epithelial basement membrane. Neurospheres were prepared from embryonic and adult mouse enteric neuronal stem/progenitor cells (ENCC) and demonstrate collagen XVIII and agrin expression within the neurospheres. Chick-mouse intestinal chimeras were generated by implanting preganglionic mouse gut into the embryonic chick coelom. The results show that chick ENCCs colonize the mouse graft and produce collagen XVIII and agrin. Using tripe-choice assays, we demonstrate that in contrast to collagen XVIII, agrin strongly inhibit the migration of multipotent ENCCs on fibronectin by interfering with cell-substrate adhesion. We conclude that ENCCs modify their microenvironment by producing multiple HSPGs which may regulate ENCC migration by modulating the adhesiveness of ENCCs to their substratum.

PROJECTIONS OF GNRH NEURONS TO HYPOTHALAMIC DOPAMINERGIC NEURONS ARE MAINTAINED DURING LACTATION

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Gonadotropin releasing hormone (GnRH) neurons provide neuronal input to the preoptic area (POA) and the arcuate nucleus (Arc), which are involved in the regulation of neuroendocrine functions and associated behaviours. In these areas, GnRH axons target neurons producing dopamine (DA), which highly co-localizes with kisspeptin (KP) in the POA, but appears in completely distinct subpopulations of neurons in the Arc. Vast amount of data supports that the DA and/or KP producing neurons play location-specific roles in the control of GnRH and prolactin secretion.
The aim of the present study was to reveal whether GnRH axonal connections with DA and/or KP producing neurons in the POA and Arc show alterations in lactating animals, compared to non-lactating mice, and to mothers separated from their pups.
Confocal microscopic analysis was carried out on immunohistochemically triple-labelled brain sections to trace juxtapositions of GnRH-immunoreactive (IR) varicosities on KP- and/or DA-secreting, tyrosine hydroxylase (TH)-IR neurons in mouse brains. In agreement with previous studies, KP expression was found to be reduced in lactating mice; there was a significant reduction of KP co-localization with TH in preoptic neurons (16.08±5.01% of all IR neurons, compared to 57.79±5.01% of non-lactating females; $p< 0.001$, ANOVA, post hoc Bonferroni). Removing the pups for 24h did not restore the KP production in DA neurons (17.28±4.58%). In spite of these changes in the co-localization level of DA and KP, the percentage of all innervated KP or TH cells of the POA did not show significant differences in the three animal models investigated (innervation of KP and TH neurons was, respectively: non-lactating 24.13±3.29% and 27.97±3.73%; lactating 19.63±2.91% and 18.87±3.42%; pups removed 13.22±1.93% and 17.16±4.20%). Similarly, the percentage of Arc dopaminergic neurons contacted by GnRH axon varicosities did not show significant differences (non-lactating 44.26±1.49%; lactating 37.57±5.16%; pups removed 33.5±3.00%).
The findings indicate that GnRH neurons directly innervate POA and Arc KP and dopaminergic neurons and the connections with these target cells are maintained during lactation when the pulsatile secretion of GnRH secretion is temporarily suspended.

Supported by the National Science Foundation of Hungary (OTKA K101326). The authors wish to thank Barna László for his excellent technical assistance.

SUCKLING-ACTIVATED NEURONS AND THEIR NEUROCHEMICAL IDENTIFICATION IN THE BRAIN OF RAT PUPS

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A critically important activity of mammalian young is suckling especially in rodents where pups are born blind and undeveloped. To understand the neural mechanisms related to suckling we performed a mapping study using the c-fos technique. After reuniting the litters at postnatal day 13 (PND13) with the dams for 2h following 17h of separation, suckling started within 5 min. A significant increase in the number of Fos-immunoreactive (Fos-ir) neurons was found in the insular cortex, the central nucleus of the amygdala (CAm), the paraventricular (PVN) and supraoptic hypothalamic nuclei, the lateral parabrachial nucleus (LPB), the nucleus of the solitary tract (NTS), and the area postrema. Double labeling experiments demonstrated the activation of calcitonin gene-related peptide-ir (CGRP-ir) neurons in the LPB, corticotropin-releasing hormone-ir (CRH-ir) but not oxytocin-ir neurons in the PVN, and tyrosine hydroxylase labeled noradrenergic neurons in the NTS. In the CAm, Fos-ir neurons did not contain CRH but were apposed by CGRP-ir fiber terminals. For comparison, pups fasted for 17h were refed with dry food at PND19 without returning them to their mothers. At this age, all pups can consume dry food and drink water. Dry food induced Fos activation in all brain areas activated by suckling, too. The degree of activation was higher following dry food consumption than suckling in the insular cortex, and lower in the supraoptic nucleus and the NTS. Furthermore, some brain areas not activated by suckling showed increased activation by dry food, such as the accumbens, arcuate, and dorsomedial hypothalamic nuclei, and the lateral hypothalamic area. Thus, neurons activated by suckling may participate in homeostatic regulations with an activity pattern different from that after refeeding with dry food.

Grant support: NKFIH OTKA K116538 and the KTIA NAP_B_13-2-2014-0004 Research Program.

PRESYNAPTIC NANOSCALE RECEPTOR/EFFECTOR RATIO CONTROLS NEUROTRANSMITTER RELEASE PROBABILITY AT HIPPOCAMPAL GABAERGIC SYNAPSES

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The subsynaptic molecular scheme that translates anterograde neurotransmission into postsynaptic signaling events via nanoclustered ionotropic receptors has recently been worked out in great details at both excitatory and inhibitory synapses. In contrast, our understanding of the biological principles that determine how retrograde synaptic communication drives presynaptic signaling processes via metabotropic receptors remains rather limited. Technical limitations including the lack of appropriate methods for molecular quantification within the functionally distinct nanodomains of an axon terminal in conjuction with precise measurements of the concurrent physiological processes represent major challenges. Here we introduce a novel approach, which makes the direct correlation of nanoscale molecular imaging data with the respective morphological and physiological parameters possible even at the single synapse level. We applied this approach to test the hypothesis that retrograde synaptic control of GABA release probability by cannabinoid signaling is not determined by the overall metabotropic receptor levels homogeneously distributed throughout the extrasynaptic surface of the axon terminal, but instead, it is primarily controlled at the level of the nanoarchitecture of the active zone i.e. how many sub- and perisynaptic cannabinoid receptors can directly inhibit the release machinery as an effector system. Paired whole-cell patch-clamp electrophysiological recordings were made between *post-hoc* identified presynaptic CB_1-positive interneurons and postsynaptic CA1 pyramidal neurons. After anatomical reconstruction of both cells, pairs linked with a single synaptic connection were further analyzed by correlated confocal and STORM super-resolution microscopy. The nanoscale molecular distribution of presynaptic CB_1 receptors in relation to the active zone visualized by bassoon immunolabeling was quantified in the identified synaptic connection and the molecular data was correlated with the electrophysiologically measured synaptic parameters. Importantly, extrasynaptic CB_1 receptor levels did not covary with neurotransmitter release probability, synaptic efficacy or synaptic potency. On the other hand, the data uncovered a strong inverse correlation between the number of CB_1 receptors concentrated in the close vicinity (100-150 nm) of the active zone and the rate of neurotransmitter release. This correlation was disrupted by application of the CB_1 receptor inverse agonist AM251. These findings are consistent with the possibility that persistent cannabinoid receptor activity controls GABA release probability and its impact is strongly influenced by the quantitative relationship between sub- and perisynaptic CB_1 receptors and their nearby molecular effectors within the active zone.

POSSIBLE NEURONAL BACKGROUND OF ADAPTIVE BEHAVIOR DECODING ENVIRONMENTAL STIMULI

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Invasion species represent a real threat for the ecosystem because of their aggressive distribution, changing the natural balance of the surroundings by outplacing the indigenous species. Zebra mussel, *Dreissena polymorpha*, which is originally a native species in the lakes of southern Russia, is typical example for it. It has incidentally been brought in and then quickly distributed worldwide in many countries. In Hungary *D. polymorpha* appeared in Lake Balaton in 1930 and within a few years it has invaded the Lake. At present, together with the even more aggressive quagga mussel (*Dreissena bugensis*), it is the ruling bivalve species. The question arises what might be the basis of this fast and successful invasive behavior. One, maybe a less considered possibility to look for, is the nervous system, commanding both early and adult adaptive behaviors which determine the optimal responses to environmental challenges. For capturing chemical signals from the surrounding the role of early sensory cells located mainly in the embryonic apical organ has already been assumed. In our present study, applying immunohistochemistry, we have investigated the development of the nervous system of different, trochophore and veliger, larval stages of *Dreissena*, with special attention to their signal molecule (serotonin [5-HT], FMRFamide [Fa]) content and projection systems. The first apical 5-HT- and Fa-immunoreactive (IR) cells, respectively, were found as early as in 16-18 hours free living trochophores, possessing a thick sensory dendrite projecting to the surface, followed later (32-60 hours veliger stage) by the appearance of additional 5-HT-IR sensory neurons in the posterior region and the stomach wall. At 48-60 hours veliger stage, the apical sensory cells displayed already their full morphology, including long proximal processes. A similar developmental staging and wiring system was found to be characteristic for the Fa-IR system, but lacking the stomach sensory neuron and including lateral interneurons. Following the application of 5-HTergic pharmacological agents, including either synthesis precursor (5-HTP), or inhibitor (pCPA), positive and negative changes, respectively, in the larval swimming activity were induced. This kind of interventions, as well as increased salinity were accompanied by parallel alterations of the 5-HT and FMRFa immunofluorescence intensity of the nervous system. Our findings suggest that the larval *Dreissena* nervous system and behavior are suitable targets for studying the effect of different environmental cues, in order to reveal the cellular and systems background of the successful adaptive behavior of this species in the aquatic ecosystem.

HISTOLOGICAL, CYTOLOGICAL AND MOLECULAR GENETIC FINGERPRINTS OF BRAIN REGENERATION IN THE EARTHWORM *EISENIA ANDREI* (CLITELLATA, ANNELIDA)

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The process of regeneration is an omnipresent biological phenomenon, however only few animals (e.g. planarians, Annelid polychaetes and certain earthworm, newts and salamander) are able to recover lost parts of the central nervous system. For example the earthworm *Eisenia andrei* is capable of fully regenerating the suprapharyngeal ganglion (its brain) within a three week period. The regenerated brain seems to be identical with the original one in size, form, organization of the cellular network, and both dorsoventral and anteroposterior patterning. New structures form adequate connections to old (intact) neurons located in the subesophageal or more fare ganglia. In this study we utilized the earthworm *Eisenia andrei* to explore the histological, cytological and molecular genetic basis of brain regeneration.
Following the extirpation of the brain at the 3rd hour an accumulation of free-floating cells were seen in the stead of the brain. Most of them were identified as coelomocytes (eleocytes and amoebocytes) but several neoblasts (stem cells of earthworm), characterized by high mitotic activity, occurred there, too. These cells formed a loose cellular network known to be regeneration blastema. Later on, between the 3rd and 7th days, a large quantity of axons from the circumpharyngeal connectives emanated into the blastema and formed a network to which blastemal cells attached and first they proliferated then differentiated. During differentiation growing of the neural processes and formation of cell junctions, both gap junctions and chemical synapses, were observed suggesting that the neurons and glial cells of the new brain developed from blastemal cells.No migrating cells were seen in circumpharyngeal connectives so neural origin of the neurons of the regenerated brain was excluded. Changes in the mRNAs expressions were studied by means of quantitative PCR using the first three ganglions of the ventral nerve cord and the regenerating blastema. We found that the expression of mRNA of Neuromacin was significantly increased in the regenerating nervous system. The expression of Neuromacin was transcriptionally most active at the 5th day of regeneration. Our results suggest that Neuromacin is involved in the later stages of regeneration (cell proliferation and differentiation namely axon growing and formation of cell junctions both gap junctions and chemical synapses).

POPULATION ACTIVITY STATISTICS DISCRIMINATES SOURCES OF VARIABILITY IN V1 SPIKE TRAINS

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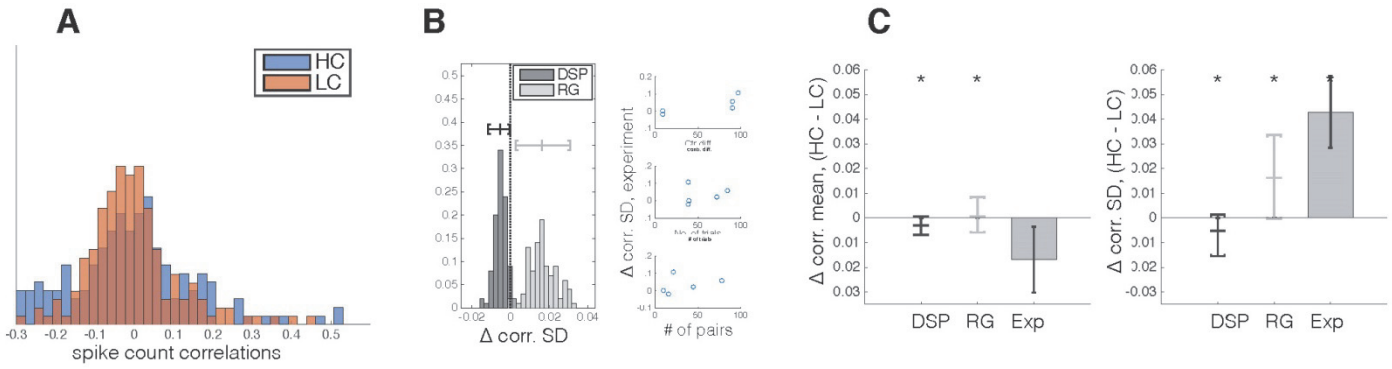
Neural response variability may stem from different physiological sources, which carry different implications about cortical computations. However, various widespread modelling approaches make very different assumptions about these sources. As response variability and covariability change along with stimulus statistics, their sources are discernible in settings where models make conflicting predictions about these statistics. Here we investigate two widely used models of variability, the Doubly Stochastic Poisson (DSP) model, originating stochasticity both from membrane potential dynamics and spike generation, and the Rectified Gaussian (RG) model that assumes minimal stochasticity in spiking, deferring stochasticity to the level of membrane potentials. We demonstrate that they can both reproduce single-cell statistics of responses to standard manipulations of stimulus properties, such as orientation and contrast. However, predictions of the two models contradict about the way the correlation of a pair of neurons changes in response to such manipulations. To compare such predictions to data without having to rely on very precise receptive field characterization, we aim to reconstruct population-level statistics with simulated DSP and RG populations. We use publicly available multielectrode recordings from the V1 of awake macaques viewing grating stimuli at different orientations and contrast levels. We show that the population distribution of spike count correlations significantly widens with increasing stimulus contrast, which dependence is captured by the RG but not the DSP population model. Our analysis also points out that the population average of correlations is insufficient to characterize their stimulus dependence without also reporting the spread of the distribution. We argue that it is possible to discern sources of neural variability based on population statistics, and they are compatible with a correlated source of stochasticity at the membrane potential, but at odds with high private variability introduced by Poissonian spiking.



A, Distributions of spike count correlations in high (HC) and low contrast (LC) stimulus conditions recorded from a population of V1 neurons. **B**, *Left*: Distributions of change in the standard deviation of spike count correlation distributions between HC and LC conditions in simulated DSP and RG populations *Right*: The same difference in the data across sessions is positively correlated with both the amount of available data (number of trials and unit pairs) and the difference between the two contrast levels used in the session. **C**, *Left*: Changes in the mean spike count correlations between HC and LC conditions are small in populations of DSP and RG model neurons and in recordings. *Right*: Change in the standard deviation of spike count correlation distributions between HC and LC conditions is compatible between the RG and the experimental population, but not with the DSP population.

CYTOKINE PRODUCTION INDUCED BY OXIDATIVE STRESS IN PRIMARY RAT ASTROCYTES

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Introduction: Hepatic encephalopathy (HE) is a neuropsychiatric syndrome, caused by liver insufficiency and/or portosystemic shunting. The acute form of HE is characterised by brain edema and the associated cerebral hernia, which represent a potentially lethal complication. Astrocyte swelling is one of the most important component of the brain edema (cytotoxic edema), however the pathomechanism of this process is not fully understood. It has been shown that both oxidative stress and neuroinflammation contribute to the astrocyte swelling, mainly by microglia.
Hypothesis: It is currently believed that microglia causes astrocyte swelling, thus the latter cell type is a victim, which bears the detrimental effects of microglia. On the contrary, we hypothesize that astrocytes are also responsible for the pathogenesis of HE by neuroinflammation due to oxidative stress.
Materials and methods: Primary rat astrocyte cultures were prepared from brains of 1-2-day-old Sprague Dawley rat pups. After the cultures became confluent, were incubated with cytosine arabinoside, followed by L-leucine-methyl ester in order to generate astrocytes cultures devoid of contaminating microglia. These cultures consisted of at least 99% astrocytes as was determined by glial fibrillary acidic protein immonocytochemistry. Incubation of cultured astrocytes with graded concentrations of hydrogen peroxide (H_2O_2) for 1 h caused oxidative stress. It was detected by measuring the fluorescence of 2′,7′-dichlorofluorescein (DCF) which is derived from 5-6-Chloromethyl 2′,7′-dichlorodihydrofluorescein diacetate (CM-H2DCFDA). The number of living cells was assessed by propidium iodide staining. Afterwards the production of tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-10 was detected by ELISA kits with using the chosen H_2O_2 concentration for 1 h.
Results: Based on the preliminary data, the elevation of oxidative stress was increased with the H_2O_2 concentration in a dose-dependent manner. For cytokine assays, we determined the lowest concentration of H_2O_2 that caused significant oxidative stress, but relatively low cell death. Applying this particular H_2O_2 level, i.e. as a result of oxidative stress, both Il-10 and TNF-α production was elevated in the primary rat astrocyte cultures in our experimental setup. However Il-6 production did not show notable changes.
Conclusion: Cytokine production induced by oxidative stress was varied with the types of cytokines. Thus, it could be supposed that not only microglia, but also astrocytes have key role in the pathogenesis of acute HE by causing neuroinflammation. The nature of the underlying mechanisms and the details of these astrocyte-derived phenomena, are however still not completely understood.

Experiments were funded by grants OTKA115613 to A. Zsarnovszky (National Research, Development and Innovation Fund, Hungary); and 11475-4/2016/FEKUT grant of the Hungarian Ministry of Human Resources.

FUNCTIONAL MRI IN NILE CROCODILES

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Crocodilians are crucial for understanding the evolutionary history of amniote neural systems as they are the closest extant relatives of modern birds and share the same stem amniote ancestor with mammals. Although the crocodilian brain has been investigated anatomically, functional studies are lacking. Here we employed fMRI, never previously used in poikilotherms, to investigate crocodilian forebrain properties. Juvenile Crocodylus niloticus were placed in a 7T MRI scanner and BOLD signal changes recorded during presentation of visual (flickering light at 2-8 Hz) and auditory (simple: chords centered around 1000 or 3000 Hz, complex: classic music) stimuli. Visual stimulation increased BOLD signals in rostral to mid-caudal portions of the dorsolateral dorsal ventricular ridge (DVR). Presentation of simple auditory stimuli led to signal increase in two areas of the dorsocentral DVR, while complex stimuli activated additional regions of the mediocentral DVR. Activation patterns during visual and simple auditory stimulation resembled the projection fields of diencephalic sensory fibers, similar to birds. The recruitment of additional, presumably higher sensory areas, reflects observations made in birds and mammals, in which stimulus dependent, hierarchical processing has been reported. Our results indicate that structural and functional aspects of sensory processing have been conserved during the evolution of sauropsids, and that basic principles of hierarchical processing may have been present in the common ancestor of mammals and sauropsids. Our study shows that fMRI can be applied to gain insights into the neural processing and potential cognitive abilities of poikilotherms.

HUMAN INDUCED PLURIPOTENT STEM CELLS MEDIATE TISSUE SPARING WITH MODERATE FUNCTIONAL IMPROVEMENT AFTER SPINAL CORD CONTUSION INJURY IN RATS

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Spinal cord injury results in irreversible tissue damage followed by very limited recovery of function. In this study we investigated whether transplantation of human induced pluripotent stem cells (hiPSCs) into the injured rat cord is able to prevent the secondary spinal cord damage, induce regeneration and functional recovery.
hiPSCs were grafted intraspinally one week after a thoracic (T11) spinal cord contusion injury performed in Fisher 344 rats. Control animals underwent contusion injury without hiPSC transplantation. Locomotor analysis of the injured animals was performed by using open field tests (BBB) and our detailed kinematic analysis system. Two months after the injury the retrograde tracer Fast Blue was applied distal to the injury to determine the extent of propriospinal axonal sparing/regeneration. Immunhistological analysis was performed to investigate the fate of the transplanted cells and the environment of the injury.
Grafted animals showed significantly better and faster functional recovery after contusion injury. Morphologically, the contusion cavity at the epicenter was significantly smaller in grafted animals than in controls. The amount of spared white matter was significantly greater in grafted cords, but no remarkable difference was found in the extent of gray matter rescue in grafted animals compared with controls. Retrograde tracing studies showed statistically significant increase in the number of propriospinal neurons in different segments of the spinal cord above the injury. The hiPS cells partially integrated into the host tissue and differentiated to neurons. The extent of regeneration and functional improvement was inversely related to the amount of CS-56 molecules around the cavity and to the astrocytic and microglial reactions in the injured segment early after injury.
These data suggest that grafted hiPS cells prevent the secondary spinal cord damage and are able to induce moderate functional recovery in the acute phase of spinal cord injury.

MICROGLIA ACTIVATION IN THE SPINAL DORSAL HORN IN A MOUSE MODEL OF BONE CANCER PAIN

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Objective: Cancer pain is a significant clinical problem where the therapy is still an unresolved issue. Therefore, appropriate animal models are important to investigate the underlying complex neuro-immune processes and to develop new treatment strategies. The aim of our study was a multifactorial examination of chronic cancer pain including sex differences, functional and morphological parameters, as well sensitization mechanisms with special emphasis on microglia activation in a mouse osteosarcoma model. This is particularly interesting, since according to recent data, microglia cells and astrocytes can play a role in central sensitization and the maintenance of chronic pain.
Methods: We used 3-month-old adult male and female Balb/c mice, which were treated with intratibial injection of mouse osteosarcoma cells (K7M2), saline-injected (sham-operated) mice served as controls. Changes of the mechanonociceptive threshold of the hindpaw measured by dynamic plantar aesthesiometry, dynamic weight bearing and spontaneous nocifensive behaviors were observed repeatedly during the studies. Tumor growth was monitored in vivo by measuring the knee diameter and microCT scanning of the tibia. Two or four weeks after the injection, mice were anesthetized and transcardially perfused with 4% buffered paraformaldehyde to study microglia and astrocyte activation in pain-related brain regions (somatosensory cortex, periaqueductal gray matter) and L4-L6 spinal dorsal horn by Iba1 and (GFAP) immunohistochemistry, respectively.
Results: Significant, about 40-45% drop of the mechanonociceptive threshold (mechanical hyperalgesia) and 80% decrease of weight bearing on the tumor cell-injected limb developed along with the appearance of spontaneous pain (flinching and guarding of the paw) from the third week of the experiment. Increased knee diameter was accompanied by the characteristic osteoplastic abnormalities on the microCT. Immunopositivity of the microglia marker Iba1 significantly increased in the unilateral superficial spinal dorsal horn regions 2 weeks after the injection, but not in other pain-related brain areas. GFAP immunopositivity was not altered in any regions at either timepoint. Significant differences were not found between any parameters of male and female mice.
Conclusion: This is the first study investigating osteosarcoma-induced nociceptive alterations with a complex functional and morphological approach in an *in vivo* mouse model. Significant mechanical hyperalgesia, spontaneous pain-related behaviors and structural bone changes develop similarly in both sexes. Microglia activation in the spinal dorsal horn at an earlier timepoint, when hyperalgesia reaches its maximum, might have a role in maintaining chronic pain.

Support: EFOP-3.6.1-16-2016-00004, KTIA_NAP_13-2014-0022, GINOP-2.3.2-15-2016-00048, GINOP-2.3.2-15-2016-00050. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

ROLE OF THETA RHYTHMIC SIGNALING FROM HIPPOCAMPUS TO LATERAL SEPTUM DURING EXPLORATORY BEHAVIOUR

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Theta oscillations (5-12 Hz in mice) occur during exploration. Longstanding questions about the role of the hippocampus and theta oscillations in locomotion via rhythmic coordination of specific brain circuits remain unanswered. During locomotion theta frequency changes with running speed, however the relationship between theta synchronization and motor output is complex. Hippocampal theta rhythm is modulated via the medial septum. The medial septum is considered the fundamental theta pacemaker. Medial septum parvalbumin-positive GABAergic cells synapse onto interneurons in the hippocampus CA1 and rhythmically release pyramidal cells from inhibition. The lateral septum is the main subcortical output region of the hippocampus, yet little is known about their interactions. The lateral septum is a key element in circuits governing expression of innate behaviours according to environmental context. A major lateral septum target, the lateral hypothalamus, comprises the diencephalic locomotion region, that provides downstream motor circuits with direct commands for movement. Combining optogenetic control of hippocampal theta oscillations with electrophysiological recordings in mice, we show that hippocampal theta oscillations regulate locomotion. Higher theta regularity causes more stable and slower running speeds during exploration and is accompanied by more regular theta-rhythmic spiking output of pyramidal cells. Theta oscillations are coordinated between the hippocampus and the lateral septum. Chemo- or optogenetic inhibition of this pathway reveals its necessity for the hippocampal regulation of running speed. Moreover, theta-rhythmic stimulation of lateral septum projections to the lateral hypothalamus replicates the reduction of running. These results suggest that changes in hippocampal theta synchronization are translated into rapid adjustment of running speed via the lateral septum.

DETECTING CAUSALITY FROM MANIFOLD DIMENSIONS

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Determining causal relations from time series observations is a non-trivial task and recently gained paramount interest in many scientific fields including neuroscience. The knowledge of causal relations is particularly important in the treatment of patients with drug-resistant epilepsy, where the only treatment option is the surgical removal of epileptic focus. In this case, clearly, there is no opportunity to execute experiments and the least invasive solution is to implant electrodes into brain tissue and try to localise the epileptic focus from extracellular field potential observations.
When we observe correlation between two time series it does not determine the exact causal relation between the variables. That it is possible that one is a cause of the other or vice versa or both and it is also possible that there is a hidden common cause triggering the correlation between the two variables.
In the ‘50-s Norbert Wiener proposed a predictive principle for determining directed causal relations from time series observations. In the next decade the first practical implementation was made in an autoregressive modelling framework by Clive Granger. In the early 21st century transfer entropy was introduced as nonparametric method for determining causal relations using the very same Wiener principle. A few years later George Sugihara et. al. published a causality detection method using the dynamical systems abstraction. It is based on Takens' theorem and the topological equivalence of manifolds in state-spaces reconstructed with time-delay embedding from time series.
However these methods can be used for retrieving directed causal information from observations there have been no single method existed yet that could identify all causal cases, for example none of the aforementioned methods can detect a hidden common cause.
Here we present a new causality detection method which uses manifold dimensions to determine causal relation from time series data. This method can identify independence, directional and circular causal effects as well as hidden common causes properly by assigning probabilities to each possible case.
We show our method's performance on simulated examples and analyse the applicability on neurophysiological measurements.

This work was supported by the Hungarian National Research, Development and Innovation Office (NKFIH) grant no. K113147, the Flag-ERA Human Brain Project CANON grant no. NN118902, the Hungarian National Brain Research Program KTIA NAP 13-1-2013-0001, and KTIA NAP 12-2-201.

CAUSAL RELATIONSHIP BETWEEN LOCAL FIELD POTENTIAL AND INTRINSIC OPTICAL SIGNAL IN EPILEPTIFORM ACTIVITY IN VITRO

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The temporal structure of directed causal relationships were examined between the local field potential (LFP) and the intrinsic optical signal (IOS) during induced epileptiform activity in *in vitro* cortical slices by a new version of Sugihara's causality analysis method. In order to reach this, Sugihara's method have been extended to analyse both the temporal changes of connection strength, both the delay of the causal effect between data series. Two components of the IOS signal have been distinguished, a faster, activity dependent component which changes its sign depending on transmitted and or reflected light application, and a slower component, which is negative in both cases. We have found a delayed causal effect from LFP to faster IOS with 500 ms delay, although the correlation was close to zero between the two signals at that time delay. We have shown, that the overall strength of the causal connections between the two signals increased during the slow development of the epileptiform activity. Based on these observations, a simple model have been set up to describe the dependency of the IOS on the LFP power and fast component of the IOS is reconstructed, based on the LFP signal. The causality analysis have been cross validated on a model with known causal dependency and effect delay. Besides the actual results, this study demonstrates, that it is possible to calculate time dependent and delayed causality between two data series with drastically different time scales.

This research was supported by the grant ERA-NET FLAG-ERA, Human Brain Project, NKFIH NN-118902: CANON project and National Research, Development and Innovation Office NKFIH under grant no. K-113147 and National Brain Research Program grant no. KTIA_13_NAP-A-IV/1,2,3,4,6.

COMPARATIVE PROTEOMIC STUDY OF HOMOCYSTEINE TOXICITY IN CEREBRAL ISCHEMIA AND ISCHEMIC TOLERANCE

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Homocysteine is a side product of amino acid methionine metabolism. Its elevated level of is believed to be one of the independent risk factors for developing ischemic stroke by accelerating atherosclerotic changes. Homocysteine increases production of reactive oxygen species by various mechanisms and thus contributes to oxidation of proteins, lipids and nucleic acids. We studied the effect of hyperhomocysteinemia on outcome of global ischemia and ischemic preconditioning, a procedure, that should minimize neuronal death after ischemia by inducing ischemic tolerance.
The study was carried out on homogenates of rat´s brain cortexes, from which were created proteomic profiles by 2 dimensional electrophoresis. Proteins with densitometric fold change grater then 1,5 in comparison to control group were considered significantly changed. In total there were 43 such proteins and we managed to identify 21 of them.
According to our findings in hyperhomocysteinemic animals when compared to control group was induced downregulation of malate-dehydrogenase shuttle, pyruvate kinase and aconitase. These enzymes are all connected to cellular energy production and thus we can speculate that increased level of homocysteine impairs cellular energy metabolism.
In ischemic model we identified two ATP synthase subunits and both were downregulated in ischemia, ischemia/reperfusion samples and also in preconditioning/ischemia/reperfusion condition. Downregulation of ATP synthase might indicate dysfunction in ATP production in ischemia.
In ischemia/reperfusion and preconditioned tissue, there was upregulation in citrate cycle enzyme aconitase, probably reflecting increased cellular need to produce energy during reperfusion period.
Increased expression of phosphatidylethanolamine-binding protein in ischemic tissue, protein regulating acetylcholine secretion and cellular proliferation, could be a protective response to increased oxidative stress.
We did not find any significant changes in energy metabolism between ischemic tissue and ischemic tissue with interference by ischemic preconditioning, which might be caused by insufficient sensitivity of protein profiling and small sample size.

This work was supported by grant VEGA 1/0128/16 and BioMed Martin ITMS code: 26220220187

PLURIPOTENT STEM CELL-BASED DISEASE MODELLING - DIGEORGE SYNDROME

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Human pluripotent stem cells (PSCs) can be differentiated into derivatives of all three germ layers thus can develop into all cell types. Therefore PSCs give us the opportunity to study early stages of embryonic development, can be used for drug and toxicity testing, and disease modelling. In addition, PSCs can potentially be used for cell therapy in regenerative medicine. Among PSCs, we distinguish embryonic stem cells (ESC) that are derived from the inner cell mass of the blastocyst, and induced pluripotent stem cells (iPSC) that are generated from somatic cells via reprogramming process by forced expression of certain transcription factors.
Our aim is to investigate in vitro cellular phenotypes in a complex multi-organ diseases, the 22Q11 deletion (or DiGeorge - DG) syndrome. DG syndrome is the most common micro-deletion syndrome associated with a broad range of developmental features affecting the cardiovascular, nervous and immune systems. The syndrome is associated with a 20- to 30-fold increased risk of schizophrenia, making this microdeletion the strongest known link between any genetic anomaly and schizophrenia. Theses abnormalities are caused by genetic deletions affecting about 60 genes. Primary candidate gene in the deleted regions is the DG syndrome critical region gene 8 (DGCR8), which encodes for a component of the microprocessor complex essential for biogenesis of microRNAs, which are key regulators controlling diverse cellular functions in eukaryotes.
To model this disease, first we generated iPSCs from peripheral blood samples of a DG syndrome trio, where both the mother and the child were affected, and the father was unaffected. Reprogramming of blood mononuclear cells to pluripotent state was performed by forced expression of four transcription factors - Oct3/4, Sox2, Klf4 and c-Myc - using Sendai virus vector. IPSC are being differentiated into cardiac and neural cell types relevant to the disease by mimicking the in vivo developmental program. During this process we compare phenotypes of disease affected and control cells.
Second, to investigate the role of DGCR8 in the evolvement of different symptoms of the DG syndrome, we established a DGCR8 knock-out hESC, by removing exon 3 of DGCR8 gene. This genetic modification was implemented by CrispR/Cas9 system, which enables a considerably precise genome editing. This genetic modification may cause several measurable changes in phenotype and function of derived mature cell types (e.g. cardiomyocytes and neurons). Examination of these „*in vitro* anomalies" by immunocytochemistry, Ca-imaging, and patch-clamp technique may bring us closer to understanding the molecular mechanisms underlying the complex symptoms of the DG syndrome. This *in vitro* model system will also be suitable for drug screening and pharmacological testing.

This study has been funded by the National Brain Research Program of Hungary (KTIA_NAP_13-1-2013-0001, KTIA_NAP_13-2014-0011).

ESTABLISHING TEMPORAL AND SPATIAL INPUT INTEGRATION RULES OF DIFFERENT HIPPOCAMPAL INHIBITORY NEURONS USING HIGH SPEED, PATTERNED OPTOGENETIC STIMULATION

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Inhibitory neurons (IN) have a crucial role in shaping the activity of the hippocampal formation, as different types are activated specifically during distinct behaviour-associated network states. INs are diverse in many features, like dendritic geometry, distribution of inputs and variations in ion channels, assigning each type a specific integrative property. If we want to understand how the underlying mechanisms shape the network activity, it is essential to clearly define the integrative functions of inhibitory cell types.
Most studies addressing this question used the same input (i.e.: pathway, subset of axons or synapse) for repeated activation, therefore the possible effects of short-term plasticity contaminated the characterization of temporal properties. Furthermore, spatial and temporal interactions among different inputs is another important element of integration that we want to examine.
In this study, we present an approach for detailed characterization of neuronal integrative properties, using optogenetics combined with a digital micro-mirror device (DMD). We developed a software suite that can stimulate selected inputs with different spatial and temporal combinations and record the neuron's response automatically.
We tested many viral vectors and mice lines to optimize ChR2 expression in principal neurons, and recorded from INs in vitro. First, we create a response map for the recorded neuron. Then we selectively stimulate the response areas (5-10) in spatially and temporally (as quick as 0.5 ms stepping) patterned combinations, that enables us to examine parameters like short-term plasticity of individual inputs and spatiotemporal integration properties of INs.
With further experiments, we are aiming to reveal how neurons respond to in vivo recorded spike sequences distributed over many inputs. Similar experiments can be run on neurons expressing ChR2 directly to study the integration rules of membrane currents. Finally we plan to compare how IN types differ from each-other in their integrative properties.

EFFECTS OF CHRONIC MEMANTINE TREATMENT ON OKADAIC ACID INDUCED MEMORY IMPAIRMENT AND CHANGES IN NEUROTRANSMITTER ACTIVITY IN THE SEPTO-HIPPOCAMPAL SYSTEM

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Background: Several types of animal models have been developed to investigate Alzheimer's disease. Cerebral or intracerebroventricular (ICV) okadaic acid (OA) administration induces Alzheimer's disease-like phenotype in rats. Memory impairment induced by intracerebroventricular injection of OA has been reported, accompanied by remarkable neuropathological changes including hippocampal neurodegeneration.
Objective: The present study was designed to explore involvement of neurotransmitter activity dysfunction in septo-hippocampal system in OA-induced memory impairment. The effects of chronic memantine treatment were also assessed on OA induced alterations in above parameters associated with memory deficit.
Methods: The effects of bilateral ICV administration of OA on memory function were studied in the water maze task taxing different strategy of spatial memory function. OA-induced changes in neurotransmitter activity in the hippocampus and in medial septal nucleus were evaluated by immunostaining. Rats were given daily intraperitoneal injections of memantine (5 mg/kg) for 13 days starting from the day of OA injection. OA dissolved in aCSF (20ng/µl) and injected in volume of 10µl on each side. Animals were divided into four groups: control rats injected i.p. with saline - Contr(S) or memantine - Contr(M) and OA - injected rats treated i.p. with saline - OA(S) or memantine - OA(M).
Results: The results of behavioral studies showed that OA injected rats acquired the visible platform version of the water maze task but failed to learn the platform location in space indicating hippocampal-dependent spatial memory impairment. The results of immunohistochemical studies showed a significant reduced AChE staining in hippocampus in OA(S) group as compared to sections obtained from Contr(S) and OA(M) groups. The results showed that ICV microinjection of OA decreased the number of AChE sensitive neurons in different regions of the hippocampus and decreased the number of ChAT and parvalbumin sensitive GABAergic neurons in the medial septum.
Conclusions: Chronic administration of memantine significantly attenuated OA induced spatial memory impairment and the OA-induced neuropathological changes in the hippocampus and in the MS. It is suggested that ICV injection of OA may impair the hippocampus-dependent spatial memory through damaging the cholinergic and GABA-ergic projections between the MS and the hippocampus and the septo-hippocampal dysfunction may be at least part of the underlying mechanisms of OA induced spatial memory deficit. OA induced changes reported in the present study have been observed in patients with Alzheimer's disease, and therefore reinforce the importance of this model for the investigation targets of new therapeutic strategies. It may also lead to a better understanding of the fundamental neurobiology of memory.

CEREBELLAR-NETWORK DYSFUNCTION AND COGNITIVE IMPAIRMENT IN PROGRESSIVE SUPRA NUCLEAR PALSY

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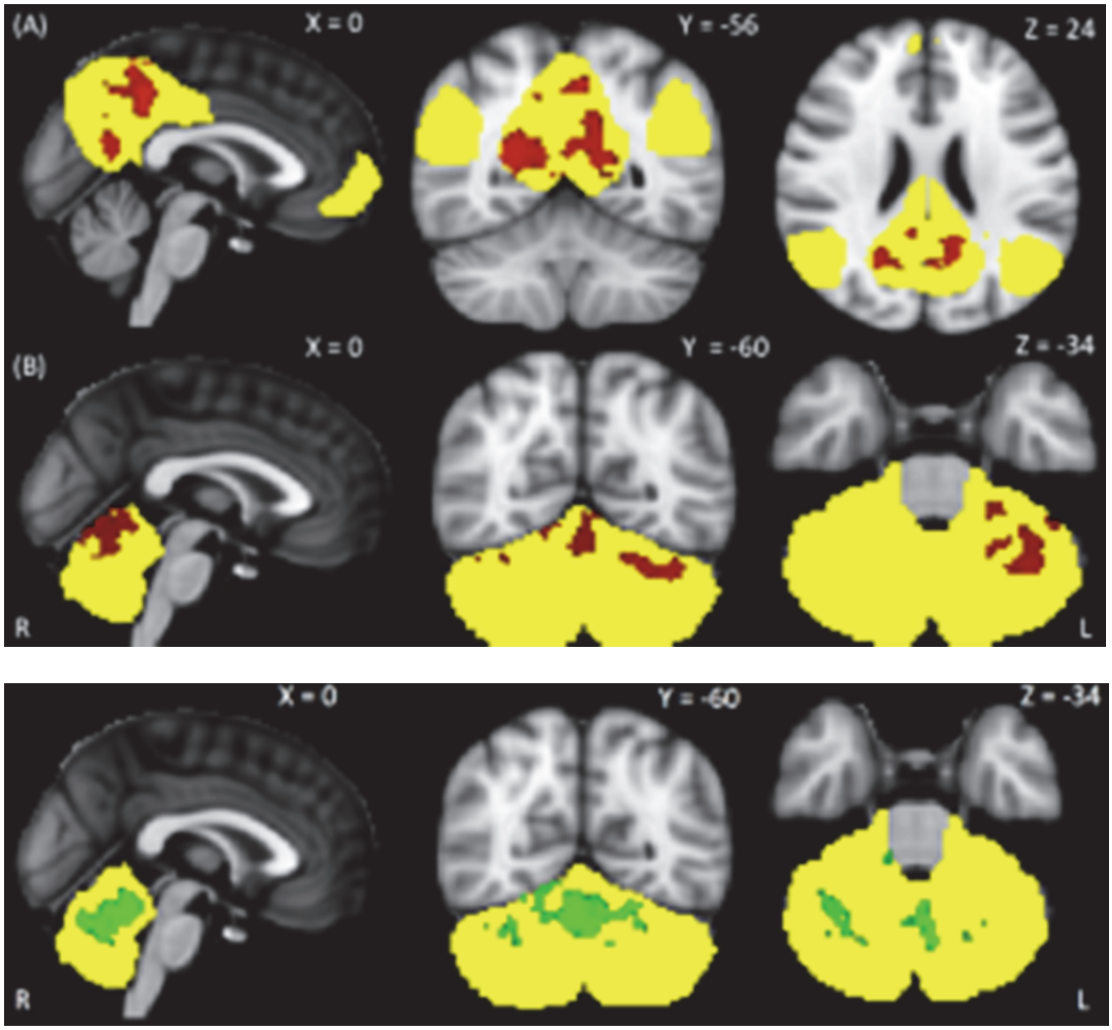
Background: Pathological and MRI-based evidence suggests that multiple brain structures are likely to be involved in functional disconnection between brain areas. Few studies have investigated resting-state functional connectivity (rsFC) in progressive supranuclear palsy (PSP). In this study, we investigated within-network rsFC abnormalities in PSP.
Methods: Twenty patients with PSP, and sixteen healthy subjects underwent a resting-state fMRI study. Resting-state networks (RSNs) were extracted to evaluate within-network rsFC using the FSL Melodic software.



Results: Increased default mode network (DMN) and cerebellar within-network (CBN) rsFC was observed in PSP as compared to healthy controls (Figure 1). Within-network cerebellar rsFC positively correlated with mini-mental state evaluation (MMSE) scores in patients with PSP (Figure 2).
Conclusion: This study provides evidence of functional brain reorganisation in PSP. Increased within-network rsFC may represent a higher degree of synchronisation in damaged brain areas. Positive correlation between cerebellar rsFC and MMSE scores may represent the cognitive impairment in PSP.

AMYGDALA NRG1-ERBB4 IS CRITICAL FOR THE MODULATION OF ANXIETY-LIKE BEHAVIORS

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Anxiety disorder is one of the most common psychiatric diseases (28% lifetime prevalence) and contributes to the etiology of major depression&substance abuse and schizophrenia. Despite its high prevalence, the underlying molecular mechanisms of anxiety remain unclear. Moreover, classical 1,4-benzodiazepines as standard treatment for anxiety disorders, are not consistently effective, which is due to their significant side effects. Therefore, better therapeutic strategies require a deeper understanding of anxiety pathophysiological mechanisms. The amygdala is important for manifestation and modulation of anxiety. However, relatively little is known regarding the mechanisms that control the amygdala inhibitory activity that is involved in anxiety. We provide evidence that neuregulin 1 (NRG1) and its receptor ErbB4 tyrosine kinase in the amygdala are critical for the modulation of anxiety-like behaviors through a GABAergic mechanism. We found that almost all ErbB4 receptors in the basolateral amygdala (BLA) were expressed in GABAergic neurons. Neutralizing endogenous NRG1, pharmacological inhibition of ErbB4, or knocking down ErbB4 in the BLA of mice enhanced anxiety-like behaviors and, concomitantly, inhibited GABA release, while it had no effect on glutamatergic transmission, indicative of a necessary role of NRG1-ErbB4 signaling in the regulation of anxiety and GABAergic neurotransmission. In contrast, no effects of exogenous NRG1 on these behaviors and synaptic transmission were observed, suggesting that the activity of endogenous NRG1 is at a saturated level in the amygdala. Interestingly, the expression level of both NRG1 and ErbB4 was down-regulated specifically in the amygdala of high-anxiety mice and the administration of NRG1 into the BLA alleviated their anxiety and reduced GABAergic neurotransmission. These observations indicated that NRG1-ErbB4 signaling is critical to maintaining GABAergic activity in the amygdala and thus to modulating anxiety-like behaviors. Together, our findings identify a novel pathophysiological mechanism for anxiety. Because NRG1 and ErbB4 are susceptibility genes of schizophrenia, our studies might also help to explain the potential mechanism of emotional abnormality in schizophrenia.

HIPPOCAMPUS ENDOTHELIN1 DECREASES EXCITABILITY OF PYRAMIDAL NEURONS AND PRODUCES ANXIOLYTIC EFFECTS

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As it is known, anxiety disorder is one of the most common psychiatric diseases (28% lifetime prevalence) and contributes to the etiology of major depression, substance abuse and schizophrenia. Despite its high prevalence, the underlying molecular mechanisms of anxiety remain unclear. Moreover, classical 1,4-benzodiazepines as standard treatment for anxiety disorders are not consistently effective, which is due to their significant side effects. Therefore, better therapeutic strategies require a deeper understanding of the pathophysiological mechanisms of anxiety.
The hippocampus play an important role in development and/or maintenance of pathological anxiety. However, the mechanisms that control the neuronal activity of hippocampus in anxiety are still not clear. We found that Endothelin1 (ET1) mRNA in the hippocampus was down-regulated in mice anxiety-like bahaviour. Neutralizing endogenous ET1 in the CA1 of hippocampus enhanced anxiety-like behaviors. We next revealed that most ET1 and its receptors in the CA1 were expressed in pyramidal neurons, and the ET1 signaling pathway directly regulated the excitability of CA1 pyramidal neurons and glutamatergic synaptic neurotransmission. Finally, we proved that infusing exogenous ET1 into the CA1 alleviated the anxiety susceptibility. Together, these results indicate that ET1 signaling is critical in maintaining the excitability of glutamatergic neurons in the hippocampus, and thus to modulating anxiety-like behaviors. Because ET1 is a risk factor for ischemic stroke, our findings might also help to explain the potential mechanism of emotional abnormality in stroke.

AMYGDALAR ENDOTHELIN-1 REGULATES PYRAMIDAL NEURON EXCITABILITY AND AFFECTS ANXIETY

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An abnormal neuronal activity in the amygdala is involved in the pathogenesis of anxiety disorders. However, little is known about the mechanisms. High-anxiety mice and low-anxiety mice, representing the innate extremes of anxiety-related behaviors, were first grouped according to their anxiety levels in the elevated plus maze test. We found that the mRNA for endothelin-1 (ET1) and ET1 B-type receptors (ETBRs) in the amygdala was down-regulated in high-anxiety mice compared with low-anxiety mice. Knocking down basolateral amygdala (BLA) ET1 expression enhanced anxiety-like behaviors, whereas over-expressing ETBRs, but not A-type receptors (ETARs), had an anxiolytic effect. The combined down-regulation of ETBR and ET1 had no additional anxiogenic effect compared to knocking down the ETBR gene alone, suggesting that BLA ET1 acts through ETBRs to regulate anxiety-like behaviors. To explore the mechanism underlying this phenomenon further, we verified that most of the ET1 and the ET1 receptors in the BLA were expressed in pyramidal neurons. The ET1–ETBR signaling pathway decreased the firing frequencies and threshold currents for the action potentials of BLA pyramidal neurons but did not alter BLA synaptic neurotransmission. Together, these results indicate that amygdalar ET1-ETBR signaling could attenuate anxiety-like behaviors by directly decreasing the excitability of glutamatergic neurons.

EFFECT OF ATORVASTATIN ON INFLAMMATORY RESPONSE AND MOTORICAL ACTIVITY OF HINDLIMS AFTER SPINAL CORD INJURY

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Spinal cord injury (SCI) is a serious clinical problem associated with lifelong disability and a range of associated complications. Primary damage of spinal cord causes tissue necrosis, swelling and bleeding. Induction of a cascade of secondary pathophysiological mechanisms, such as ischemia, apoptosis, lipid peroxidation, free radical production and inflammatory response occur early after SCI. Inflammatory response is a complex process that appears within a very short time interval after injury. It affects numerous cell populations and a large number of non-cell mediators. The aim of our study was to limit the inflammatory response at the lesion site by post-SCI administration of Atorvastatin (ATR), and to decrease the extent of SCI pathology. Adult Wistar rats (n=24), used in the study were divided into 6 experimental groups: 1-3) Th9 compression (weight of 40g/15 minutes) and survival for 4, 24 hours, and 6 weeks; 4-6) Th9 compression + ATR (5 mg/kg, i.p.) and survival for 4, 24 hours, and 6 weeks. At the end of survival, spinal cord was cut into 1 cm block (lesion site) and 1 cm block cranially as well as 1cm caudally from the epicenter of injury. Spinal cord sections were used for immunohistochemical determination of macrophages (ED-1), astrocytes (GFAP), microglia (IBA-1) and outhgrowing axons (GAP-43). The level of IL-1ß was determined in blood serum (Elisa), taken from each animal at regular intervals throughout the survival periods. Post-operative neurological impairment was tested using BBB open field locomotor test, one day after surgical procedure, and continued weekly for 6 weeks of survival. Strong inflammatory response was noted early after Th9 compression. The level of IL-1ß was 40±1.33 pg/ml in the blood serum of intact animals and it was significantly elevated 4 hours after SCI (490±32.08 pg/ml). We noted strong infiltration of macrophages 24 hours after injury in the white (250± 20.42) and grey (300± 22.96) matter. ATR treatment decreased the level of IL-1ß release after 4 hours almost to control level (50±2.94 pg/ml), and significantly reduced the number of infiltrated macrophages into the white (100±33.35) and grey (150±40.16) matter at the lesion site 1 day after SCI. Although a single dose of ATR markedly reduced the activation of astrocytes and microglial cells, and increased the number of overgrowth axons at the lesion site 6 weeks after SCI, no significant improvement was noted in neurological activity of hindlimbs. We suggest, that neuroprotective effect of ATR (5 mg/kg, i. p.) seen early after SCI could be crucial for improvement tissue regeneration, when combined with cell grafting and/or gene therapies, or other therapeutical strategies aimed at creating an optimal growth environment.

Supported by APVV grant No. 15/0766 and VEGA grant 0168/17.

MOTOR CORTEX PLASTICITY AND BEHAVIOR IMPROVEMENT PROMOTED BY TREADMILL EXERCISE IN AN INITIAL PHASE OF PARKINSON DISEASE RAT MODEL

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Parkinson's disease (PD) is the second most common eldery neurodegenerative disease, and promote disabilities. Exercise has been described as a good intervention for PD. The objective of this study was analyze if treadmill exercise protocol, a non-pharmacological tool, can promote plasticity in the motor cortex, area that excitability is altered in the PD. Then, rats were subjected to the unilateral PD model induced by striatal 6-hydroxydopamine and a treadmill exercise protocol (3x / week for 40 minutes). The behavioral responses was investigated with cylinder test in two moments of the study, before and 10 days after the induction of the PD model. The changes in structural and synaptic proteins were determined by immunostaining of synaptic proteins (synapsin and synaptophysin) and structural (neurofilaments and MAP-2) 10 days after induction model of PD. The immunohistochemistry data were correlated with neurofunctional changes in motor cortex assessed by method of positron emission tomography (PET) marked with radiopharmaceuticals Fludesoxiglicose-(18F) ([18F]FDG), which assesses the activity of glucose in the brain of these animals.

The data obtained so far in the project reveal that the treadmill exercise protocol is able to promote dopaminergic protection and neuroplasticity in motor cortex even after only 10 days of exercise. Our data showed that that was an improvement in motor behavior with decreased asymmetry in the cylinder test in animals injected with 6-OHDA and trained. In addition, there was a neuroprotective effect on dopaminergic neurons of the substantia nigra. The data from sedentary parkinsonism animals revealed an increased in the expression of MAP-2 in primary and secondary motor cortex, suggesting an excitability cortical increased, being reversed by the exercise protocol. In addition, the [18F]FDG data corroborate the staining data and showed changes in the motor cortex between the groups. Thus, this study suggest that this exercise protocol could be normalize the cortical excitability, and improve the motor behavior that occur in initial phase.

THE ROLE OF DIRECT VESTIBULO-TRIGEMINAL CONNECTIONS IN PREY-CATCHING BEHAVIOR OF FROG

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The majority of the jaw muscles in frogs are supplied by the trigeminal motoneurons. We have previously described that the primary vestibular afferent fibers, conveying information about the movements of the head, established close appositions on the motoneurons of trigeminal nerve. The aim of our study was to reveal the spatial distribution of vestibular close appositions on functionally different trigeminal motoneurons. In common water frogs the vestibular and trigeminal nerves were simultaneously labeled with different fluerescent dyes and the possible direct contacts between vestibular afferents and trigeminal motoneurons were identified with the help of DSD2 attached to an Andor Zyla camera.
In the rhombencephalon an overlapping area was detected between the incoming vestibular afferents and trigeminal motoneurons along the whole extent of the trigeminal motor nucleus. The vestibular axon collaterals formed 138±30 close appositions with dorsomedial and ventrolateral dendrites of trigeminal motoneurons. The majority of direct contacts were located on proximal dendritic segments closer than 300 μm to the somata. The identified contacts were evenly distributed on rostral motoneurons innervating jaw closing muscles and motoneurons supplying jaw opening muscles located in the caudal part of trigeminal nucleus.
We suggest that the identified contacts between vestibular axon terminals and trigeminal motoneurons may constitute one of the morphological substrates of a very quick response detected in trigeminal motoneurons during head movements and are important elements of sensory modulation of prey-catching behavior in the frog.

USE OF EMBRYONIC STEM CELLS FOR DOPAMINE REPLACEMENT IN PARKINSON´S DISEASE

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Studies in animal models of Parkinson´s disease (PD) have shown that transplanted dopamine neuroblasts can restore dopaminergic neurotransmission in the grafted striatum and reverse PD-like motor impairments. Open-label clinical trials in patients with PD have shown that dopamine neuroblasts obtained from fetal human midbrain tissue can survive and function over many years in the brain of PD patients, restore striatal dopamine release, and provide sustained and long-lasting improvements in motor behavior. The ethical and practical problems associated with the use of fetal tissue is a serious obstacle to further developments of this approach. Further progress, therefore, is critically dependent on the development of transplantable dopamine neurons from stem cells. The most promising results so far have been obtained using pluripotent stem cells, ESCs or iPSCs, as starting material. Recently developed and optimized protocols allow efficient generation of midbrain dopamine neurons from human ES cells that survive well following transplantation to the striatum, in the absence of any contaminating tumor-forming cells, and differentiate into genuine midbrain dopamine neurons of both A9 and A10 subtypes. In recent experiments performed in immunosuppressed and immunodeficient rats we have shown that the hESC-derived neurons grow to form an extensive axonal terminal networks in appropriate striatal, limbic and and cortical targets and reverse PD-like motor impairments. The results indicate that transplantable and fully functional midbrain dopamine neurons can be generated from human ES cells, ready to be used in patients.

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NEUROBIOLOGICAL BASIS OF ADAPTABILITY: NEUROTRANSMITTER AND BEHAVIORAL PROFILE OF TWO SPECIES OF LIZARD, THE ITALIAN WALL LIZARD, PODARCIS SICULUS AND THE DALMATIAN WALL LIZARD, PODARCIS MELISELLESIS

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The Italian wall lizard, *Podarcis siculus* and the Dalmatian wall lizard, *Podarcis melisellensis* are two lizards from the family of *Lacertidae*. When this two species share same habitat, *P. siculus* outcompetes *P. melisellensis* as a dominant competitor, usually leading to extinction of *P. melisellensis*. We investigated the behavior of these two species of lizards, *Podarcis siculus* and *Podarcis melisellensis* in a new environment. In order to do so, we observed habituation period in the open field test and 8-arm radial maze. Habituation is an extremely simple form of learning, in which an animal, after a period of exposure to a stimulus, stops responding. We had 28 specimens of each species, 14 females and 14 males. Each experiment lasted between 15 and 23 minutes. Parameters of interest were: latency time, time spent in the central vs. marginal area and returning into hiding place. We tested for the behavioral differences between the species and sexes. After behavioral testing, the animals we sacrificed and levels of monoamines (5ht, DA, NA) in the brain was analyzed with HPLC. *P. melisellensis* showed bolder behavior, spending more time in open spaces, while *P. siculus* was more agile and found food quicker and in general learned faster than *P. melisellensis*. Consistent with literature, females were bolder than males. Specific traits correlated with neurotransmitter levels. Each species adapted in a different way to the same environment. We propose these two species of lizards as a good model for understanding the neurobiological basis of adaptive behavior, warranting further research.

IMMUNOHISTOCHEMICHAL STUDY OF REORGANIZATIONAL PROCESSES IN THE HUMAN FETAL CINGULATE GYRUS

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The transient fetal zones of the presumptive cingulate gyrus in humans differ from the rest of the medial telencephalic wall, suggesting some specificity in timing of the morphogenetic processes such as neuronal and glial migration, differentiation, afferent axon ingrowth and reorganization in this region. The aim of this study was to elucidate these regional specificity correlating spatial-temporal expression pattern of neuronal, glial and extracellular immunohistochemical markers among transient zones of particularly the future anterior and midcingulate region in comparison with other limbic areas and other regions of the telencephalic wall. Analysis included 27 post mortem fetal human brains, aged from 17 post conception weeks (PCW) to 40 PCW, stained by Nissl method, neuronal (MAP2), glial (GFAP) and extracellular neurocan (NCAN) and tenascin-C (TNC) immunohistochemichal markers. The MAP2 immunoreactivity in the cortical plate of the cingulate gyrus is visible before 20 PCW, but pyramidal-like cellular staining appears clearly just at 20 PCW. The early signs of lamination of the cortical plate of cingulate gyrus are seen at 33 PCW, coincidentally with the appearance of mature astrocytes morphology in the cortical plate shown by GFAP. The NCAN, a proteoglycan proven to play a number of roles in the brain development, is expressed in the cortical plate of the future cingulate region stronger than in the rest of the medial telencephalic wall before 22-23 PCW and at the beginning of late fetal period (33 PCW). By the end of gestation, distribution of the NCAN is the same in cingulate gyrus as it is in the rest of the medial telencephalic wall. Significant differences in TNC immunoreactivity between the presumptive cingulate region and other telencephalic areas were found. In the marginal zone expression of TNC was up-regulated during the mid-gestation in comparison to the other brain regions, while in the same period TNC expression in the subplate zone (SP) was down-regulated. The TNC immunoreactivity in the subplate zone (SP) shows decreasing gradient from deep to superficial, and differences in the timing and level of expression from anterior to posterior cingulate areas, appearing later in mid and posterior areas. Expressions of TNC and NCAN coincide in the intermediate zone from 17 PCW until 28 PCW and in the cortical plate from 20 PCW until 33 PCW. Altogether, these findings imply on one side involvement of these extracellular molecules in autocrine stimulation of astrocyte/oligodendrocyte differentiation and possible axon guidance for projecting subplate neurons, and on other side possible role in the ingrowth of afferents, that will be focus for further research of the insights from fetal human cingulate gyrus.

Keywords: limbic cortex, cortico-cortical connections, tenascin C, neurocan.

This work was supported by projects: Croatian Science Foundation (CSF):No.7379 and CSF:DOK-2015-10-3939, UNIZG-BM0054 and HIMRICO-ADRIS.

MUSCLE ACTIVATION IN BODYWEIGHT EXERCISES

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Our study examines the process of conditional skill development, maximal strength development using bodyweight exercises. Strength training with own bodyweight doesn't include external load, only uses the weight of the body in different positions, where the leverage between the supporting/hanging point and the center of mass of the body are changed. The most common strength training protocols use the percentage of maximal strength, with the one repetition maximum tested first. It is a simple method, when the trainer can calculate the quantity of the load (e.g.: 70% of 1RM) according to the specific training plan. That method can be easily used on weight training, but with bodyweight exercises, it is much more complicated, science exercises on different difficulty levels require different positions and movement patterns (e.g.: normal push up – one arm push up.). These different positions put joints in specific positions, so the exercises are similarly strengthening the muscles, but make them work in different ways. Also, it is hard to measure the actual load on the muscle. In our study, we aimed to measure the difference of forces created in muscles in push up variations, from easy to difficult exercises. To measure the peak load of the muscles, we used noraxon EMG on 12 muscles (Triceps Brachii, Biceps Brachii, Deltoid Anterior, Pectoralis Major, Rectus Femoris, Trapesius, Latissimus Dorsi, Erector Spinae, Rectus Femoris, Gluteus Maximus, Biceps Femoris, Gastrocnemius). The exercises were: 1. normal push up, 2. elevated feet push up, 3. diamond push up, 4. assisted one arm push up, 5. one arm push up. All participants did the push ups for metronome, the protocol included 8 seconds / 1 push up (4s on the negative, and positive part of the movement). There were 5 male participants, age between 22-28 (24,9) years. Our hypothesis was that differences between the measured muscle peak activations of different push up variations mirror the difficulty levels of the exercises. Although, there was no significant difference, our results suggests that there is a tendency in muscle peak activations and the difficulty levels of push ups are directly proportional to the exercise difficulties. The percentage distribution of muscle activations in the push up variations can be calculated. Our main goal is to increase the number of studied participants, because probably the low case number is the reason of the non significant differences. Changing the protocol might be also a solution, since participants need to perform these exercises in a very trained fashion with different speed and accuracy.

CROSS-FREQUENCY COUPLING IN THE HUMAN HIPPOCAMPUS

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Background: Cross-frequency coupling (CFC) of multiple neuronal rhythms could be a general mechanism used by the brain to perform network level dynamical computations underlying voluntary behaviour. It is known, in the hippocampus theta phase modulates gamma and HFO amplitude, but the hippocampal theta oscillations in humans have different properties compared with rodents. So, in the present study we analysed the general CFC characteristic in human epileptic hippocampus (Hc) during evoked events, in vivo.
Methods: We used laminar multi-electrodes to record local field potential (LFP) by cortical electrical stimulation (0.2 ms; 5-15 mA; 0.5 Hz). Hippocampal regions were reconstructed based on histological assessment: Cornu Ammonis 2-3 (CA2-3), Dentate Gyrus (DG), Subiculum (Sub). Phase-amplitude coupling (PAC) was calculated based on modulation index (MI) on 1 s window for control and evoked events.
Results: We analysed the interaction of frequencies between 10 to 1000 Hz grouped by CA2-3, DG, Subiculum. The regions show a slightly different coupling pattern (modulating/modulated frequency): 10-35 Hz/15-320 Hz (CA2-3); 10-56 Hz/47-475 Hz (DG) and 10-35 Hz/46-500 Hz (Sub). The volume of this effect is the following based on maximal MI values: 0.4 (DG), 0.25 (Sub) and 0.14 (CA2-3). Statistical analysis ($p<0.01$) resulted in a significant difference between active and background activity.
Conclusion: Our results demonstrate that phase-amplitude coupling is a prominent feature of the oscillatory LFP in epileptic hippocampus. These phase-amplitude modulations were distinct for different high-frequency bands modulated by slow gamma (10-50 Hz) and for different during evoked events and control, which similar to previous findings.

ROSEHIP CELLS: A NOVEL NEURON TYPE IN THE HUMAN CEREBRAL CORTEX

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Unbiased surveys of neocortical cell types provided insight about the overall number and diversity of cell types in rodents, but the cellular complexity of the human neocortex is relatively poorly understood. We set out to systematically analyse layer I of the human cerebral cortex in search for cell types absent from the rodent microcircuit.
We developed an initial database of whole cell recorded, biocytin filled interneurons in layer I of 350 µm thick slices of non-pathological human samples prepared from surgically removed pieces of parietal, frontal and temporal cortices. Light microscopic examination of each cell in the database identified neurons with previously described morphological features like neurogliaform cells. However, systematic analysis also revealed an emerging group of layer I interneurons having large, rosehip-like individual axonal boutons forming very compact, bushy arborizations confined to layer I (rosehip cells). Bouton volumes of rosehip cells were significantly larger than those of neurogliaform cells. Rosehip cells were immunopositive for cholecystokinin, however, none of these cells were positive for the CB1 cannabinoid receptor and these results were confirmed by single cell digital PCR. Anatomically identified rosehip cells responded to current injections with stuttering or irregular spiking firing pattern and membrane oscillations and firing of rosehip cells were tuned to beta and gamma frequency. Multiple whole cell recordings showed that local inputs to rosehip cells appear to be predominantly GABAergic, however, rosehip cells also received local excitatory inputs from layer II-III pyramidal cells sporadically. In turn, monosynaptic output connections triggered by identified rosehip cells rarely innervated interneurons and the output of rosehip cells were predominantly directed towards layer III pyramidal cells. Previous studies on rodent cortical interneurons containing CCK show functional presynaptic expression of the CB1 cannabinoid receptor, however, in line with our results of single cell digital PCR, application of the CB1 receptor antagonist AM251 was ineffective in modulating rosehip cell evoked IPSPs. Two-photon imaging combined with dual whole cell recordings showed that rosehip inputs simultaneous with backpropagating pyramidal action potentials were effective in suppressing Ca^{2+} signals only at sites which were neighbouring to the putative synapses between the two cells, no effect of rosehip cells was detected at dendritic sites relatively distal to putative synapses.
Our results identify a human neuron type absent from the rodent microcircuit with unprecedented morphological and neurochemical features. Rosehip cells specialize in providing tightly compartmentalized control of distal dendritic Ca^{2+} electrogenesis of human pyramidal cells enforcing inhibitory microdomains in dendritic computation.

INDIRECT REGULATION OF T-TYPE CALCIUM CHANNEL WITH MIRNA IN RAT BRAIN

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T-type calcium channels are important players in neural activity in the brain. They are in charge of pacemaking in thalamus and cerebellum as well as calcium signaling in various types of neurons. Expression levels of T-channels were shown to change during ontogenesis and in pathologies such as absence epilepsy or neuropathic pain. MicroRNAs (miRNA) regulate gene expression by interacting with target set of mRNAs leading to translational abruption and mRNA degradation. Up to date there are few miRNA studied to regulate ion channel expression and no such pathways known for calcium channels.

We look throughout miRNA databases and found miRNA rno-mir-1 to have two seeding sites on T-type channel $Ca_v3.2$ mRNA 3'-untranslated region (3'-UTR). Free energy of both sites was high enough to predict RNA-RNA interaction of these molecules (-19,4 kcal/mole and -16.6 kcal/mole). By means of RT-qPCR we measured levels of rno-mir-1 in rat thalamus and cortex area where $Ca_v3.2$ mRNA expression was simultaneously assayed. There were higher amounts of rno-mir-1 in samples where $Ca_v3.2$ mRNA levels were low and vice versa which indicated negative correlation between two RNA types.

To assay possible interaction in vitro we cloned rno-mir-1 into pSilencer vector and 3'UTR into pcDNA3 construct containing $Ca_v3.2$ cDNA. Both constructs were expressed in HEK293 cells in parallel with scrambled RNA vector controlled $Ca_v3.2$ channel expression. Functional testing of calcium current density by patch-clamp experiments showed no difference in miRNA expressing cells. According to these results we propose indirect regulation of $Ca_v3.2$ T-type channel expression with rno-mir-1 in rat brain.

FORSKOLIN-INDUCED LTP LEADS TO ENLARGEMENT OF POSTSYNAPTIC DENSITIES AND DENDRITIC SPINES THAT CONTAIN SPINE APPARATUS

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Synaptic strengthening is one of the molecular phenomena underlying long term memory formation. Long-term potentiation (LTP) of synapses leads to both structural and functional remodeling of the postsynaptic density (PSD). As PSDs of excitatory synapses are located on dendritic spines, changes in dendritic spine ultrastructure accompany synaptic remodeling. It is known that the size and strength of the synapse and the volume of the dendritic spine positively correlate with each other. One can also observe the presence or lack of SER in the dendritic spines. A specialization of SER that may be present is the spine apparatus. This organelle build from SER stacks is necessary for proper learning and memory formation to occur. In our study we have used 3D electron microscopy to visualize organotypic hippocampal slices that underwent chemically induced LTP (cLTP). Organotypic hippocampal slice culture (OHC) is an in vitro model of the hippocampal formation which in animals is responsible, among others, for spatial memory formation. The cLTP protocol (forscolin 50 μM, picrotoxin 50 μM, rolipram 100 nM) allows for most (if not all) of the synapses in the CA1 of the OHC to get potentiated. Therefore, it is possible to study structural plasticity at a level of population of dendritic spines.
In this study we have 3D reconstructed 256 dendritic spines from 8 OHCs (4 in cLTP and 4 in control - DMSO group) and performed stereological synapse counting in electron microscopy datasets from these slices. We have used serial block-face scanning electron microscopy (SBEM).Thus far, 3D EM employed in slice cultures was based on a labor-intensive serial sectioning transmission electron microscopy (ssTEM). SBEM is based on installation of an ultramicrotome inside a scanning electron microscope and allows obtaining 3D data of high quality relatively fast. Among other phenomena, we have found a specific increase of volume of dendritic spines that have spine apparatus and a profound increase of PSD volume among all types of dendritic spines. Noteworthy, the postsynaptic densities increased in volume relatively more than the dendritic spines. Overall, this study thoroughly characterizes the ultrastructural changes induced by cLTP.

ACTIVITY OF IDENTIFIED FAST SPIKING INTERNEURONS DURING DOWN-TO-UP STATE TRANSITIONS IN NATURAL SLEEP

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Slow wave sleep (SWS) is characterized by interchanging periods of up (active) and down (silent) states in the brain. Driven by multiple subcortical and intracortical inputs, a brief period (50-100 ms) of elevated activity characterizes the transition from down to up states (delta wave end - DWE) in the cerebral cortex. It is assumed that networks of excitatory and inhibitory neurons respond with firing at this transitional period, however, the activity of anatomically identified principal cells and interneurons is not known during the DWE in drug free animals. Here, we analyzed the activity of fast-spiking interneurons (FSIs) recorded and labeled in freely behaving animals (Averkin et al. 2016) during transitions from down-to-up states. These FSIs were classified based on their relative recruitment during spindle oscillations: descending phase related cells and spindle trough related cells. We found a sequential activation of these cell classes during DWEs. Spindle descending phase related cells fired first during DWEs at frequencies higher relative to other cells. Activity of spindle trough related cells peaked after DWEs with latencies of ~7 ms relative to spindle descending phase cells. We reported earlier that spindle trough related cells show differential high frequency activation pattern around the trough of spindle cycle (spindle ripple cells, spindle high gamma cells, simple spindle cells, complex cells), but ripple- and high gamma band modulation of these cell classes was absent during DWEs. We conclude that FSI subpopulations show a stereotyped activation sequence during down-to-up state transitions and sleep spindles. Extracortical inputs recruit spindle descending phase cells first in both sleep stages followed by the activity of spindle trough related cells which appear to be preferentially involved in local and sleep stage dependent generation of ripple and high gamma oscillations.

A MUTATION IN THE TRANSCRIPTION FACTOR ZFHX3 ALTERS ANXIETY AND CAUTIOUS-LIKE BEHAVIOUR IN MICE

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Introduction: The transcription factor ZFHX3 shows a highly localised expression pattern in adult mouse brain. Given its high expression in the hypothalamic circadian suprachiasmatic nucleus, we established that a mutation in the gene can lead to disrupted circadian behavioural rhythms. Interestingly, in addition to strong expression in the suprachiasmatic nucleus, ZFHX3 expression is also enriched in the central nucleus of amygdala (CeA), an important structure related to emotion, fear and anxiety. Therefore, we investigated whether ZFHX3 was associated with an anxiety-related phenotype.
Materials and Methods: To investigate the role of ZFHX3 in anxiety behaviour, we have used heterozygous (HET) female and male mice with a mutation in *Zfhx3* and compared them with wild-type littermates (WT). The use of the light-dark box and elevated-0 maze allowed one to assess aspects of anxiety in these mice.
Results: In females, no differences were found in the time spent in the light compartment of the light-dark box or in the open-arm of the elevated-0 maze. Conversely, HET male behaviours in these tests indicated a reduced anxiety relative to WT males. Upon further investigation, both female and male HETs showed a reduction in "cautious" behaviour toward the light part of the light-dark box compared to WT mice. Interestingly, WT females also showed an increase in "inhibition behaviour" toward the light part of the light-dark box compared to male WTs but, surprisingly, female HETs showed a reduction of this behaviour equivalent to that of HET and WT males (no difference was observed between male genotypes).
Conclusion: ZFHX3 plays a role in 1) anxiety in males only; 2) "inhibition behaviour toward anxiogenic area" in females only; 3) "cautious behaviour" in both sexes.
Ongoing studies: Anxiety, risk-taking and inhibition behaviours are known to implicate the CeA. To investigate whether ZFHX3 in the CeA plays a role in the behaviours mentioned above we are using the Cre-Lox system to delete *Zfhx3* in mouse CeA by viral vector injection. Subsequently, behaviours will be assessed in experimental and control groups. In parallel, we will investigate the cellular and molecular mechanisms and consequences of *Zfhx3* deletion in the CeA.

BENFOTIAMINE EXERTS ANTIINFLAMMATORY PROPERTIES IN ACTIVATED BV-2 MICROGLIA IN VITRO

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Aims: Microglia, the immune cells of the central nervous system (CNS), quickly activate in response to changes in tissue homeostasis and produce proinflammatory mediators. Although activated microglia has an important role in eliminating potential threats to the CNS, they often cause bystander damage to healthy neurons and other cells. Thus, suppression of microglial activation is a therapeutic strategy in neurodegenerative diseases. Benfotiamine (S-benzoylthiamine-O-monophosphate) is a synthetic analogue of vitamin B1 in use for treatment of diabetic neuropathies. Considering that benfotiamine shows antiinflammatory and antioxidative properties, the aim of this study was to examine its potential in suppressing microglial activation in vitro.
Methods: Experiments were performed on BV-2 microglial cell line activated with lipopolysaccharide (LPS), which is a well-established model of immune cell activation. BV-2 cells were pretreated with benfotiamine, activated with LPS (1 μg/ml) for 24 h and their viability, morphology and levels of proinflammatory markers were evaluated.
Results: Benfotiamine does not alter cell viability but suppresses morphological changes induced by LPS activation, reduces cell surface area and reorganization of actin filaments and presence of microprojections in activated microglia. Benfotiamine decreases levels of proinflammatory markers in activated BV-2 cells: it suppresses NO production by decreasing RNA and protein levels of iNOS, lowers RNA and protein levels of COX-2, and suppresses Hsp70. Analysis of signaling pathways showed that benfotiamine exerts its effects in part by decreasing Akt activation.
Conclusions: Benfotiamine shows immunosuppressive properties in activated microglia in vitro and should be examined further as a promising therapeutic for neurodegenerative diseases.

NOVEL, SOMATOSTATIN-MEDIATED PERIPHERAL ANTINOCICEPTIVE MECHANISM MEDIATED BY STIMULATION OF CAPSAICIN-SENSITIVE NOCICEPTORS

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Our previous research had uncovered that somatostatin released from capsaicin-sensitive nerve endings could exert systemic anti-inflammatory effects. Somatostatin is also known to have an antinociceptive effect, therefore the aim of the present study was to investigate whether stimulation of capsaicin-sensitive nerve endings had antihyperalgesic effects in rat models of nociception. Experiments were performed on Wistar female rats (150-200 g). Thermal hyperalgesia was induced by plantar incision, while neuropathic mechanical hyperalgesia was provoked by sciatic nerve injury. Nerves on the contralateral hind limb were transected to prevent the contribution of spinal and supraspinal modulatory mechanisms on the investigated response. The contralateral hind paw was stimulated by intraplantar injection of capsaicin or percutaneous application of mustard oil. For the investigation of the mechanism underlying the remote antihyperalgesic effect, prior desensitization of nerve endings or denervation of the contralateral paw was performed or animals were pretreated with antagonists of somatostatin (cyclosomatostatin i.p.) or opioid receptors (naloxone i.p.). In addition, somatostatin plasma levels were measured by radioimmunoassay 10 min after capsaicin application. Stimulation of capsaicin-sensitive nerve endings on the contralateral paw elicited a dose-dependent reduction of incision-induced thermal and nerve injury-induced mechanical hyperalgesia. The response was detectable 10 min and lasted at least until 40 min after treatment. The antihyperalgesic effect was prevented by desensitization or degeneration of nerve endings of the contralateral paw. Pretreatment with cyclosomatostatin inhibited the development of the antihyperalgesic effect nearly completely, while naloxone produced a partial reversal. A significant rise of plasma somatostatin levels was also detected after capsaicin injection into the contralateral paw.
In conclusion, our results showed that activation of capsaicin-sensitive nerve endings induced an antihyperalgesic effect on remote parts of the body via release of somatostatin and endogenous opioids. The novel modulatory mechanism is initiated at the peripheral nerve endings without the contribution of the central nervous system.

Supported by National Brain Research Programme A (KTIA_NAP_13-1-2013-0001), University of Pécs Medical School grant KA-2015-20 and EFOP-3.6.1.-16-2016-0004. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

ROLE OF THE TRPA1 RECEPTOR IN THE CUPRIZONE-INDUCED EXPERIMETAL DEMYELINATION MODEL

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Introduction: Cuprizone-induced experimental demyelination model is an accepted animal model of multiple sclerosis. A copper-chelating compound induces oligodendrocyte apoptosis, and demyelination with astrocytosis and microgliosis. Several exogen irritants and endogen reactive molecules can activate the Transient Receptor Potential Ankyrin 1 (TRPA1) receptor, which is expressed on primary sensory neurons and astrocytes. Our aim was to investigate the role of TRPA1 receptor in gene-deleted mice in the cuprizon model.

Methods: 8-week-old male wild-type (TRPA1 WT) and TRPA1 gene-deleted (TRPA1 KO) mice were fed with 0,2 % cuprizone for 6 weeks. In vivo tests (mechanonociceptive threshold, motor performance, spontaneous activity) and MRI experiment were performed on a weekly basis. Demyelination of the corpus callosum was evaluated using Luxol fast blue staining and electron microscopy.

Results: Cuprizone treatment induced marked demyelination in WT compared to TRPA1 KO mice. Morphological changes detected with MRI analysis revealed reduced myelin loss in gene-deleted animals, at each examined time point. Behavioral tests show no severe neurological deficit in the cuprizone-treated groups, but an increased exploratory behaviour was detected. Significantly increased rearing behaviour was induced by cuprizone treatment in WT mice, which was much less pronounced in TRPA1 KO mice.

Conclusions: We can conclude, TRPA1 receptors expressed on astrocytes promotes the cuprizone-induced myelin loss. TRPA1 antagonists might be potential therapeutic targets for demyelinating diseases.

Supported by the ÚNKP-16-4-I. New National Excellence Program of the Ministry of Human Capacities, KTIA_NAP_13-1-2013-0001, GINOP-2.3.2-15-2016-00048, EFOP-3.6.1.-16-2016-0004. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

MOTOR CORTICAL CONTROL OF THALAMUS PROJECTING INHIBITORY NEURONS IN THE BRAINSTEM

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Our previous data demonstrated that glycine transporter2 (GlyT2) positive inhibitory neurons in the pontine reticular formation (PRF) project to the intralaminar nuclei of the thalamus (IL). Selective optogenetic activation of PRF-IL fibers evoked immediate behavioural arrest for the duration of stimulation. The strong motor response was surprising given that PRF has been implicated in arousal but not in motor control. In this study, we aimed to examine the inputs of the PRF inhibitory cells possibly carrying motor signals and the impact of these inputs on the activity of PRF inhibitory neurons. Retrograde tracer injected into the PRF labelled L5 pyramidal cells located selectively in the cingulate and secondary motor (M2) cortices as well as neurons in the deep cerebellar nuclei. This connectivity is consistent with a possible motor function but inconsistent with the presumed role in arousal. Injections of AAV-DIO-ChR2-mCherry into the M2/cingulate cortex of RBP4-Cre/Glyt2-eGFP double transgenic mice revealed that the entire PRF receives L5 inputs. Within PRF cortical afferents contacted mostly thin distal dendrites. Cortical inputs to GlyT2 cells were confirmed at the EM level. Juxtacellular recording and labelling in PRF under ketamine/xylazine anaesthesia demonstrated that rhythmic activity of Glyt2 cells was tightly linked to slow cortical oscillation and was disrupted upon spontaneous desynchronization. Pharmacological inactivation of the cortex led to decreased irregular firing of the GlyT2 neurons, whereas photoactivation of M2 L5 cells evoked short latency action potentials with high probability in PRF. These experiments together indicate strong motor cortical control over PRF-GlyT2 cells. In in vitro preparation optogenetic activation of M2 fibers reliably produced purely glutamatergic synaptic responses in PRF-GlyT2 cells. Both AMPA and NMDA receptors were functional at these synapses, which showed non-depressive behaviour during stimulation trains. Our results indicate that synchronous higher order motor (M2) cortical activity can reliably activate inhibitory neurons of the PRF, thus likely convey a behavioural signal computed by frontal, motor cortical regions. PRF GlyT2 cells in turn transfer this signal to the IL thalamus affecting thalamocortical and thalamostriatal activity. These data suggest that the PRF could be involved not only in arousal but motor control as well.

CHARACTERISING MEDIAL PREFRONTAL CORTEX NEURONAL ENSEMBLE RECRUITMENT PATTERNS DURING APPETITIVE CONDITIONING

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Learned associations between food and the cues that predict their availability are encoded by a sparsely distributed set of neurons coined 'neuronal ensembles' in brain areas such as the medial prefrontal cortex (mPFC). This area contains excitatory pyramidal cells that control various appetitive behaviours, and their activity is modulated by local inhibitory interneurons. In laboratory animals, exposure to food-associated cues trigger conditioned appetitive responses (e.g. food-seeking) and activate both of these neuronal populations in this area, suggesting that associative representations are stored in these cue-activated neurons. To date, very little is known about how mPFC pyramidal cell and interneuron ensembles are recruited during the formation of appetitive (food-cue) associations.
Hence, the aim of this study was to examine pyramidal and interneuron recruitment patterns during the formation of food-cue associations in the dorsal mPFC, using a Pavlovian conditioning procedure with sucrose. To that end, we generated *Fos-GFP x GAD-tdTomato* transgenic mice that express GFP in strongly activated neurons, and the red fluorescent protein 'tdTomato' in interneurons. Thus, the activated pyramidal cells (GFP+/tdTomato-) and interneurons (GFP+/tdTomato+) are readily identified.
We imaged the dorsal mPFC using a microprism-based *in vivo* 2 photon (2P) imaging procedure that is better-suited for imaging deeper cortical structures located in brain fissures than conventional cranial windows 2P imaging methods. Investigations are underway into characterising the pyramidal cell and interneuron activation patterns during sucrose conditioning.

HORIZONTAL CELL SIGNALING IS MEDIATED BY CALCIUM-DEPENDENT VESICULAR GABA RELEASE IN THE MAMMALIAN RETINA

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Horizontal cells mediate feedback inhibition of photoreceptors, which is important in the generation of receptive field properties of early visual neurons. However, the cellular mechanisms regulating horizontal cell signaling in the outer retina are poorly understood. Our studies show that mammalian horizontal cell tips have small clear-core synaptic vesicles and they express a full complement of synaptic proteins including the vesicular GABA transporter (VGAT) and the calcium sensor, synaptotagmin-2. These cells also express SNARE proteins, including SNAP-25, VAMP, syntaxin and complexin, and they produce voltage-gated calcium channel currents. Together, these findings are consistent with calcium-dependent vesicular exocytosis of GABA from horizontal cell tips. Vesicle cycling in horizontal cell processes was tested using a synaptic vesicle-labeling assay. This assay uses a fluorescent, C-terminus directed VGAT (VGAT-C) antibody that binds to the luminal domain of VGAT when the vesicle fuses with the plasma membrane. Vesicle exocytosis and recycling were found to be depolarization- and calcium-dependent. To test whether horizontal cell vesicular release is involved in signaling to photoreceptors, inhibitory neurotransmission was eliminated by the selective deletion of VGAT using a horizontal cell Cx57-VGAT$^{-/-}$ mouse line. Calcium imaging of photoreceptor signals in response to depolarization allowed us to show that feedback inhibition of photoreceptor calcium channels was absent in the Cx57-VGAT$^{-/-}$ mice. This result supports the idea that vesicular release of GABA from horizontal cells is required for feedback inhibition of photoreceptors. Additional calcium imaging studies in wild-type mice and rats showed that the actions of GABAergic agents on feedback signals to photoreceptors were pH sensitive and inconsistent with direct inhibition by GABA of photoreceptor responses. To test if GABA acts indirectly, patch-clamp recordings of horizontal cells revealed muscimol activation of ionotropic $GABA_A$ receptors consistent with our finding of high levels of $GABA_A$ receptor subunit expression at horizontal cell tips. Since $GABA_A$ receptors have high bicarbonate permeability, receptor activation can regulate the pH in the photoreceptor synaptic cleft, resulting in a modulation of photoreceptor calcium channel activation. Current findings are consistent with a model of horizontal cell signaling by a GABAergic autocrine action at $GABA_A$ receptors to influence synaptic cleft pH and inhibition of photoreceptor responses.

NOVEL KINETIC MODEL OF $GABA_AR$ ACTIVITY BASED ON SINGLE-CHANNEL RECORDINGS REVEALS THE ROLE OF SPONTANEOUS GATING, SINGLY BOUND STATES AND THE IMPACT OF A_1F64 MUTATION AT THE ORTHOSTERIC BINDING SITE

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GABA type A receptors ($GABA_ARs$) play a major role in mediating inhibitory transmission in the CNS. They are pentameric, ligand-gated ion channels, most commonly composed of two α_1, two β_2 and one γ_2 subunit. In the past there have been a number of studies aimed at understanding the kinetics of $GABA_AR$ activity. Recent evidence indicates a need to include an intermediate step between binding and gating, namely flipping, in kinetic models describing $GABA_A$ receptor function. We found that the flipping transition is critically affected by mutation of the α_1F64 residue, which is located in the agonist binding site in a position of direct interaction with GABA. To get a further insight in the kinetic properties of $GABA_ARs$ as well as specific impact of α_1F64 mutation on receptor gating we have performed patch-clamp, single-channel recordings of activity evoked by a wide range of agonist concentrations as well as spontaneous activity of wild-type receptors ($\alpha_1\beta_2\gamma_2$) and $GABA_ARs$ with α_1F64 residue substituted with cysteine, alanine and leucine. We observed relatively frequent spontaneous short (1 ms) openings. On the contrary, increasing agonist concentration revealed WT $GABA_AR$ activity characterized by prolonged bursts with long openings and very brief closures (mainly below 1 ms). This type of activity was becoming more prevalent as [GABA] increased, which was not observed for α_1F64C mutants. Altogether, receptors with mutated α_1F64 residue had markedly different closed time distributions than it was observed for WT, similarly intraburst open times for saturating or high [agonist] were significantly shorter (WT > α_1F64L/A > α_1F64C). These differences were most apparent in the case of α_1F64C $GABA_ARs$. Subsequently, we performed model simulations, which suggested that flipping transition does not occur in the case of spontaneous activity but it is involved in the activity of singly and doubly bound receptors. The kinetic model that emerges from aforementioned analysis and simulations comprises spontaneous, singly and doubly bound receptor conformations which accurately reproduces activity of $GABA_ARs$ evoked by a wide range of GABA concentrations. Another important finding was that in mutated receptors both flipping and opening/closing transitions were affected, which suggests a major impact of α_1F64 mutation on $GABA_AR$ gating.

Supported by NCN grant DEC-2013/11/B/NZ3/00983 and ministry grant Pbmn135.

NANO-PHYSIOLOGY OF SMALL GLUTAMATERGIC AXON TERMINALS

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Axons broadcast action potential activity to synaptic terminals that translate this digital signal to analog postsynaptic responses in thousands of follower neurons. Albeit this digital/analog conversion constitutes practically half of the primary neuronal computation in the brain, its principles remain elusive. Our knowledge about the axonal signaling comes mostly from studies of axon terminals that have unusual morphology, such as the extremely large mossy fiber terminals (GMFT) of the dentate gyrus granule cells. However, similar insightful direct electrophysiology was not possible for small axons, which constitute the large majority of synapses of the CNS.
Using an improved electrophysiology now we gained direct access to and obtained direct recordings from small terminals of mossy fibers (sMFTs), whose size is equivalent of the majority of the cortical synapses and which are functionally segregated from the GMFTs also by their distinct postsynaptic target cell preferences. Because the sMFTs and GMFTs are formed on the same axons, we asked the questions (1) how size-dependent axonal mechanisms contribute to their distinct physiological functions, (2) whether axonal signaling is governed by the same principles in the small axon terminals as in the unusually large axonal structures and (3) what are the optimal conditions for direct patch clamp recordings from these small neuronal structures.
For these aims, we directly compared the better known active and passive membrane properties of the GMFTs with those of the sMFTs. Specifically, we determined the ionic currents that generate their specific spike waveforms and that serve as the functional link between the dynamic presynaptic activity and synaptic responses. Furthermore, taking advantage of the resolution of electrophysiology signals together with novel voltage-imaging methods we explored the technical limitations imposed by the small recording pipette in the small biophysical environment of sMFTs.
Thus, we showed that direct axonal recording provide unprecedented insights into general axonal signaling principles and hippocampal functions.

PRO-COGNITIVE EFFECTS OF MEMANTINE ARE POTENTIATED WITH α7-nAChR AGONIST PHA-543613 IN A SCOPOLAMINE-INDUCED TRANSIENT AMNESIA MODEL IN RATS

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Neurocognitive disorders (NCD) associated with progressive brain degeneration esp. in Alzheimer's disease (AD) and schizophrenia pose major public health issues worldwide. The NMDAR antagonist memantine and cholinesterase inhibitors (e.g., donepezil) are available for the treatment of dementia, however, these agents have only symptomatic effect, so it is important to develop new therapeutic strategies. The α7-nAChR is a promising drug-target for improving cognitive deficits in NCD. However, there is also an increasing interest in combination therapies, that have shown greater efficacy in many pre-clinical animal models than the monotherapies. In the present study we investigated the combined effects of memantine and α7-nAChR agonist PHA-543613 (PHA) at different doses, on spatial working memory of rats. Our aim was to get a better improvement in the cognitive performance of the animals by the co-administration of the two drugs then by mono-treatments.
The experiments were conducted with male Long Evans and Wistar rats in a scopolamine induced transient amnesia model. Spatial working memory of the animals were tested in a T-maze spontaneous alternation paradigm. Investigations started with dose range finding experiments to determine sub-effective and effective doses of memantine. In our first experiment with Long Evans rats, a lower dose of memantine (0.003 mg/kg) was combined with PHA (0.1 mg/kg). Memantine, when administered alone, did not improve significantly the alternation performance of Long Evans rats compared to scopolamine treatment, however, it was effective in combination with PHA. In the second experiment the higher dose of memantine (0.03 mg/kg) alone reversed the amnestic effect of scopolamine, but the combined treatment was not effective. In Wistar rats, memantine was also combined with PHA (0.3 mg/kg) in lower (0.1 mg/kg) or higher (0.3 mg/kg) doses. The 0.1 mg/kg dose of memantine significantly increased the alternation rate, however, the combined treatment did not further improve the performance of the animals. In contrast, memantine at the dose of 0.3 mg/kg did not reverse the scopolamine-induced performance deterioration, however, it successfully improved scopolamine induced memory deficit in combination with PHA.
To sum up, results suggest that the α7-nAChR agonist PHA enhanced the effects of memantine only when memantine was used at sub-effective doses. Since the antagonism of memantine on the α7-nAChR has been reported in several studies, we assume that the direct action of memantine on α7-nAChR may also play an important role in the mechanism of additive interaction between memantine and PHA. The additive effects of memantine and the α7-nAChR agonist may also be beneficial in reducing the dose of each substances and provide a more effective approach for the treatment of NCD.

REGULATION OF THE MOSSY FIBRE-CA3 CONNECTIVITY BY BCL11B/CTIP2

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The hippocampus is an important brain structure involved in spatial memory, learning processes and emotional behaviours. Granule cell neurons of the dentate gyrus form the primary gateway for information entering the hippocampus. These granule cells project their axons, the mossy fibres, toward the CA3 region where they form highly specialized synapses on large dendritic spines, the thorny excrescences of CA3 pyramidal cells. The hippocampal mossy fibre system has been well established to be critical for the processing of information in the hippocampus.
The zinc-finger transcription factor Bcl11b/Ctip2 is expressed throughout life in post-mitotic granule cells of the dentate gyrus, but is absent from CA3 pyramidal neurons. Previously, we demonstrated that Bcl11b/Ctip2 is a key regulator of postnatal hippocampal development. Forebrain-specific ablation of Bcl11b/Ctip2 results in defects in cell proliferation and differentiation of granule cells as well as an impaired organization of the mossy fibre tract. To determine the specific role of Bcl11b/Ctip2 specifically in the adult hippocampus, we use an inducible knockout system. The selective ablation of Bcl11b/Ctip2 in the adult forebrain results in a reduced number of thorny excrescences, ultrastructural changes of the mossy fibre bouton, as well as a strong and progressive loss of glutamatergic synapses in the CA3 stratum lucidum. Moreover, we observe a dramatic decrease of mossy fibre LTP in the CA3 and impaired spatial learning after mutation of Bcl11b/Ctip2 in adulthood. Together, our data provide strong evidences of the essential role of the transcription factor Bcl11b/Ctip2 for the regulation of the hippocampal mossy fibre-CA3 connectivity in adult mice.

CHEMOGENETIC STIMULATION OF BED NUCLEUS OF STRIA TERMINALIS MODULATES CONTEXTUAL FEAR MEMORY CONSOLIDATION

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A growing body of evidence highlights the differential role of bed nucleus of stria terminalis (BNST) and amygdala in anxiety-like behavior versus fear learning, respectively. This canonical functional dichotomy is challenged by emerging data indicating the involvement of the BNST in different phases of fear learning, such as fear extinction and stress-induced relapse of extinguished fear. Here we used a chemogenetic approach to study the role of BNST in anxiety-like behavior, as well as consolidation and generalization of fear memory in mice. We expressed the excitatory hM3Dq designer receptor exclusively activated by a designer drug (DREADD) in the BNST and stimulated with clozapine-N-oxide (1) during open field and social interaction tests; and (2) immediately after auditory fear conditioning to interfere with fear memory consolidation. Controls were mice expressing fluorophore viruses (no active hM3Dq) in the BNST. To dissect the differential role of BNST in fear learning, we measured both context- and cue-dependent fear recall, and generalization from one context to another. BNST stimulation increased social investigation time in the social interaction test (a pro-social/anxiolytic effect), but was ineffective in the open field test. Stimulation of the BNST during fear memory consolidation resulted in decreased fear memory recall in a context-specific manner. Namely, freezing was decreased by BNST stimulation when exposed to the shock context, but it was unaltered in a different context. Similarly cue-dependent fear recall and fear extinction characteristics were unaltered. In summary, our results indicate that BNST is involved in fear learning related to contextual elements, and modulates anxiety-like responses in a challenge-dependent way (i.e. highly stressful social challenge versus low-stress exploratory challenge).

NOVEL CARBON TIPPED SINGLE OPTRODE FOR COMBINED ELECTROPHYSIOLOGICAL AND ELECTROCHEMICAL RECORDINGS IN VIVO

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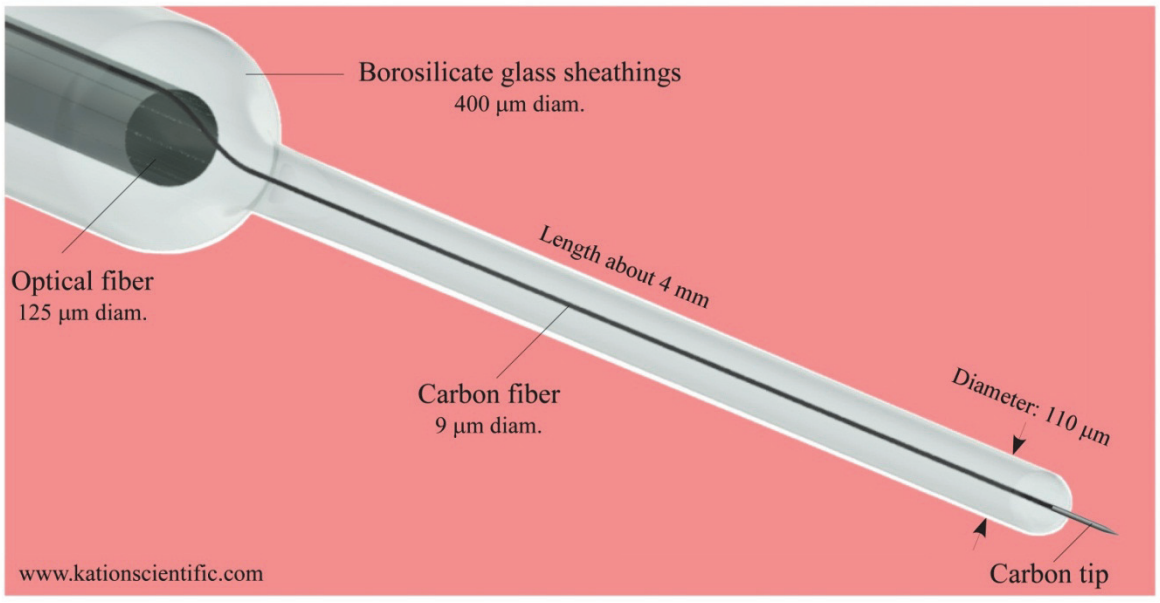
Optical electrodes (optrodes) in neuroscience are used to transmit light into the brain of a genetically modified animal to evoke and record electrical activity from light sensitive neurons. Most currently used single optrodes are bifurcated structures that may cause extensive tissue damage when inserted into the live brain and their metal tips are capable of recording spikes or field potentials only. Our novel solution integrates a light transmitting optical fiber and a lead element carbon fiber into borosilicate glass sheathings in a co-axial configuration what ends in a conical, micrometer-sized tip. The protruding carbon fiber allows recordings of neuronal spikes and field potentials as well as electrochemical or micro-biosensor signals.
A 125 µm quartz optical fiber and a 9 µm (diameters) single carbon fiber are pulled in borosilicate glass capillary tubing using a programmable puller. At sections of the pull, vacuum is applied to form a hermetic seal around the fibers. The pull is arranged so that carbon fiber runs along the optical fiber in the stem of the microelectrode and it extends over the optical fiber in the final few millimeters of the tip (see figure). Lastly, the tip is formed by controlled remove of the glass and by etching the protruding carbon fiber into a sharp tip at the required length. Light transmission is tested by measuring the power of light projected through the glass tip. Electrical performance is estimated by measuring impedances of the carbon tip.
On the top of the detailed description of this groundbreaking new optrode, application examples are also presented here. This includes in vivo recordings of light-evoked single unit spike activities from genetically transfected neurons and detecting responses to dopamine of the carbon tip in fast cycling voltammetry in vitro.
Our cutting edge single optrode may open up new avenues in optogenetics as the minimalized tissue damage and the function of carbon tip may permit never seen before combined recordings of electrical and electrochemical or micro-biosensor signals from the brain.



Acknowledgment: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

COMPARING RANDOM DOT STEREOTESTS WITH THE LANG TEST IN THE OPHTHALMOLOGICAL PRACTICE

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Introduction. Amblyopia, defined by poor visual acuity in one of the eyes is accompanied by a range of functional deficits and also compromises binocular vision. According to international literature, the currently available stereotests are not sensitive enough to detect anomalies leading to amblyopia. The aim of this research was to test the dynamic random dot stereotest E (DRDSE) in the clinical practice compared to the gold standard Lang II stereotest.
Methods. In this study, a total of 122 children (4 to14 years) were recruited from the pediatric ophthalmology outpatient clinic in Pécs, Hungary. The patients were either healthy or were diagnosed with one or more of the following eye conditions such as amblyopia, anisometropia, strabismus, hyperopia, myopia, astigmatism, and heterophoria. All of them went through a regular ophthalmologic examination and were also tested with the DRDSE.
Result. Altogether 97 children with one or more from the above mentioned eye disorders were included in the study. We examined the most common simultaneously occurring diagnoses, and put the patients into groups on this basis. Group No.1. included patients with astigmatism and myopia. Altogether 16 patients (16.4%) belonged to this group. Further groups were: astigmatism and hyperopia in 9.2% (n=9); hyperopia with convergent strabismus in 7.2% (n=7). The pass-fail ratio of these patients were examined with DRDSE and Lang II tests. The ‘astigmatism+myopia' group failed DRDSE in 32%, whereas Lang II only in 18%. The fail ratio in the ‘astigmatism+hyperopia' group was 66.7% in contrast with Lang's 33%, respectively. Finally, 100% of the ‘hyperopia+strabismus' group failed DRDSE, and only 85% of them did not pass the Lang test.
Discussion. Our data suggests that the DRDSE, compared to the well known Lang II stereotest, would be a more sensitive and reliable method for the screening of amblyopia or other eye disorders potentially leading to amblyopia.

This project was supported by the Hungarian Brain Research Program "KTIA_NAP_13120130001" and OTKA K108747, EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

EFFECTS OF CHRONIC STRESS ON NEW EXPERIENCE-INDUCED BRAIN ACTIVITY: DECREASED FOS EXPRESSION IN THE RAT BRAIN CORTEX

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Chronic stress produces deficits in learning and memory accompanied by alterations in neural activity. We assessed the effects of daily stress on new experience-induced brain activity. The adult male Long-Evans rats (250...350 g) were singly housed, maintained on a 12-12 h light-dark schedule with ad libitum access to food and water. On the first day all rats were assessed on open-field test. Then rats were exposed a single 5-min of inescapable electric footshock (15×10s, AC, 50 Hz, 1 mA) daily for 2 weeks ("stressed group") or left unhandled except for weighing during this period ("unstressed control group"). Both groups were placed in a novelty context for 5-min on the last day of experiment. We then compared numbers of Fos-immunoreactive cells in control unstressed and chronically stressed rats in 1 hr 30 min after of 5-min novelty exposure. c-Fos protein detection was performed with the well-established immunohistochemical diaminobenzidine technique in the retrosplenial and motor cortices; the posterior, anterior and medial portions of the paraventricular nucleus of the thalamus; the basomedial, basolateral and central amygdaloid nucleus. These areas were chosen based on prior our reports of their Fos reactivity to acute stress (Bulava et al., 2016). In animals previously exposed to two-week period of intermittent electric footshock we found that Fos expression in the retrosplenial and motor cortices was significantly lower as compared to unstressed rats (Mann-Whitney $z = 2.3$, $p = 0.02$; Effect size $r = 0.81$). This is effect was higher in animals that showed reduced stress resistance in the «open field» test on the first day of experiment. Thalamic and amygdalar nuclei had similar patterns of Fos induction in both groups.
The results indicate brain system reorganization due to the chronic stress, which probably causes functional changes in brain cortex and may contribute to stress-induced changes in learning and memory.

This research was supported by RSF grant #14-28-00229.

AUDITORY PROCESSING DURING SLEEP SPINDLES

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Sleep spindles are 7-14 Hz oscillations occurring most frequently in stage II. sleep. They are hypothesized to be involved in memory consolidation, as well as having a sleep protective function, though their overall role is still unclear. Also, while the basic cellular mechanisms of spindle generation are relatively well known, the cortical population patterns elicited by spindles are unexplored, largely due to the large variability between individual spindle events. To overcome this obstacle, we analyzed spindles evoked by sensory simuli, that may be taken as a fixed point of analysis.
Recently we found that under urethane anesthesia auditory stimuli can evoke sleep spindles in a stimulus specific manner. Moreover, population activity during spindles contains information about the ongoing stimulus, but the representation is markedly different from activity during stimulus induced slow waves. In our current work, we extend these experiments to chronically implanted, freely moving/sleeping rats. We find that out results hold under natural sleep, confirming sleep spindles as a new type of information encoding packet.

CHARACTERIZATION OF PUPILLARY RESPONSE IN „SCHIZOPHRENIA-LIKE" (WISKET) RATS

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Patients with schizophrenia show significant alterations of autonomic regulation including pupillomotor control. The aim of this study was to reveal the potential alterations in pupillary light reflex (PLR) in anesthetized animals of a new "schizophrenia-like" substrain (named WISKET) showing behavioural disturbances related to schizophrenia.
Methods: Two experimental groups of male rats were studied: naive WISTAR rats without any treatments (n=14) and WISKET rats (n=20) after postweaning social isolation and subchronic ketamine treatment for between age of 4-7 weeks. At the age of 3 months animals were anaesthetised with **chloral-hydrate** (200 mg/kg ip.) and accommodated to dark for 10 minutes. The direct response of PLR was recorded with a modified digital camera (Nikon D700) under infrared illumination. Intensive light stimulus was applied into the eye with an infrared flashlight. For off-line analysis an evaluation algorithm was developed as a custom written program in MATLAB MathWorks Software R2015a. In order to correct for any variabilities in the distance from the camera to the animal's eye, the relative pupil diameter was determined as the ratio of the iris' diameter (relative diameters, expressed in %).
Results: The initial and the redilated pupil diameters did not differ between the two groups, however the minimal pupil diameter was significantly larger in WISKET rat compared to control rats. The amplitude and the degree of the constriction showed close to significant differences between the two groups. Other contraction parameters did not show alterations between groups, although the variance of duration of contraction was higher in WISKET rats. The dynamic redilation parameters showed slower trend in WISKET rats compared to control animals.
Discussion: In conclusion, the WISKET animals revealed some alterations in the direct pupillary response for light stimulus, suggesting an imbalance in the autonomic nervous system. Thus, WISKET rat substrain might be an appropriate model for simulating either behavioural or autonomic symptoms.

POSTERIOR HYPOTHALAMIC „TIMING CELLS" ACTIVITY AS A POSSIBLE MECHANISM OF FREQUENCY PROGRAMING OF HIPPOCAMPAL THETA RHYTHM IN RATS

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Hippocampal formation (HPC) theta rhythm is one of the best examples of neural synchrony in the mammalian brain. HPC theta field potentials in rats consist of high-amplitude (up to 2 mV), almost sinusoidal waves in 3-12 Hz frequency range. It is well-known that the pathway of theta generation originates in the nucleus reticularis pontis oralis (RPO), then RPO projects to the supramammillary nucleus (SuM) of the posterior hypothalamic region (PHr), and finally through the medial septal area (MS) to HPC and other limbic structures. This tract is called the ascending brainstem-hippocampal synchronizing pathway.
It has been established that HPC theta frequency is paced by the MS region but the PHr is also known to be partially involved in the process at least during immobility. Recently, it was shown that the posterior hypothalamic nuclei not only act as a rely/modulator in the ascending synchronizing system but can also generate local theta oscillations independently of the HPC (in vivo and in vitro studies). Furthermore, theta-related cells in the PHr were found to be similar types to those found in the HPC. In addition, a new type of cells has been found in the posterior hypothalamic region and based on its very regular pattern of firing and possible pacemaker role these cells were termed "timing".
Forty four in vivo experiments were performed using 44 anesthetized Wistar rats and 56 in vitro experiments were conducted using brain slices taken from 56 Wistar rats. Theta rhythm and accompanying cell discharges were induced by carbachol (cholinergic agonist) administration. Recordings were performed using a single electrode located in the SuM nucleus of the posterior hypothalamus. The relation of neuronal firing to local field theta rhythm was investigated according to an earlier developed cell discherge classification for the HPC.
Thirty seven theta-related neurons were recorded in vivo and 14 theta-related neurons were recorded in the in vitro condition. A new neuron type termed "timing cell" was recorded: ten times in vivo and 27 in vitro. These cells demonstrated a very regular pattern of discharges in a steady frequency in the theta band (3-12 Hz).
Gathered experimental data suggests that theta-related neuronal activity recorded in the posterior hypothalamic region resembles well-documented patterns of theta-related cell discharges in the hippocampal formation maintained in in vitro and in vivo conditions. The role of posterior hypothalamic "timing cell" activity is discussed regarding hippocampal theta rhythm frequency programing.

[Supported by NCN grant 2013/11/B/NZ4/04872]

COORDINATED GAMMA OSCILLATIONS IN THE LATERAL SEPTUM AND THE LATERAL HYPOTHALAMUS DRIVE FOOD SEEKING

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Cortical cognitive processing involves gamma oscillations, which support memory, attention, cognitive flexibility and sensory responses. These functions crucially contribute to feeding behavior, however, the underlying neural mechanisms are unknown. Lateral hypothalamus (LH) is crucial for regulation of feeding, yet little is known about the regulation of LH by top-down inputs from cognitive control regions. Top-down forebrain innervation of LH is provided, to a large extent, by inhibitory inputs from the lateral septum (LS), a key region for governing innate behaviors according to environmental context; LS is connected, in turn, with cortical networks. Here we combined optogenetics and electrophysiological high-density recordings in mice during spontaneous behavior in a free-access feeding paradigm. We found that LS and LH displayed prominent gamma oscillations (30-90 Hz) which entrained neuronal activity within and across the two regions (Carus-Cadavieco et al., Nature, 2017). When mice engaged in approach to the food zone, the power of gamma oscillations in LS and LH matched the time required to reach the food zone, but not the drinking zone. Optogenetic gamma-frequency stimulation of somatostatin-positive (LS-SST) projections to LH facilitated food-seeking, i.e. shortened latency to reach the food zone but not the drinking zone or a control zone. It also increased probability of entering the food zone prior to food-free zones, located in other corners of the enclosure. Optogenetic inhibition of the LS-SST-LH pathway during food approach reduced food seeking. LS inhibitory input enabled separate signaling by LH neurons according to their feeding-related activity, making them fire at distinct phases of the gamma oscillation. In contrast to increased food intake during optogenetic stimulation of LH Vgat cells, food intake during gamma-rhythmic LS-LH stimulation was not changed. Accordingly, we identified that LS-LH gamma-rhythmic input regulate activity of function-selective subgroups of LH cells in a different way than activation of LH Vgat neurons. Upstream, we identified medial prefrontal cortex projections providing gamma-rhythmic inputs to LS, leading to improved performance in a food-rewarded learning task. Overall, our work identifies a novel top-down pathway, which utilizes gamma synchronization to guide activity of subcortical networks and to regulate feeding behavior by dynamic reorganization of functional cell groups in hypothalamus.

PROTEIN LEVEL OF CHEMOKINE SDF-1α IN HIPPOCAMPAL ORGANOTYPIC CULTURES IS DOWN-REGULATED AFTER PRENATAL STRESS PROCEDURE AND MODULATED BY THE ANTIDEPRESSANT DRUGS

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Introduction: The disturbances of cytokines and chemokines expression in the central nervous system may be involved in pathological processes leading to mental disorders, including depression. Among these changes, the level of CXCL12/SDF-1α (stromal cell-derived factor-1 alpha) is crucial both during brain development and in the adult brain, due to its ability to influence migration, differentiation and proliferation of neuronal and glial cells, thus, its proper action contributes to maintaining homoeostasis in the brain.
Aim of the study: The aim of present study was to determine the impact of prenatal stress procedure (the animal model of depression) on the protein level of SDF-1α in hippocampal organotypic cultures. What is more, the effect of lipopolysaccharide (LPS) stimulation on above-mentioned parameter was evaluated. We also examined the influence of antidepressant drugs (venlafaxine, tianeptine) on the SDF-1α level in those conditions.
Materials and methods: Pregnant Sprague-Dawley rats were subjected daily to three sessions of restraint stress from the 14^{th} day of pregnancy until the delivery. Control pregnant rats were left undisturbed in their homecages. Hippocampal organotypic cultures were prepared from 7-8 day old animals. Hippocampi isolated from control and prenatally stressed rats were sectioned into 350 μm slices and transferred onto porous membrane inserts of 6-well culture plates. Cultures were maintained for 7 days. Then, slices were stimulated with the drugs (tianeptine at the concentration of 10 μg/ml, venlafaxine at 1 μg/ml) for 30 minutes and then treated with LPS (1 μg/ml) for 24 hours. The protein level of SDF-1α was measured using commercially available ELISA kit.
Results: The obtained data showed that the prenatal stress procedure significantly decreased the protein level of SDF-1α in hippocampal organotypic culture. The stimulation with LPS caused higher secretion of SDF-1α in both examined groups. However, the SDF-1α level after LPS treatment was lower in hippocampal organotypic cultures obtained from prenatally stressed animals comparing to the control group. Both used drugs regulated changes in the SDF-1α level caused by prenatal stress, as well as LPS treatment.
Conclusions: To sum up, our results concerning the SDF-1α level indicate that prenatal stress may have a significant influence on chemokine homoeostasis in the hippocampus, and consequently for a proper functioning of the central nervous system. What is more, prenatal stress has an impact on the sensitivity of hippocampus to LPS stimulation. It may be suggested that the therapeutic efficacy of the antidepressants is related to normalisation of some chemokines levels in the animal model of depression.

This research was supported by the grant no. 2013/09/B/NZ7/04096, National Science Centre, Poland.

MODELLING THE EFFECT OF LPS AND MINOCYCLINE ADMINISTRATION WITH 1H-NMR METABOLOMICS

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Recent research suggests that inflammation may be involved in the pathophysiology of psychiatric disorders. The use of anti-inflammatory compounds, such as minocycline, in the treatment of mental illness, therefore, has gained notable interest. However, the mechanisms underlying the psychotropic properties of minocycline remain elusive.
^{1}H-NMR spectroscopy has proven to be a useful tool to differentiate between healthy and disease states through the analysis of metabolic profiles. Hence, we are interested in investigating if peripheral administration of an inflammatory agent or minocycline alone would result in detectable changes in polar brain metabolites, and whether minocycline administration would attenuate inflammation-related metabolite changes.
Methods: Male CD1 mice received either a single or repeated (7days) intraperitoneal injection of minocycline (50ug/kg) or saline. Brain tissue and plasma were harvested two hours after the single injection. Animals that had received repeated injections of minocycline also received an injection of LPS or saline on the 7th day, and samples were collected 24 hours later.
Polar metabolites were extracted with perchloric acid, and NMR samples were prepared by dilution in phosphate buffer (D_2O, pH7.4). NMR spectra were obtained with a Bruker AVIII 700MHz spectrometer, and processed with MestreNova. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to determine differences in the metabolic profiles between treatment groups.
Results: An acute dose of minocycline resulted in detectable metabolite changes in plasma, but not in brain. The plasma model generated by OPLS-DA was significantly more accurate at predicting which treatment group a test sample belonged to compared to a randomly generated model (accuracy +23.3%, $p<0.05$). The key variables identified in building this model are peaks associated with lipoproteins.
In the repeated minocycline injection study, OPLS-DA modelling separated the LPS-alone group (Sal/LPS) from control (Sal/Sal) in both the hippocampus (accuracy 67.1%) and the frontal cortex (accuracy 85.3%). The key variables identified are peaks associated with glutamine and glutamate. Repeated minocycline injections did not result in detectable metabolite changes in brain. Unlike the Sal/Sal and Sal/LPS groups, significant differences were not detected between the minocycline-alone (Mino/Sal) and both-treated (Mino/LPS) groups, suggesting that minocycline may have an interaction effect with LPS.
We have shown brain metabolite changes following a single peripheral injection of LPS. While we have yet to detect significant changes in polar brain metabolites after acute and repeated injections of minocycline, the lack of difference between the Mino/Sal and Mino/LPS groups suggest that minocycline is having an effect. As minocycline-related changes in plasma were associated with lipoproteins, it is possible that minocycline mainly affects lipid-phase metabolites.

INVESTIGATING THE ROLE OF VIP+ INTERNEURONS IN LEARNING-RELATED PLACE CELL DYNAMICS IN HIPPOCAMPAL AREA CA1

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Decades of accruing evidence have landed strong credence to the idea of the hippocampus being an indispensable part of the episodic memory formation, storage and retrieval processes. Specifically, in the CA1 subregion a substantial percentage of the pyramidal neurons, now called place cells, exhibit a firing pattern strongly modulated by environment location, thus acting as neural anchors of spatial memories. However, in the brain, no player is acting alone, and it is no surprise that pyramidal cells are tuned by a wide variety of local-network interneurons. Among those, interneuron-targeting, disinhibitory interneurons, expressing vasoactive intestinal polipeptide (VIP) have recently been proposed to play an important role in mice spatial learning behavior during a goal oriented task. It is hypothesized that one mechanism by which VIP cells affect spatial learning is the alterations in place field properties.
To examine this hypothesis we developed a biophysically constrained network model of the CA1 region that consists of 100 cells. More specific, the network includes 80 pyramidal cells and 20 interneurons. Specifically, there are five classes of interneurons, namely basket, bistratified, axo-axonic, OLM and VIP. All neuron models were validated against experimental data regarding basic electrophysiological, connectivity and input properties. To simulate place cell formation in the network model, we generated grid cell input from the Entorhinal Cortex (EC) and the CA3 regions, activated in a realistic manner as observed when an animal transverses a linear track. Some of the data used to simulate the grid-like inputs are taken from in vivo experiment, such as the animal's speed and the path it follows. Realistic place fields emerged in a subpopulation of pyramidal neurons (10-20%), in which similar EC and CA3 grid cell inputs converged onto distal/proximal apical and basal dendrites. The tuning properties of these cells are very similar to the ones observed experimentally in awake, behaving animals.
Ongoing work aims to assess the role of VIP interneurons in the formation and/or tuning properties of place fields. Towards this goal, we will selectively remove connections for VIPcells onto basket cells and VIP cells onto OLM cells, as well as we will lesion the whole VIP population. Given the lack of experimental data on the precise role of VIP cells in spatial memory, our modeling manipulations will provide new predictions as to the mechanistic effects of these neurons at the cellular level. These predictions can in turn guide experimental testing that will ultimately reveal whether VIP cells contribute to the formation and learning related reorganization of place cells via their disinhibitory effects on somatic and/or dendritic inhibition.

REVEALING THE PATHOLOGICAL MECHANISM OF ALZHEIMER'S DISEASE-LINKED APP D678H MUTATION USING INDUCED PLURIPOTENT STEM CELL

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Alzheimer's Disease (AD) is the most common form of dementia, primarily caused by accumulation of amyloid plaque in the brain. The main component of amyloid plaque is Aβ, which is produced by sequential cleavages of amyloid precursor protein (APP) by β- and γ- secretases. APP is a transmembrane glycoprotein that is highly expressed in the brain. The mutations of APP usually increase the Aβ production and/or aggregation of Aβ, thus cause early onset of AD. To date, more than 50 different mutations in APP gene have been identified. The APP D678H mutation that causes early onset of AD was identified from two Taiwanese families. In this study, the pathological mechanism of this mutation was examined using patient-derived induced pluripotent stem cells (iPSC), which is more closely related to the human physiological condition. To generate proper control, CRISPR/CAS9 system was used to edit the APP mutation back to normal as an isogenic control. Human iPSCs were differentiated into excitatory neuron by introducing Ngn2 using the lentiviral system. Neuron-like morphology could be observed in less than one week and mature neuron morphology in around 14 days. Both calcium imaging and electrophysiology study indicated that these iN generated action potential after 21 days. In these iNs, the increase levels of Aβ and p-tau could be detected in the presence of APP D678H mutation. The change of APP processing and synaptic dysfunction is under investigation.

ALTERATIONS OF FUNCTIONAL CONNECTIVITY IN ALEXITHYMIC PERSONALITY TYPE DURING A RESTING STATE

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The alexithymia construct is characterized by impairment of emotional processing and reduced interaction between different brain areas during various experimental conditions. Yet little known about permanent alteration of functional connectivity associated with alexithymia in resting state. The aim of current study was to investigate the resting state cortical networks of alexithymic personality type. 232 volunteers, first-third year students from the Taras Shevchenko National University of Kyiv aged 18 to 24 years participated in this study. EEG was registered during the rest state (3 min). We estimated the interhemispheric and intrahemispheric average coherence across all EEG segments in all frequencies from 0.2-45 Hz. To determine the level of alexithymia we used 26-item Toronto Alexithymia Scale (TAS-26). Alexithymic personality type was found in 43 volunteers (TAS-26 total score ≥74, alexithymia group, AG). A control group consisted 113 subjects with low alexithymia (TAS-26 total score ≤62, non-alexithymia group, NAG). 85 participants formed intermediate group (IG, TAS-26 total score 62> and <74). In background EEG activity during the development of the alexithymia variations in EEG spatial synchronization were observed in low- and high-frequency EEG components. Alexithymic personality type includes breaking of interhemispheric anterior frontal-frontal (alpha1,2-subband) and formation central-temporal links (alpha1-subband) (awareness and cognitive processing of incoming information). We demonstrated left lateralization of intrahemispheric links in alpha3 (occipital-parietal area) and beta (central area) subbands (inner image formation, external attention). Inter and intrahemispheric coherence in low-frequency EEG components (theta2-subband) indicates the influence of alexithymia on attention focusing, working memory, and emotional processes. As such, topographical reorganization of functional connectivity under alexithymia had specific features reflecting information and emotion-activating processes.

SPATIAL MEMORY IMPAIRMENT AND HIPPOCAMPAL CELL LOSS INDUCED BY OKADAIC ACID

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In the present study, we evaluated and compared effect of intracerebroventricular (ICV) and intrahippocampal bilateral microinjection of okadaic acid (OA) on spatial memory function assessed in one day water maze paradigm and hippocampal structure in rats. Rats were divided in following groups: Control(icv) - rats injected ICV with aCSF; Control(hipp) - rats injected intrahippocampally with aCSF; OAicv - rats injected ICV with OA; OAhipp - rats injected intrahippocampally with OA. At the end of the behavioral experiments OA treated and control rats were deeply anesthetized with pentobarbital and perfused through the ascending aorta with 300 ml saline followed by 600 ml 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4)). The surviving pyramidal cells in the hippocampus of rats were visualized by Nissl staining. The number of the hippocampal pyramidal cells in Nissl staining sections was counted at X 400 magnification. Nissl staining of hippocampal sections showed that the pyramidal cell loss in OAhipp group is significantly higher than that in the OAicv. The results of our behavioral experiments showed that all rats exhibited a decreased latency to find the hidden platform across the eight training trials and OA treatment did not affects probe-test performance 30s after training. In marked contrast, the present experiments indicate that OA treatment affects probe-test performance 24 h after training. These findings suggest that OA treatment did not affect learning process and short-term spatial memory but induced impairment in spatial long-term memory. OA-induced spatial memory impairment may be attributed to the hippocampal cell death. Based on these results OA induced memory deficit and hippocampal cell loss in rat may be considered as a potential animal model for preclinical evaluation of anti-dementia drug activity.

2D AND 3D CA2+ IMAGING COMBINED WITH ELECTROPHYSIOLOGY OF HIPPOCAMPAL NEURONS IN VIVO

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Hippocampal rhythms are driven by interactions between excitatory pyramidal cells and inhibitory interneurons. During network activities synaptically activated dendritic segments may participate in the formation of the neuronal engram.
In our previous studies, we demonstrated the existence of dendritic regenerative activities in fast spiking parvalbumin interneurons in vitro. Until now, the dendritic integration mechanisms of functionally working hippocampal neurons remain elusive in vivo, partially because of the difficulties of complex imaging and electrophysiological technics. Here, hippocampal in vivo two-photon imaging and simultaneous ipsilateral hippocampal multi-channel recordings will be presented. Using in vitro and in vivo 2D and 3D random-access point scanning methods together with in vitro glutamate uncaging and pharmacological manipulation we show complex 3D Ca2+ events at different subcellular regions of hippocampal neurons. We also show the dependence of these events on voltage-gated Ca2+ channels in vitro.
We developed new surgical and imaging technics combined with electrophysiology in order to understand the mechanisms of hippocampal coincidence detection and neuronal functions during input-output formation and conversion in vivo in deep brain areas.

THE INVESTIGATION OF 3 SYNAPTIC INPUTS ON FROG SPINAL MOTONEURONS BY DIFFERENT METHODS INCLUDING 3D COMPUTER RECONSTRUCTION OF THE INDIVIDUAL CONNECTIONS.

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The motoneuron is the final common path for the control of skeletal muscle contraction; thus it integrates information from various sensory, supraspinal and intraspinal pathways. In this work we compare the data of different structural and functional parameters of lumbar motoneuron control in frogs. Monosynaptic EPSPs were recorded in three lumbar motoneurons after intracellular stimulation of dorsal root afferent, reticulospinal and propriospinal fibers. HRP or neurobiotin filled microelectrodes were used both for fiber stimulation and EPSPs recording in the isolated brainstem-spinal cord preparations. The mean values of EPSP amplitude were 154, 735 and 830 uV respectively.
Then, histochemical procedures, light microscopic investigation and 3-D computer reconstructions (Eutectic Neuronal System) of serial sections were made. Quantal analysis of transmitter release in each one of afferent fiber-motoneuron pairs was used to estimate the release parameters in three labeled synapses. The comparison of functional and structural characteristics of these contacts showed a rather close correspondence between the number of contacts and the number of binomial units.

Supported by the grants: NSF - 31-45729.95, the RFBR project 15-04-05782 and the BR Program of RAS Presidium.

GENETIC RISK AND PROGNOSTIC MARKERS IN PATIENTS WITH MULTIPLE SCLEROSIS

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Background: We still do not know why in some patients with Multiple Sclerosis (MS), disease course is „benign“ and in the others, it is „malign“. The individual differences in MS susceptibility and disease course are probably the result of the interaction of environmental, genetic and epigenetic factors. Vitamin D has a large scale of immunomodulatory and antiinflammatory effects and its role in MS is still not completely clear. Interleukin 7 receptor alpha chain (*IL-7Ra*) can be involved in etiopathogenesis of MS by liganding of interleukin 7, a cytokine involved in T-cell activation during autoimmune reaction.
Aims: The aim of our study was to analyse the association of several single nucleotide polymorphisms in vitamin D receptor (*VDR)* gene and *IL-7Ra* gene with the risk and disability progression of MS.
Material and methods: The *VDR* gene polymorphisms TaqI, ApaI, BsmI, FokI and *IL-7Ra* gene polymorphism rs6897932 were analysed in a group of 270 MS patients and 303 healthy controls from central and northern regions of Slovakia. Genotype determination was performed by the restriction analysis. The disease disability progression was evaluated using Multiple Sclerosis Severity Score (MSSS).
Results: As the risk factors of MS development, we identified allele C and genotype CC of polymorphism rs6897932 in *IL-7Ra* gene and genotype Ff of rs10735810 (FokI) in *VDR* gene. Allele T of rs6897932 in *IL-7Ra* gene (in both genotypes, CT and TT) and genotype BB of rs1544410 (BsmI) in *VDR* gene were identified as protective factors against MS development. As the risk factor of rapid MS disability progression, we identified allele C and genotype CC of rs6897932 in *IL-7Ra* gene and number of copies of haplotype t-A-B-F (C-T-A-C) in *VDR* gene. The protective effect against rapid disease disability progression was observed in carriers of allele T of rs6897932 in *IL-7Ra* gene, especially in individuals with genotype TT.
Conclusions: The results of our study point out the role of genetic factors in the modulation of the MS susceptibility and disease course. The panels of these markers together with other already used clinical markers could be used in individual management of MS patients, especially in genetically predisposed individuals.

The work was supported by the grants APVV-15-0107, VEGA 1/0287/16 and MZ SR 2012/30-UKMA-7.

SLEEP SPINDLES AS AN EARLY BIOMARKERS OF AGING DURING NEURODEGENERATION IN RAT

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Our previous study has shown that the sustained sigma amplitude augmentation within the motor cortex during Rapid Eye Movement (REM) sleep presents the earliest sign of aging in the rat model of Parkinson's disease cholinergic neuropathology. Sleep spindles, as the hallmarks of non-rapid eye movement sleep, are the EEG oscillations in sigma frequency range (10.1-15 Hz) associated also with epilepsy, schizophrenia, stroke, brain injury, learning and memory, and neuronal plasticity. Since the sleep spindles result from interactions of the thalamic reticular, talamocortical, hippocampal and cortical neurons, their characteristics may reflect the integrity of these circuits or serve as the biomarkers of their dysregulations.
The aim of this study was to analyze all possible differences between the control spindle activity and spindle activity in the bilateral pedunculopontine tegmental nucleus (PPT) cholinergic denervation during REM. We analyzed the differences in spindle density, duration, and frequency, in order to explain the new REM phenomenon (REM enriched with sigma activity) evidenced in our former study.
We performed the experiments in two experimental groups of adult Wistar rats, chronically instrumented for sleep recording: the physiological controls, and the bilateral PPT lesioned rats. During operative procedure for the EEG and EMG electrodes implantation, under ketamine/diazepam anesthesia, the PPT lesions were done by microinfusions of 100 nl 0.1 M ibotenic acid. We recorded spontaneous sleep in 4.5 months old rats, and after conventional amplification and filtering, the analog signals were digitized (sampling frequency 256/s). We applied Fourier analysis using MATLAB 6.5, and differentiated REM 10 s epochs in all 6 h sleep recordings. In order to identify the spindles we have combined the automatic spindle detection with visual validation of detected spindles and their exctraction. Namely, the first step for automatic spindle detection was to filter the EEG signals between 11 Hz and 17 Hz band, for further implementation of a algorithm using a complex Morlet wavelet. For the analysis of spindle dynamics we used the spindles with minimal duration of 0.5 s. Statistical analysis was done using Kruskal Wallis ANOVA with post hoc Mann Whitney U test.
We have evidenced the augmentation of spindle density of different pattern in the motor cortex during REM in 4.5 months old rats with PPT cholinergic neuronal loss ($z = -2.12$; $p = 0.03$). In addition to the higher density of sleep spindles, the bilateral PPT lesion prolonged their duration and reduced their intrinsic frequency ($z \geq -10.45$, $p \leq 10^{-3}$).
We have demonstrated in young adult Wistar rats with impaired PPT cholinergic innervation the alterations of spindle activity during REM, that presented the earliest biomarker of their aging.

EFFECT OF SEPTAL INACTIVATION ON PATH INTEGRATION

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Path integration is the process of updating position by monitoring translational and rotational information, in an egocentric reference frame, based on idiothetic information. Recently discovered grid cells are a strong candidate for playing a critical role in path integration. Populations of grid cells can, at least theoretically, provide two types of spatial information: position in the environment and distance to or from a reference point, such as a start or goal location.
The medial septum is considered an important source of theta generation. Theta signal is crucial to the activity of grid cells, since by inactivating the medial septum, theta power decreases substantially in medial entorhinal cortex, leading to grid fields destabilization[1].
It has been shown that both medial entorhinal cortex lesions and grid fields destabilization by medial septum inhibition, lead to deficit in distance evaluation. Also, head direction cells activity may have a causal role on the angular component of path integration. However, head direction cells destabilization leads to grid field distortion.
Despite these evidences, is not yet clear if grid cells have a role in path integration. Considering that septal inactivation leads to theta power decrease and grid fields destabilization, we designed an experiment aiming at understanding the effect of septal inactivation on path integration, both on its distance and angular components. For this purpose we established a homing task, based on computing a return path to a home base after foraging for food in an environment lacking any specific external cues. In alternating test sessions, the medial septum was inhibited via muscimol injections or control injections. The test was performed by two separate groups of animals, either in light or dark condition. To test the effect of multiple muscimol injections, at the end of the behavioral experiment, we assessed the LFP in the medial entorhinal cortex, while the rats were freely moving in a cylindrical arena.

PROGRESSION AND PERSISTENCE OF THE NEUROTOXICITY INDUCED BY MDMA ON THE DOPAMINERGIC SYSTEM IN MICE AND ASSOCIATION WITH NORADRENERGIC, GABAERGIC, AND SEROTONERGIC DAMAGE

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The amphetamine-related drug 3,4methylenedioxymethamphetamine (MDMA) is known to induce neurotoxicity at the level of the dopaminergic system in mice. In order to characterize how the number of administrations influenced the severity of MDMA-induced damage to dopaminergic pathways and to describe the localization and persistence of this effect, we evaluated the changes in tyrosine hydroxylase (TH) and dopamine transporter (DAT) in different regions of the mouse brain. Moreover, we investigated whether the dopaminergic damage was associated with noradrenergic, GABAergic, and serotonergic damage, by evaluating the changes in noradrenaline transporter (NET), glutamic acid decarboxylase-67 (GAD-67), and serotonin transporter (SERT). Mice received 14, 28 or 36 MDMA administrations (10 mg/kg twice a week) and were sacrificed at different time-points (post natal days 85, 110, 138, or 214) for immunohistochemical evaluation. Mice that received 28 administrations showed reduced levels of TH-positive nigral neurons and of DAT-positive fibers in caudate-putamen (CPu) and medial prefrontal cortex (mPFC). These mice also displayed increased NET-positive hippocampal fibers, reduced GAD67-positive neurons in CPu and hippocampus and reduced GAD67-positive fibers in mPFC. Mice that received 36 administrations displayed, together with the effects already observed in mice that received 28 administrations, also reduced THpositive striatal, cortical and hippocampal fibers. The reductions in dopaminergic and GABAergic markers persisted at 3 months after MDMA discontinuation. Finally, MDMA never modified the levels of SERT. These results provide further insight into the localization and persistence of MDMA-induced toxic effect on dopamine, and show that this effect is associated with GABAergic but not noradrenergic or serotonergic damage.

DEVELOPMENTAL DIFFERENCES IN DOPAMINE D2HIGH RECEPTORS

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The involvement of dopaminergic systems in many psychiatric illnesses has resulted in the pharmacological targeting of dopamine transmission as the primary treatment strategy for many disorders. This can be problematic as the responsiveness to dopaminergic drug administration varies across ontogeny. For example, administration of the irreversible dopamine receptor antagonist, EEDQ, decreases basal and dopamine agonist-induced activity in adult rats but enhances activity in preweanling rat. Moreover, the psychostimulant cocaine produces different levels of activity in preweanling rats after acute and chronic treatment as compared to adult or adolescent rats. The cause of this difference is unclear, however, it is possible that differences in the maturity of dopamine D2 receptors is at least partially responsible. In a series of experiments, we examined age-dependent differences in the response to inactivating dopamine receptors with EEDQ and to the acute administration of cocaine. Response was measured as changes in locomotor activity and dopamine receptor binding properties in the dorsal striatatum. Our results showed that that EEDQ's ability to enhance the locomotor activity of preweanling rats was primarily due to the inactivation of D2 receptors. Consistent with this finding, only drugs that directly or indirectly stimulated D2 receptors produced a potentiated locomotor response in EEDQ-treated rats. As expected we found that preweanling rats were more sensitive to the behavioral effects of cocaine than older age groups and the young rats had a significantly greater percentage of dorsal striatal D2(High) receptors than adolescent or adult rats. These results show that dopamine receptor inactivation caused dramatically different behavioral effects in preweanling and adult rats, thus providing additional evidence that the D2 receptor system is not functionally mature by the end of the preweanling period. Moreover, our receptor binding data suggest that increased percentages of D2(high) receptors may be responsible for these age dependent differences.

THE EFFECT OF KISSPEPTIN (1-5) FRAGMENT ON ANXIETY

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Introduction: Kisspeptin belongs to the RF-amid family, members of which share the N-terminal sequence and regulate several neuroendocrine functions. Kisspeptin binds with high affinity to its cognant receptor GPR54 and with lower affinity to the neuropeptide FF receptors. Previously, we demonstrated that kisspeptin-13 (KP13), an endogenous derivative of kisspeptin, possesses an anxiogenic and pronociceptiv effect. In the present experiment our aim was to investigate if the kisspeptin(1-5) fragment (KP1-5) has similar effect as KP13.
Methods: Different doses of KP1-5 were injected to the lateral ventricle of adult male Wistar rats. 30 minutes later we performed a computerized open field test to assess the peptide's effect on explorative behavior. The observed parameters were horizontal (distance travelled) and vertical (number of rearings) locomotion, immobility time and central activity (time and distance travelled in the center of the arena). Furthermore, we investigated the effect of KP1-5 on amygdalar GABA release in a superfusion study. We had removed the amygdala of rats and incubated the prepared slices with tritium labelled GABA. Then they were put into the superfusion system and after KP1-5 treatment basal and electric stimulated GABA release was assessed.
Results: In the open field test KP1-5 decreased the time spent and distance travelled in the center of the arena. Furthermore, it triggered a significant decreased in vertical locomotion and immobility time. The superfusion study revealed that KP1-5 caused a significant decrease in amygdalar GABA release upon electric stimulation, but did not alter basal neurotransmitter release.
Conclusion: Based on our results KP1-5 has an anxiogenic effect similar to KP13. However, the mechanism of action might differ as in contrast to KP13 KP1-5 blunted the amygdalar GABA secretion whereas KP13 increased it. Different receptor affinity profile of the two peptides might mediate this action.

This work was supported by the Hungarian Brain Research Program-KTIA_13_NAP-A- III/8.

SIMULTANEOUS ATTENTION TO VISUAL AND AUDITORY INFORMATION UNDER FATIGUE

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Mental fatigue induced by increasing Time-on-Task (ToT) has been found to affect various forms of cognitive operations. However, only a very few studies have addressed the effects of fatigue in tasks with non-visual sensory stimuli, and even no studies have aimed at examining ToT-related changes on bimodal information processing. Therefore, in one experiment we tested how simultaneous attention to visual and auditory stimuli was affected by ToT. Healthy human participants (N = 20) performed a bimodal (visual-auditory) 2-back task adapted for the ToT-paradigm. They were asked to attend at visual and auditory stimuli simultaneously and needed to find the match between the actual visual or auditory stimulus and the stimulus shown two trials earlier. The experiment had three phases. The pre-task phase involved an activity-sleep monitoring (by wrist actiwatch) during the night prior to the experiment as well as the completion of questionnaires referring to general sleepiness and fatigue. In ToT phase, the bimodal 2-back task was performed for an hour without rest (900 trials in total). Reaction time, error rates, and psychophysiological markers of motivation and fatigue as skin conductance, skin temperature, heart rate and blink rate were continuously recorded. Finally, in the post-task phase, after a resting period of 12 min, a 12-min-long block of trials was performed. Results suggest significant fatigue (i.e. ToT) related changes in bimodal information processing.

Supported by NKFI-120012, EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

BRAINAREAR: A TOOL FOR ELECTROPHYSIOLOGICAL ANALYSIS AND FUNCTIONAL PATTERN DETECTION

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The recent development in multielectrode array technology enables recording of potential with a high spatio-temporal resolution and to use more sophisticated experimental setups. There is a parallelly emerging need for more complex quantities describing the activity and spatial interaction of neuron population and visualization.
We aim to target this demand by the BrainAreaR, a software with graphical user interface developed in R. Besides generating images of simple quantities as raw and filtered data, power and frequency spectrum, kernel source density distribution map and coherence is also calculated. One of the main features is the coherence-clustering which can be used for identifying functionally cohesive neuron population. This might be of special interest for those interested in changes of population level interactions under various paradigms.
An application of this software is demonstrated on data from freely moving rat recorded by a 4x8 channel foli based multielectrode with 1 mm interelectrode distance. The result of the coherence clustering is a map that shows strong similarity to the underlying anatomical cortical area map (more on F. Fedor's poster).

This work was supported by the Hungarian National Research, Development and Innovation Office (NKFIH) grant no. K113147 and NKFIH 120143, furthermore by the Hungarian National Brain Research Program KTIA NAP 13-1-2013-0001 and KTIA NAP 13-2-2015-0004.

METABOLIC EFFECTS OF INTERLEUKIN-1β MICROINJECTION INTO THE CINGULATE CORTEX OF THE RAT

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In the present series of experiments, metabolic effects of the primary cytokine interleukin-1β (IL-1β) was examined after its administration into the cingulate cortex of male Wistar rats. The cingulate cortex, as the part of the limbic system, is known to play important role in the central regulation of feeding and meatbolism. In our extracellular single neuron recording experiments, the presence of neural cells which alter their firing rate in response to IL-1β has been revealed.
Microinfusion pump controlled bilateral microinjection of IL-1β was delivered via guide cannulas implanted above the target area during a stereotaxic operation one week earlier. The measurements were performed 20 minutes after the microinjections. Blood glucose levels of the animals were examined in a glucose tolerance test (GTT) after 12 hours of fasting. Blood samples were obtained from the tail vein of the rats right before the cerebral micrionjections and 9, 18, 30, 60 and 120 minutes after the intraperitoneal glucose load. The plasma levels of total cholesterol, HDL, LDH, triglycerides and uric acid were measured with a cold chemistry photometer after 12 hours of fasting, similar to the GTT.
Blood glucose levels of the IL-1β treated animals were higher throughout the GTT, but these values did not reach the level of significance. In contrast, significantly lower plasma concentrations of HDL and total cholesterol were measured in the cytokine treated rats. LDL, triglyceride and uric acid levels did not differ significantly compared to the control group.
Our results indicate that IL-1β mediated processes in the cingulate cortex play important role in the central regulation of metabolism.

Supported by: PTE ÁOK KA 2013/34039/1; EFOP-3.6.1-16-2016-00004. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

SPATIAL MEMORY TRACE ENCODING BY CELL ASSEMBLIES IN THE HIPPOCAMPUS AND BEYOND

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The hippocampus is important for the encoding and stabilisation of certain types of memories, including spatial memories. Hippocampal cells, the so-called place cells, encode the current location of the animal, collectively forming a cognitive map of allocentric space. Sleep periods in the hippocampus have been suggested to be involved in the consolidation of memories by replaying waking memory traces and transferring them to other brain areas for long-term storage. The aim of my research has been to examine how brain circuits interact and code for such spatial memory traces in the hippocampus and in interconnected brain areas such as the entorhinal cortex.
Our past work showed that place cell assemblies reorganise to disproportionally represent newly-learned reward location in open environments. Now we also show in a complex maze task that hippocampal neurons encoded a combination of short- and long-term memory traces and the formation of the new memory code is gradual. We further examined mnemonic coding in the entorhinal cortex. Here, we found that spatially-selective cells, such as the grid cells, reflect short-term spatial memory features on a maze task. Furthermore, entorhinal cell not always represent current locations, but they can transiently express entire movement trajectories. During sleep, trajectories were also replayed, suggesting a hippocampus-independent pathway for consolidation.
To better understand circuit operations during learning, we examined the role of inhibition in spatial coding. We found that, in the hippocampus, reward learning also reorganises the activity of inhibitory neurons and triggers changes in the strength of individual pyramidal cell-interneuron connections. Therefore, plastic changes take place in inhibitory circuits during spatial learning. Moreover, inhibition in hippocampal networks can lead to lasting changes in the coding of place cells: optogenetic inhibition of place cells caused the destabilisation of their place fields and the formation of new spatial firing fields. Overall, our research revealed means by which short- and long-term spatial memory traces are encoded in the hippocampus and the entorhinal cortex, and demonstrated the role of inhibitory circuits in these processes.

INPUT-SPECIFIC CLUSTERING OF VOLTAGE-GATED POTASSIUM CHANNEL SUBUNITS IN THE SENSORY THALAMUS, QUANTITATIVE ANALYSIS BY LOCAL MORAN'S I TEST

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Voltage-gated ion channels are either distributed homogeneously or accumulate in high-density clusters, endowing neurons with unique electrical properties. Employing a novel method, local Moran's I test, to detect spatial inhomogeneities in immunolabeled sections, here we describe highly specific accumulation of Kv4.2/4.3 subunits on the large caliber proximal dendrites of thalamocortical neurons. We found that Kv4.2/4.3 subunits were selectively clustered on membrane surfaces that are in direct contact with large, subcortical, glutamatergic axon terminals in thalamic nuclei that receive sensory inputs of all modalities. Our quantitative approach allowed us to detect significant differences of Kv4 ion channel clusters in three distinct ascending somatosensory pathways, carrying different types of whisker information. We also found increase in clustering during the maturation of the somatosensory system. Finally, lesion of the source of the sensory fibers resulted in a loss of Kv4.2/4.3 clusters. Our results demonstrate tight association of specific sensory afferents and postsynaptic Kv4 ion channel clusters indicating an active role of transient, A-type K+ current in setting the time course of sensory stimulus-evoked EPSPs and modifying the dynamics of thalamocortical information transfer.

THREE MAIN STRATEGIES UTILIZED BY TYROSINE HYDROXYLASE POSITIVE AMACRINE CELLS TO TARGET POSTSYNAPTIC NEURONAL PARTNERS IN THE MAMMALIAN RETINA

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Dopamine (DA) is released in a paracrine manner in the vertebrate retina by tyrosine hydroxylase expressing amacrine cells (THACs) and diffuses to retinal neurons to act on their activity, responsiveness and electronic coupling to adapt retinal circuits to daylight vision. Rod pathway AII ACs have been considered as THAC primary targets due to a characteristic ring-like axo-somatic innervation they provide to AII cell somata. Dopamine and GABA have been thought co-released at these sites to specifically modulate AII cell excitability and electrical coupling. Here, we show evidence that THAC axo-somatic innervation is neither specific for AII cells nor show evidence to GABAergic synaptic interactions in light microscopic observations. We found a number of non-AII ACs whose somata were located inside of THAC axonal rings. This latter population included electrically isolated starburst ACs as well as W-S2/3 ACs that form a dense electrically coupled array. In addition, ACs that are tracer coupled to retinal ganglion cells were often found in juxtaposition to axo-somatic rings. This finding implies that, though not exclusively, but THAC axo-somatic inputs serve the dopaminergic modulation of AC gap junction conductance. In addition, we found that vesicular GABA transporter 1 (VGAT1) immunoreactive plaques were often localized at THAC rings and some of these were also in a juxtaposition with GABAARa1 immunolabeled postsynaptic sites. This first suggested, that THAC axo-somatic rings are GABAergic synaptic sites, however statistical analyses based on Neurolucida reconstructions showed that the frequency of such triple or double colocalizations are not higher than those found in non-ring THAC AC sites. These observations highly suggest that, contrary to widely accepted hypotheses, THAC axo-somatic rings are not specialized synaptic contact sites.

This work was supported by OTKA (K105247) and the Hungarian Brain Research Program (KTIA_NAP_13-2-2015-0008), EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

HOW MOUSE GENETICS REVEAL NOVEL TREATMENT OPTIONS TO PARKINSON'S DISEASE

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Parkinson disease (PD) is a neurodegenerative disorder characterized by dopaminergic neurons affected by inflammatory processes. Current medications only provide symptomatic relief but fail to halt the dopaminergic neuronal death. Studies from experimental animal models have provided crucial insights into the molecular mechanisms in disease pathogenesis and recognized possible targets for therapeutic interventions. Post-mortem analyses of brain and cerebrospinal fluid from PD patients show the accumulation of proinflammatory cytokines, confirming an ongoing neuroinflammation in the affected brain regions. The tetracycline-derivative doxycycline (α-6 deoxy-5-hydroxytetracycline) has been shown to be neuroprotective in *in vitro* and *in vivo* models of neurodegenerative diseases. The proven reliability and safety of the medication suggests its potential as an effective and inexpensive treatment to protect or at least mitigate the central nervous system from neurodegenerative diseases such as PD. It has been suggested that the modulation of astrocyte and microglial activation could prevent neuronal demise and thus the progression of neurodegeneration. We hypothesize that doxycline could exert a neuroprotective effect by suppressing astrocyte and microglial activation induced by the neurotoxin 6-hydroxydopamine (6-OHDA), a preparation with similarities to PD. We investigated in striatal 6-OHDA lesioned mice the effects of a doxycycline given chronically either orally or subcutaneously at sub-antibiotic concentrations. To assess the protective mechanism conveyed by doxycycline we quantified using immunoreactive labeling tyrosine hydroxylase (neurons), glial fibrillary acid protein for astrocytes and cell surface marker macrophage antigen complex-1 for microglial cells. Brain regions containing cell bodies and fibers of dopamine in the nigrostriatal pathway were evaluated. Neuroinflammation indicators were sampled by Western blot analysis of metalloproteinase-3, cycloxygenase-2 and caspase-3 from the striatum. Chronic treatment with doxycycline, either administered orally or injected subcutaneously at sub-antibiotic concentrations, mitigates the loss of dopaminergic neurons in the substantia nigra compacta and nerve terminals in the striatum. This protective effect was associated with a decrease in the astrocyte and microglia response to the neurotoxin 6-OHDA in the globus pallidus and substantia nigra compacta, and a decrease of the astrocyte reaction in the striatum. No change in the expression of MMP-3, Caspase-3 and COX-2 was observed. Our results suggest that doxycycline blocks 6-OHDA neurotoxicity *in vivo* by inhibiting microglial and astrocyte expression. This action of doxycycline in nigrostriatal dopaminergic neuron protection is consistent with a role of glial cells in PD neurodegeneration.

EPIGENETIC ASPECTS OF VASOPRESSIN-DEFICIENT BRATTLEBORO RATS' SCHIZOPHRENIA-LIKE BEHAVIOR

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Previous studies have shown schizophrenia-like behaviors in vasopressin-deficient Brattleboro rats, and other studies have suggested that epigenetic changes may also be involved in the development of schizophrenia. Therefore our aim was to find out whether epigenetic changes contribute to schizophrenia-like behavior of this strain. Behavioral and epigenetic characteristics of vasopressin-deficient rats were compared to wild type controls. Locomotion was tested by telemetry, cognitive function was studied by novel object recognition, social recognition and social avoidance test, attention was measured by pre-pulse inhibition (PPI) test. DNA methyltransferase1, DNA methyltransferase3a, as well as catechol-O-methyltransferase, glutamate decarboxylase, vesicular glutamate transporter 1, serotonin 2A, brain-derived neurotrophic factor mRNA levels in prefrontal brain region and hippocampus were studied by qRT-PCR. Working with MTA SZBK, histone3 (H3) and H4 acetylation (Ac) were investigated by western-blot. Then, specifically, H3 lysine9 (K9) acetylation was studied by immunohistochemistry in the prefrontal cortex, hippocampal regions, lateral septum and nucleus accumbens.
Impaired cognitive, social and attention behavior of vasopressin-deficient rats confirmed schizophrenia-like symptoms in our local colony. There were no major significant alterations in the studied mRNA levels. The pan-AcH3 immunoreactivity was lower in prefrontal region and elevated in the hippocampus of vasopressin-deficient animals. We found lower immunoreactivity in the dorsal peduncular prefrontal cortex and the ventral lateral septum and increased AcH3K9 immunopositive cell number in CA1 region of vasopressin-deficient animals.
In conclusion, we confirmed that Brattleboro rat is a good preclinical model of schizophrenia. The appearance of schizophrenia-like behavior was accompanied by H3 acetylation changes in the prefrontal region and hippocampus. This may contribute to disturbances of many schizophrenia-related substances leading to development of schizophrenia-like symptoms. Our studies confirmed that not a single gene, rather fine changes in an array of molecules are responsible for the majority of schizophrenia cases.

OPPOSING AND STAGE DEPENDENT EFFECT OF PACAP1-38 ON RETINAL APOPTOSIS

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The pituitary adenylate cyclase activating polipeptide (PACAP), an ubiquitous and pleiotropic regulator has been in the focus of neurotoxicity research due to its significant neuroprotective potential. Although the literature proving its repressive effect on the apoptotic machinery in various neurodenerative models is vast, the abscense of a precise analysis of its status in the normal development is striking.
We performed quantitative analyses on caspase activity upon 100, 50, 25 or 1 pmol intravitreal PACAP injection during early postnatal development of the rat retina. Wistar rats were treated in postnatal days P1, P3, P5, P7. Tissues were harvested 6, 12, 18, 24 or 48 hours post-injection. Activity, amount or localization of apoptotic enzymes were revealed using Apo-ONE homogeneous caspase 3/7 assay, western blot and TUNEL assay, respectively.
Surprisingly, 100 pmol PACAP had no effect on either the level of active caspase 3,7 or their activity in the retina at P1, P5 or P7. In contrast, PACAP seemed to exert a biphasic effect on apoptotic activity at P3 by significantly repressing active caspase 3/7 level in 12 and 18 hours post-injection while increasing their activity in 24 hours post-injection. Levels of activated initiator caspase 9 and 12 were induced as well. TUNEL positive cell bodies appeared throughout the inner plexiform layer in response of PACAP treatment. Injection of 1, 25 or 50 pmol PACAP could not exert anti-apoptotic effect in 18 hours. However, injection of 50 pmol PACAP resulted in a slight elevation in caspase 3/7 activity in 24 hours post-injection.
Here, we demonstrate that PACAP regulates apoptosis in a stage dependent manner during early postnatal retinogenesis, presumably, due to differential expressions of receptor isoforms. We show for the first time that PACAP not only inhibit development related apoptosis at P3 but as a long term effect it induced apoptosis even when its concentration was as low as 50 pmol. Considering the latency, it is resonable to assume that the later effect was evoked indirectly due to induction of other secreted regulator(s).

Support: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008.

THAPSIGARGIN (TG) AND TUNICAMYCIN (TM) INDUCED ER STRESS AND ITS POSSIBLE IMPACT ON MITOCHONDRIAL FUNCTION

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Introduction: Disturbances in the normal functions of the endoplasmic reticulum ER lead to an evolutionarily conserved cell stress response, the unfolded protein response (UPR), which is aimed initially for compensating of damage but can eventually trigger cell death if ER dysfunction is severe or prolonged. Increasing information accounts for the crosstalk between mitochondria and ER as decisive in many cellular events. There exists specialized connection between ER and mitochondria, called mitochondria associated membranes (MAMs), with central role in regulation of many cellular processes including mitochondrial membrane dynamics. Considering that the ER–mitochondria interface is involved in several molecular pathways, it will be important to determine its relevance to human diseases, because the changes in MAMs have already been implicated in different diseases.
Aims: Aim of our study was to observe if perturbations in protein synthesis and folding, that are associated with ER stress, can be transmitted to mitochondria. We evaluated the efect of substances causing endoplasmic reticulum stress (tunicamycin, thapsigargin) on the survival of different neuronal cell lines (SHSY 5Y, SK-N-SH), expresion of proteins controlling mitochondrial dynamics and activity of enzyme complex IV of the mitochondrial respiratory chain.
Materials and Methods: As a model, we used two different neuroblastoma cell lines SH-SY5Y and SK N SH. Cells were treated with ER stress inducers Thapsigargin (TG) and Tunicamycin (TM) in the time interval 6, 16 and 24 hours. We used methyl-thiazol tetrazolium (MTT) assay for determination the survival rate of cells with various concentrations of TG and TM. Proteins derived from cell lysates, were separated by SDS-PAGE, and their levels was confirmed by Western Blot analysis. Activity of enzyme complex IV was evaluated spectrophotometrically (550nm) after pre-incubation with 50 mM Tris-HCl (pH 8.0) and 0.01 % (w/v) n-dodecyl β-D-maltoside. Results were processed using statistical methods.
Results and conclusions: Induction of ER stress was monitored by presence of phosphorylation of eukaryotic initiation factor 2α (eIF2α). The increased concentration of TG and TM induced cell death in both types of cell lines and partially altered expression of mitochondrial fusion protein (Mfn1) which is implicated in mitochondrial dynamics. TG and TM led to significant reduced activity of the enzyme complex cytochrome c oxidase. Our findings suggests a possible association of endoplasmic reticulum stress with disruption of mitochondrial functions in neuronal cells.

ASSOCIATION BETWEEN TOOTH LOSS, COGNITIVE DECLINE AND STRUCTURAL BRAIN DIFFERENCES IN OLDER ADULTS

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Background and Aims: Poor dental health has been linked to cognitive decline, and cardiovascular disease (CVD) and inflammation seems to play a role in such an association. We aimed to examine the association of tooth loss with cognitive decline and structural brain differences taking into account CVD and inflammation.
Methods: Within the population-based Swedish National study on Aging and Care-Kungsholmen, 2715 dementia-free participants aged ≥60 were identified at baseline, and followed for up to 9 years. Cognitive function was assessed with the Mini-Mental State Examination (MMSE) at baseline and at follow-ups. A subsample (n =394) of participants underwent MRI. Information on dental status and medical history of CVD was collected at baseline. Inflammation was assessed with blood C-reactive protein (CRP). Data were analysed using mixed-effects models and linear regression with adjustment for potential confounders.
Results: At baseline, 404 (14.9%) people had partial tooth loss, and 206 (7.6%) had complete tooth loss. Partial (β: -0.21, 95% CI: -0.30 to -0.12) and complete (β: -0.49, 95% CI: -0.63 to -0.35) tooth loss were significantly associated with greater MMSE decline over time compared to those with no tooth loss. The association remained significant when stratifying by CVD, stroke and CRP level above/below 5 mg/l for participants with complete tooth loss but not in those with partial tooth loss. Furthermore, people with tooth loss had significantly lower adjusted total brain (β: -35.05, CI: -57.03; -13.07), grey matter (β: -25.34, CI: -40.94; -9.74) and hippocampal (β: -0.28, CI: -0.53; -0.03) volumes, compared to no tooth loss.
Conclusion: Tooth loss is associated with accelerated cognitive decline and neurodegeneration, and this association may not be explained by vascular disorders or inflammation.

A SYSTEMS BIOLOGICAL APPROACH TO UNDERSTAND PARENTAL BEHAVIOUR

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The hypothalamus has long been known to control maternal behaviour in rodents with the neuropeptide oxytocin playing a pivotal role. Recently, novel elements of the brain maternal network have been functionally identified as selective lesion of galanin neurons in the preoptic area of the hypothalamus eliminated, while their optogenetic stimulation elicited care of the offspring. We hypothesized that molecular changes accompany the identified alterations of neuronal functions. These molecular adaptations were addressed using systems biological tools at the mRNA as well as the proteome level, which led to the identification of maternally altered genes in different parts of the rat brain. Thus, we identified a novel neuropeptide, amylin, which appears in the rodent brain only in the preoptic area of parenting rodents. In line with major differences between parental care systems between mammalian and avian species, amylin was found in several distinct regions of zebra finch brain showing sexually dimorphic distributional pattern. A combination of neurogenomics and proteomics approaches led us to identify another system involved in the adaptation of the brain to parenting: insulin-like growth factor-I. All these molecular changes could be evoked by hormonal actions during parenting. However, maternal behaviour appears in rodents in response to pup exposure even in the absence of hormones. Therefore, we addressed maternally activated neuronal inputs to the preoptic area and identified a posterior thalamic region, which may relay somatosensory information from the pups to the mother to control maternal responsiveness. A neuropeptide, TIP39 is induced in the thalamic neurons of mother rats projecting to the hypothalamus. Synaptic innervation of oxytocin and galanin neurons by TIP39-containing nerve terminals has also been demonstrated suggesting that somatosensory neuronal input reaches these cells and may participate in the control of maternal behaviours.

Grant support: KTIA NAP_B_13-2-2014-0004 Research Program.

BEHAVIORAL IMPAIRMENTS AND ALTERATION OF EXPRESSION OF MICROGLIA AND ENDOTHELIUM-SPECIFIC GENES IN A MODEL OF ALZHEIMER'S DISEASE

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It is known that degeneration of cholinergic neurons is one of key events during development of Alzheimer's disease. Here, we studied effect of intracerebroventricular injection of toxin saporin conjugated with antibody to receptor p75 on the learning performance of rats. Ig-saporin induced a significant decrease in the number of septal choline acetyltransferase-positive neurons. In three weeks after the injection we started behavioral testing after which we performed RNAseq and analyzed expression of genes in the hippocampus. Administration of the immunotoxin led to a significant increase in the total distance moved and higher velocity in the Morris Water Maze test ($p<0.05$) and slight impairment in passive avoidance learning. We found that during probe trial, when the platform was removed from the maze, saporin-treated rats spent significantly less time in a quadrant, where the platform was during training, and swam shorter distance in it, as compared to the control animals. In contrast, locomotor and exploratory activity in the open field task did not change as compared to the control. Analysis of rat behavior in T-maze did not reveal any significant differences in the number of errors compared to the control. Analysis of differentially expressed genes in the hippocampus using RNAseq showed that the expression of majority of microglia-specific genes was elevated whereas expression of majority of endothelium-specific genes decreased. These data suggest that degeneration of cholinergic neurons leads not only to some disturbances in memory but also to alterations in functioning of microglia and vascular endothelium.

The work was supported by Grant of Russian Science Foundation No 16-15-10403.

ROLE OF FEMALE GONADAL HORMONES ON NTPDASE2 PROPERTIES IN HIPPOCAMPAL GLIOSOMES

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In the central nervous system, glial cells provide trophic, structural and metabolic support to neurons. Since NTPDase2, an ecto-enzyme that preferentially hydrolyzes ATP, is expressed by rat astrocytes, we examined extracellular adenine nucleotides hydrolysis during oestrus cycle in the hippocampal glial subcellular particles (gliosomes). We also examined whether gonadal steroid hormone deprivation, induced by bilateral ovariectomy (OVX) will affect NTPDase2 function and the role of exogenous 17β-estradiol (E2). Determination of the oestrus cycle stage was evaluated by proportion of specific cell types in the vaginal smear. The rats were submitted to OVX and three weeks after the surgery injected with a single dose of E2 (33.3μg/kg). The alteration in ATP hydrolysis were not observed while ADP hydrolysis showed cyclic fluctuations across the estrus cycle ($p<0.01$). In OVX animals, we observed significant decrease in ATP hydrolysis ($p<0.01$) compared to all three phases, while ADP hydrolysis was similar to the ADPase activity at proestrus. Immunoblotting analysis confirmed NTPDase2 as dominant ectonucleotidase in the hippocampal gliosomes, whose relative protein abundance also decreases after OVX and up-regulated after the treatment. ATP/ADP hydrolyzing ratio strongly argues in favor of NTPDase2, which is confirmed by immunoblot analysis. Since OVX might induce astrocytic responses similar to those observed after injury and affect neuronal chemistry and morphology, our finding that E2 upregulates NTPDase2, potent modulator of inflammatory reactions within the hippocampus, could represent a useful therapeutic target in human disease.

Supported by Ministry of Education, Science and Technological Development, project No 173044 and 41014.

THE ROLE OF DISTURBED LIPOPROTEIN METABOLISM IN HUNTINGTON´S DISEASE

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Previous studies have shown an involvement of cholesterol in neurodegenerative diseases such as Huntington´s diseases (HD). However, the mechanisms underlying lipid disturbances in HD and whether they are secondary or primary in the pathogenesis of the diseases is not known. Using striatal neurons expressing mutant 120polyQ Huntingtin (Htt) expressing protein, we observed that the levels of lipoprotein receptors (LDLRs) are markedly reduced compared with controls. As cholesterol uptake occurs via the lipoprotein receptors that can be influenced by various factors, we are currently studied the mechanism in more detail. We focus on the E3ligase Mylip/Idol that regulate the LDLRs via protein ubiquitination. The possibility also that the PCSK9 extracellular pathway for LDLRs degradation is increased in HD cells via lysosomal degradation, and this can be targeted by novel anti-PCSK9 monoclonal antibodies now in the clinics to treat hyperlipidemias. We have recently also shown that the cholesterol biosynthetic pathway involving SREBP signaling can be influenced by cytokines and by activation of the low affinity p75 neurotrophin receptor (p75NTR) by pro-NGF. This may suggest that neuroinflammation can influence lipoprotein receptors in vulnerable neurons in HD. The role of pro-NGF and other inflammatory factors in controlling lipoprotein metabolism in mutant Htt expressing neurons and in HD are currently under investigation.

NLRP 2 INFLAMMASOME IS UPREGULATED IN THE SUPERFICIAL DORSAL HORN OF RATS SUFFERING FROM CHRONIC INFLAMMATORY PAIN

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Intense and long-term noxious stimulation that are associated with chronic inflammation lead to central sensitization of neural circuit within the superficial spinal dorsal horn. There is a general agreement that inflammasomes play a crucial role in this process. Inflammasomes are key signalling platforms that detect pathogenic microorganisms and activating the pro-inflammatory cytokines such as interleukin-1β (IL-1β) and IL-18. Precursor of the IL-1β (Pro-IL-1β) is cleaved by the pro-inflammatory protease caspase-1 processing it to a mature biologically active form.The activation of caspase-1 occurs *via* recruitment to a multi-protein complex termed the inflammasome. In our previous results we demonstrated a significant enhancement in the IL-1β expression of astrocyte cells by using an unilateral plantar injection of complete Freund adjuvant (CFA). Based on this model we performed single immunoperoxidase stainings and showed the expression of inflammasomal proteins within the superficial spinal dorsal horn. The evaluation of double immunofluorescent labelings revealed that NLRP2 protein was abundantly expressed by astrocyte cells in chronic inflammatory pain conditions. The results indicate that NLRP2-positive astrocytes may play role in the spinal processing of pain by contributing to the production of IL-1β evoked by chronic peripheral inflammation.

This work was supported by the Hungarian Academy of Sciences (MTA-TKI 242) and the Hungarian Brain Research Program (KTI_NAP_13-1-2013-001).

SHORT-TERM HYPOXIA INDUCES PLASTICITY IN RETINOCOLLICULAR PATHWAY

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The visual system, especially retina and subcortical visual centers are extremely sensitive to hypoxia, due to their energy demands. Lesions of the visual system, as a result of injury, toxicity, degenerative and inflammatory processes, metabolic and cardiovascular disease are often mediated or accompanied by short- or long-term hypoxia.
In this study we investigated effects of short-term hypoxia on synaptic transmission in the first visual (retinocollicular) synapses in coculture. Coculture of dissociated retinal cells and superior colliculus (SC) neurons was developed as adequate in vitro model of retinocollicular pathway with easily identified couples of retinal ganglion cell (RGC) – SC neuron. Method of fast local superfusion was used for short-term (5 minutes) application of hypoxic solution on synaptically connected neurons. Pharmacologically isolated NMDA receptor (NMDAR)- and $GABA_AR$-mediated postsynaptic currents (PSCs) were evoked by generation an action potential in presynaptic RGCs and recorded from SC neurons.
In case of NMDAR-mediated evoked PSCs recording we used magnesium-free solution due to the block of NMDAR channels by external magnesium ions. Application of hypoxic magnesium-free solution resulted in a long-term potentiation (LTP) of NMDAR-mediated transmission. Furthermore, application hypoxic standard solution (2 mM Mg^{2+}) resulted in reduction in the ability of Mg^{2+} to induce a normal voltage-dependent blockade of the evoked NMDA response. Analysis of the oxygen deprivation effect on spontaneous and miniature postsynaptic currents (sPSCs and mPSCs) revealed an increase in occurrence frequency and the second peak appearance in the mPSCs histogram. Obtained estimates for quantal and binomial parameters reflected the presynaptic changes during the potentiation, independent of the release probability. Most likely this type of hypoxia induced plasticity can be caused by increasing in the average number of release sites and reducing of Mg^{2+} blockade.
$GABA_AR$-mediated synaptic transmission responded to hypoxia by a long-term depression (LTD). Analysis of sPSCs and mPSCs showed significant decrease in the occurrence frequency and in the amplitude of mPSC (quantal size) during oxygen deficiency. Estimation changes in quantal and binomial parameters showed complex of presynaptic (independent of the release probability) and postsynaptic (decrease in sensitivity of postsynaptic receptors) mechanisms during hypoxia induced LTD of $GABA_AR$-mediated synaptic transmission.
Physiological role of GABAergic retinocollicular projections is in regulation of activation and plasticity of excitatory NMDAR-mediated transmission. Thus, hypoxia induced LTD of GABAergic transmission could enhance pathological effect of LTP of NMDAR-mediated transmission in retinocollicular synapses and possibly is an additional injury which can be caused by hypoxia on transmission from the *retina to subcortical visual centers.*

INVOLVEMENT OF D1 TYPE DOPAMINE RECEPTOR IN ACCUMBENS NUCLEUS IN HEDONIC FOOD INTAKE

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Introduction Alterations in feeding reward have been recognised in obesity but primary reasons of overeating are not known. Malfunction of the accumbens nucleus (Nac), a key reward centre is emphasised by several studies. The Nac is subdivided into core and shell regions that are reported to be involved differently in feeding reward.
Aims We investigated the function of Nac subregions in feeding reward in healthy and obesity-prone rats.
Methods Obesity prone-rats (PR) were produced by intrauterine protein restriction. Sweetened condensed milk (SCM) was provided to rats to provoke reward related feeding. Consummatory behaviour and SCM-elicited Fos-reaction within the Nac were analysed. In situ hybridisation for D1- and D2-type dopamine receptors was used to detect changes in the mesolimbic dopaminergic system. D1R agonist SKF 82958 was injected into the medial shell of Nac in control animals to reveal functional importance of found morphological changes.
Results PR rats were born with lower body weight than controls and showed fast catch-up growth. Young adult PR animals weight-matched with controls and became slightly hyperphagic. They produced heightened SCM consumption, and showed greedier consummatory behaviour. SCM-evoked Fos in Nac medial shell was proportional to SCM intake within both groups. Only PRs exhibited similar relationship in Nac core. The average Fos-count however was comparable between the groups, indicating neurons in PRs had a higher activation threshold. This was associated with reduced D1 type dopamine receptor mRNA expression in the medial shell. Most of the SCM-evoked Fos-positive cells actually expressed D1R mRNA here in controls. Selective D1R agonist injected into the medial shell attenuated SCM intake.
Conclusion D1R signalling has robust impact on inhibitory control over feeding. Altered neuronal sensitivity in the Nac medial shell related to D1R malfunction may be a key factor leading to reward-driven overconsumption.

Supported by: NKFI K115422 (ZET).

HYDROGEN SULFIDE MODULATES NEURONAL FUNCTIONS IN ANIMAL MODELS OF TRIGEMINAL NOCICEPTION

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Objectives: The toxic gas hydrogen sulfide (H_2S), which is produced also in mammalian tissues, is known as an endogenous neuromodulator acting together with nitric oxide (NO) through their reaction product nitroxyl (HNO). HNO opens TRP channels of the ankyrin type 1 (TRPA1) causing release of calcitonin gene-related peptide (CGRP) from primary afferents, which is involved in the pathophysiology of primary headaches. In rodent models of meningeal nociception we asked if the interaction between H_2S and NO induces CGRP release through the activation of TRPA1 receptors concomitant with an increase in blood flow in the medulla spinalis and activation of neurons in the spinal trigeminal nucleus, where the nociceptive information from meningeal tissues is processed.
Methods: In anesthetized rats, the H_2S donor Na_2S and the NO donor DEA-NONOate were infused intravenously. CGRP content of cerebrospinal fluid (CSF) collected from the cisterna magna was measured with ELISA, blood flow of the exposed medulla spinalis was monitored by laser Doppler flowmetry and the activity of spinal trigeminal neurons was registered by extracellular recordings. Endogenous production of H_2S was inhibited by oxamic acid and NO production by L-NAME to manipulate endogenous HNO formation. TRPA1 and CGRP receptors were inhibited by HC030031 and olcegepant, respectively.
Results: Infusion of Na_2S was followed by increased CGRP levels in the CSF and increases in medullary blood flow. These responses were abolished by pre-administration of HC030031, L-NAME or olcegepant. Na_2S caused increased ongoing activity in the majority of spinal trigeminal neurons, while a smaller sample of units was inhibited, and L-NAME following Na_2S returned the activity to baseline. Systemic administration of DEA-NONOate increased neuronal activity, subsequent infusion of Na_2S facilitated the neuronal response to DEA-NONOate, and oxamic acid neutralized the stimulating effect of DEA-NONOate.
Conclusions: H_2S liberated from Na_2S causes CGRP release followed by increased blood flow in the medulla spinalis. NO facilitates these effects, most likely by forming HNO, which is able to activate TRPA1 receptor channels inducing calcium inflow at central terminals of trigeminal afferents. Spinal trigeminal neurons may be activated or (less frequently) inhibited by these mechanisms. The effects of H_2S-NO-TRPA1 signaling may be involved in the pathophysiological events of primary headaches but the role of TRPA1 activation in pain generation appears ambiguous.

This work was supported by research grants K119597 of the Hungarian National Research, Development and Innovation Office, the Alexander von Humboldt Foundation and the European Union (EUROHEADPAIN, Grant agreement no: 602633).

ADAPTIVE MECHANISMS AND CHANGE IN NEURONS MORPHOLOGY ACCOMPANY PERSISTENT ANOREXIA THROUGH THE SEROTONIN 4 RECEPTORS AFTER STRESS APPLICATION

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Adaptive decision making to eat is crucial for survival but in neurodegenerative and mental diseases, the brain persistently supports persistent changes in food intake. How the brain persists in reducing or enhancing food intake to the point of death despite the evolution of multiple mechanisms to ensure survival by governing adaptive eating behaviors in front of stress remains mysterious. In order to study the functions of 5HTR4, we previously engineered the 5HTR4 knockout mice (KO4) and evidenced their critical role in anorexia like and binge eating. Here, we show an absence of increase in the production of the phosphorylated cAMP responsive element binding protein (CREB) in the hypothalamus in KO4 mice following acute stress. As pCREB is a transcription factor, we conducted transcriptom and RQPCR analyses. We evidenced that the 5HTR4 control the expression of neurogenesis and DNA methylation factors suggesting that 5HTR4 could induce persistent morphological changes of neurons and favor a persistent restrictive food intake. Consistently, we found that 5HTR4 exert a positive control of the anorectic Brain derived neurotrophic factor in the medial prefrontal cortex, in which 5HTR4 control stress induced anorexia. The number of dendritic spines of pyramidal cells in the medial prefrontal cortex is reduced in the absence of 5HTR4, which may support the abnormal resistance of KO4 mice to chronic stress induced hypophagia. The present study shows that 5HTR4 may contribute to implement neural networks by controlling gene expression of neural growth factors that are involved in adaptive behavior to stress.

HIPPOCAMPAL CODING DURING A CONDITIONAL DISCRIMINATION LEARNING TASK: PRELIMINARY FINDINGS

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Hippocampal place cells are thought to collectively form cognitive maps that uniquely represent distinct environments. Such maps may enable flexible behaviour in the face of changing conditions within a given environment. However, it is unclear how distinct, task-defined conditions within the same spatial environment are encoded by such neurons. To address this, we designed a conditional discrimination task in which mice learned to associate two sets of LED displays, each defining a distinct ‘context’, with different reward locations within the same enclosure. This was carried out while performing multiple single-unit recordings with tetrodes implanted in the hippocampus. Mice learned to make such a conditional discrimination within one day and remembered the association in a subsequent probe session. Crucially, trial-limited optogenetic silencing of CA1 pyramidal neurons during the probe session impaired performance. Furthermore, preliminary evidence suggests that a subset of hippocampal neurons differentially represent each LED-defined context during the course of learning. Together, these results suggest that hippocampal neurons may be involved in encoding distinct, learning-defined, non-spatial contexts. This experimental paradigm should, therefore, allow the dissection of circuit mechanisms underlying the differential annotation of cognitive maps with task-defined non-spatial information and the use of such annotated maps to flexibly discriminate between distinct contexts within the same environment.

CHARACTERIZATION OF GENERAL ACTIVITY, AND STEREOTYPIC BEHAVIORS OF WISKET RATS IN LARGE HOME-CAGE WITH ENVIRONMENTAL ENRICHMENT

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Introduction: Schizophrenia is a neuropsychiatric disorder, affecting approximately 1% of the population. It is characterized primarily by positive and negative symptoms and cognitive disturbances. Clinical studies revealed that schizophrenic patients often show abnormal motor activity, and several schizophrenic animal models investigated this phenomenon. A new substrain of Wistar rats (WISKET) was developed by selective breeding after social isolation and subchronic ketamine treatment. The WISKET rats display several schizophrenia-related deficits, including disturbed sensory gating, cognitive function, thermoregulation, cortical electrophysiological abnormalities, opioid and cannabinoid receptor activation in different brain structures, and decreased acute heat pain sensitivity. Our earlier studies also showed decreased motor activity in these animals during short-lasting learning tasks and telemetric experiments in regular rat home-cage. Environmental enrichment has been shown to modify symptoms of many neuropsychiatric illnesses. In this study we tested the changes in behavior of our schizophrenia rat model in a large, complex home-cage, which provided more feasibility to move and explore in comparison to regular home cages.
Methods: Two groups of 3 months old male rats (n=9/group) were involved: naive Wistar and WISKET rats. The animals were housed separately for seven days into big cages (height:55.5, width:55, depth: 60 cm) with three floors and two shelves at 14 and 34 cm height, respectively, and are accessible by a ladder. The behaviors of the rats were recorded for 24 hours using infrared cameras, including the motor activity, the marble burying and the hoarding behaviors. From the camera recordings we gained information on the duration and frequency of the rat's behavior at both light and dark phases during off-line analysis.
Results: There were no significant differences in the volume of consumed water and food between the two groups. However, the hoarding activity (food burying) was significantly enhanced in the WISKET group, even at the later phases of experiment. Similarly, the marble burying behavior was increased. Regarding the motor activity, the WISKET animals displayed lower level of activities at both the dark and light phases, especially at the first few days of the experiment.
Conclusion: In conclusion, the disturbed behavior of WISKET rats could also be observed in a large cage with environmental enrichment, and the increased hoarding and burying behaviors may indicate enhanced stereotype activity, reinforcing our WISKET model translational relevance to neuropsychiatric disorders, e.g. schizophrenia, autism.

PARASITOID WASP MANIPULATES NOCIFENSIVE BEHAVIOR OF COCKROACH HOST

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The parasitoid wasp *Ampulex compressa* delivers a sting precisely into the cerebral ganglia of its host, the American cockroach. This sting does not paralyze the cockroach but rather reduces its ability to initiate walking or escape and its responsiveness to noxious stimuli. In the present study, we explore the mechanisms of manipulation of the nocifensive behavior of cockroaches stung by the wasp. Recordings from a single connective of the ascending fibers in the abdominal nerve cord reveal differences in the evoked response to tactile versus noxious stimuli. The response to tactile stimuli is accompanied with the firing of large-diameter fibers at the beginning of the stimulus and smaller diameter fibers at the end of the stimulus. The response to noxious stimuli is accompanied with the firing of intermediate size-fibers throughout the stimulus. These recordings were combined with electromyogram (EMG) recording from the coxal depressor muscle of the metathoracic leg to monitor the behavioral response to tactile and noxious stimuli. We found that cockroaches respond differently to tactile versus noxious stimuli. Furthermore, stung or un-stung cockroaches respond differently to noxious stimuli. To summarize, our results indicate that the modalities of touch and nociception are encoded by different fibers in the cockroach central nervous system. Moreover, although nociceptive information is carried by interneurons of the nerve cord in stung cockroaches, such information fails to trigger strong nocifensive behavior. Hence, by injecting venom into the host brain, the wasp must be manipulating the processing of nociceptive input.

MODAFINIL PRODUCES RESISTANCE OF LONG-TERM SOCIAL MEMORY AGAINST INTERFERENCE IN MICE

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Adult male mice are able to form long-term social recognition memory under laboratory conditions. The consolidation of this type of memory can be blocked by the peripheral administration of the protein synthesis blocker anisomycin at defined time points after learning. Also long-term social memory in mice was shown to be sensitive to manipulations leading to the phenomena known as retro- and proactive interference. Here we show that long term social memory of adult male mice of the C57BL/6OlaHSD strain can be blocked by exposure of unknown conspecifics at 3 and 6 h after learning (retroactive interference). Pre-exposure of an unknown conspecific 3 h before learning caused proactive interference if tested for social memory 21 h later. Subcutaneous injections of modafinil (10 mg/kg b.wt.) 30 min before learning were able to protect social memory when attempts were made to induce retroactive interference 3 and 6 h later. Similarly, treatment with modafinil suppressed also proactive interference when learning took place 3 h later. These data suggest modafinil as a potent stabilizer of social recognition memory. Subsequent studies will investigate of whether and how modafinil interacts with the protein synthesis processes thought to underlie long-term social memory in mice.

SYNESTHESIA IN BIPOLAR AND SCHIZOPHRENIC PATIENTS: A STUDY OF ITS RELATIONSHIP WITH ABSTRACT THINKING

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The neurological condition 'synesthesia' may explain the links underlying metaphor perception and comprehension of abstract concepts in humans[1]. Schizophrenia and bipolar disorders share certain similarities regarding symptomology which often inhibits and attenuates differentiating between them. Individuals with schizophrenia have more pronounced structural brain and neuropsychological abnormalities than those with bipolar disorder[2]. A unique characteristic of schizophrenics' thought and language disturbance is concretism. In other words, schizophrenic patients fail to understand metaphors[3]. On the other hand, an intellectual ability such as metaphor perception remains intact in bipolar patients.
The aim of the current study is to determine (a) if patients with schizophrenia are weaker at metaphor comprehension than bipolar and normal individuals (b) if the patients with schizophrenia are weaker in synesthesia comprehension than bipolar and normal individuals (c) if bipolar patients can understand metaphors as well as healthy people, and (d) whether bipolar patients can understand synesthesia as well as healthy controls.
Twenty-eight schizophrenic patients, 28 patients with bipolar disorder, meeting diagnostic criteria, as defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and 28 healthy controls, were analyzed in two subgroups of male and female participants, who completed Synesthesia battery[4] and a designed metaphor task.
The results of battery and the task in schizophrenic patients were significantly lower, in comparison with bipolar patients' (p<0.01). The responses to the metaphor task were more literally comprehended in the schizophrenic group as compared with the bipolar and control groups. No significant differences were observed in the results between the healthy control and bipolar group tasks.
The results revealed a strong correlation between synesthesia and metaphor recognition which could stem from co-existing common neurological structures[5]. Previous research has indicated that the formation of synesthesia occurs in the human brain before the ability to understand metaphors and abstract thinking develop[6]. Thus, this condition may determine a causal role in the ability to develop understanding abstract concepts and abstract thinking.

Keywords: Synesthesia, schizophrenia, Abstract Thinking, Metaphors, bipolar.

PAROXYZMAL DEPOLARIZATION SHIFT – IS IT AN INTERICTAL PHENOMENON IS HUMANS?

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Paroxysmal depolarization shift (PDS) was extensively studied in animal models of acute and subacut epilepsy. Based on the observation that PDS occurred between behavioral seizures it had been complemented by interictal discharges, that can be recorded in humans in the seizure onset zone (SOZ) and around. PDS is characterized by excessive discharge of large number of neurons within a certain area of the cortex. Studying human SOZ with microelectrodes revealed that selective or no neuronal firing increase happen during IIDs.
In this poster we demonstrate interictal and ictal neuronal discharge patterns in humans and compare to PDS recordings from animals. Based on similarities and discrepancies we try to conclude how neuronal hyperexcitability can be interpreted in human chronic epilepsy compared to our understanding from animal models.

NAVIGATION IN DYNAMIC ENVIRONMENTS: IMPAIRMENT OF COGNITIVE COORDINATION AND BEHAVIORAL FLEXIBILITY IN SCHIZOPHRENIA AND OBSESSIVE-COMPULSIVE DISORDER

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The assessment of cognitive functions represents a crucial step in the diagnostics and therapy of mental disorders such as schizophrenia (SZ) and obsessive-compulsive disorder (OCD). Both disorders share impairment of executive functioning affecting mental flexibility and cognitive coordination. We present the data obtained in a human analogue of the task performed on a rotating arena that demonstrated its sensitivity towards cognitive changes observed in animal models of SZ and OCD. Two versions of the virtual Carousel Maze Task (vCMT) have been created to asses [A] spatial learning and cognitive coordination in schizophrenia or [B] mental flexibility and inhibition control in OCD. 1) In the vCMT-A task used in SZ the original avoidance paradigm was adapted to a preference task. The virtual analogue thus requires subjects to navigate towards several hidden positions bound either to the reference frame of the rotating arena or the surrounding room. We will demonstrate the deficit of cognitive coordination in first episode schizophrenia patients in vCMT-A consisting of four different phases. 2) In addition, both preference and avoidance variants of the task have been created for OCD patients. We will present preliminary results obtained using these two vCMT-B task variants in unmedicated OCD patients to demonstrate the impairment of mental flexibility and inhibition control previously reported using the original carousel maze in animal model of OCD. Our results correspond well with studies testing animal models of schizophrenia and OCD in original Carousel maze paradigms, suggesting the vCMT task as a sensitive task for cognitive screening in future comparative studies.

The study was supported mainly by the projects of Ministry of Health of the Czech Republic grant number AZV 15-34524A, AZV 15-29370A and 17-30833A, and by the project Nr. LO1611 with a financial support from the MEYS under the NPU I program.

DISTINCT EFFECTS OF ACTIVATING THALAMIC AND EXTRATHALAMIC INHIBITION ON CORTICAL ACTIVITY AND BEHAVIOR

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Inhibition has a crucial role in the proper function and regulation of neuronal networks. In the thalamus, inhibition is provided by thalamic and extrathalamic (ET) sources. Thalamic inhibition originates in the nucleus reticularis thalami, (nRT) whereas ET inhibition consists of fibers arising from the basal ganglia, the zona incerta, the anterior pretectal area, the pontine reticular formation (PRF) and the ventral pallidum (VP).
Thalamic and ET inhibition have distinct anatomical and physiological properties. Previous results demonstrated that selective stimulation of nRT with single laser pulses evoked sleep spindles in the first order ventral posteromedial nucleus. However, selective stimulation of ET fibers (PRF) with a train of pulses in the higher order intralaminar nucleus evoked slow cortical oscillation and behavioral arrest. Whether these effects are specific to the type of inhibitory afferent or rather the thalamic nucleus specify the effects are presently unclear.
To answer these questions we selectively labeled nRT or ET inhibitory afferents with conditional channelrhodopsin injected into the rostral nRT or the VP of vesicular inhibitory amino acid (VIAAT)-Cre mice. We activated nRT and VP GABAergic terminals in the medial part of the mediodorsal thalamic nucleus (MDm) with trains of stimuli (5, 9, 30 Hz) in awake and sleeping states. We recorded the behavior and the LFP activity from parietal and frontal cortices.
Selective activation of nRT terminals in all frequencies resulted in large amplitude rhythmic cortical LFP activity which was prominent in the ipsilateral frontal cortex, the projection zone of MDm. The animals did not display overt behavioral response. Five and 9 Hz stimulations were effective in the awake state, but at 30 Hz the evoked response appeared in both states. The evoked oscillation displayed significant waxing and waning and considerable jitter relative to the stimulus.
Stimulation of the VP fibers in MDm also induced a rhythmic oscillation in the frequency of the stimulation which emerged in both conditions and was present in frontal as well as parietal cortical recordings, ipsi- and contralaterally. Furthermore for 30 Hz stimulation in the sleeping period the animal woke up, whereas in the awake state it elicited significant motor disturbances.
According to our data, both nRT and ET inhibition is able to recruit cortical oscillation via the MDm, probably due to a precise inhibitory control which enables thalamic cells to fire in a specifically defined time windows. Thalamic inhibition had a focal effect whereas ET inhibition evoked a wider synchronization between cortical areas even though the same thalamic cell population were entrained. The differences in the effects are presumably due to the difference in nRT and ET inhibitory action and/or the antidromic activation of ET terminals outside the thalamus. We conclude that both nRT and ET inhibition may have significant role in controlling cortical oscillations.

A THREE-HIT THEORY BASED DEPRESSION MODEL IN PACAP MUTANT MICE.

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Major depression is a common cause of chronic disability and is defined as a multifactorial disease with genetic and environmental risk factors shaping the epigenome and the adaptation ability. As currently no widely accepted animal model is available for depression, our main aim was to set up and test a new approach based on the three-hit concept of vulnerability and resilience. We combined genetic predisposition (hit 1, mutation of pituitary adenylate cyclase-activating polypeptide, PACAP gene), early-life adversity (hit 2, 180-min maternal deprivation, MD180) and chronic variable mild stress (hit 3, CVMS) in mice. Physical, endocrinological and behavioral tools were used to evaluate the model's validity according to the Willnerian criteria. Functional morphological experiments were performed to assess the chronic neuronal activity (i.e. FosB expression) of corticotropin-releasing factor (CRF) producing neurons in the bed nucleus of the stria terminalis, urocortin 1 expressing cells in the centrally projecting Edinger-Westphal nucleus and of serotoninergic cells of the dorsal raphe nucleus.

The effectiveness of CVMS was proved by changes in body- and adrenal weight as well as altered corticosterone titers. Forced swim test indicated increased depression level upon CVMS in PACAP heterozygous mice with MD180 history. This finding was accompanied by elevated anxiety level in marble burying test. CRF neurons in the oval division of the bed nucleus of the stria terminalis showed increased FosB expression, which turned out to be refractive to CVMS exposure in wild-type and heterozygous mice. Urocortin1 neurons became over-active in CMVS-exposed PACAP knock out mice with MD180 history. This suggested that the centrally projecting Edinger-Westphal nucleus contributed to the reduced depression and anxiety level of stressed knock out mice. The serotoninergic neurons of the dorsal raphe nucleus in MD180 mice lost their ability to adapt to CVMS. Based on the Willnerian construct and face validity criteria, we conclude that MD180 PACAP heterozygous mice on CD1 background upon CVMS may be used as a reliable model for the three-hit theory. Morphological results further support the model's reliability as they prove that multiple stress-recruited systems are affected, which is also in line with results of human studies. In our ongoing project the predictive validity criterion of this model by selective serotonin reuptake inhibitor treatment is being evaluated (Farkas et. al. Neuroscience. 2017 354:11-29).

Financial support: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, Research grant of the University Pécs, PTE ÁOK KA 2017-01, Hungarian Research Grant No.: OTKA PD 100706, GINOP-2.3.2-15- 2016-00050 "PEPSYS", MTA-TKI. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

A NOVEL GLIAL-NEURON CIRCUIT IN THE EXTERNAL ZONE OF THE MEDIAN EMINENCE REGULATING HYPOPHYSIOTROPIC TRH NEURONS VIA THE ENDOCANNABINOID SYSTEM

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Tanycytes are special glial cells, lining the floor and lateral wall of the third ventricle behind the optic chiasm and have an important role in the regulation of the hypothalamic-pituitary-thyroid (HPT) axis. In the external zone of the median eminence (ME), β2-tanycyte processes closely intermingle with the axon terminals of hypophysiotropic thyrotropin-releasing hormone (TRH) neurons that are adjacent to capillaries of the hypophyseal portal circulation. Since we have previously demonstrated that hypophysiotropic axons in the ME contain type 1 cannabinoid receptor (CB1R), we hypothesized that tanycytes may regulate the release of TRH via the endocannabinoid system.

To test this hypothesis, we first determined whether hypophysiotropic TRH neurons synthesize CB1R, and demonstrated that 73.4±1.5% of TRH neurons in the hypothalamic paraventricular nucleus (PVN), the region where the hypophysiotropic TRH neurons are located, expressed CB1 mRNA. Furthermore, we demonstrated that the majority of the TRH-axon terminals in the ME contained CB1R-immunoreactivity (IR), and that diacylglycerol lipase alpha (DAGLα), the enzyme synthesizing the CB1R ligand 2AG, is present in tanycytes in the ME. Triple-labeling immunocytochemistry confirmed that the CB1-IR TRH axon varicosities in the ME were closely associated to DAGLα-IR tanycyte processes.

To explore the potential physiologic significance of this anatomical association, using ME explants incubated with a TRH-degrading enzyme inhibitor (a phosphinic analogue of TRH), we demonstrated that the CB1R antagonist (AM251) markedly stimulated TRH release, whereas the CB1R agonist (WIN55-212-2) inhibited the TRH release in the presence of the DAGLα inhibitor (tetrahydrolipstatin). These results indicate that tanycytes inhibit the release of TRH from the hypophysiotropic terminals in neural-hemal zone of the ME via the endocannabinoid system. In addition, since endocannabinoid synthesis is stimulated by glutamate (Glu) in neuronal synapses, and we have shown that the hypophysiotropic TRH neurons have a glutamatergic phenotype, we used whole cell patch clamp electrophysiology to investigate whether Glu can influence the tanycytes in the ME. Application of 0.5 mM Glu markedly increased the resting membrane potential of tanycytes via both kainate and AMPA receptors, and also by TBOA-sensitive glutamate transport in the presence of TTX.

In summary, these data indicate that in the external zone of the ME, tanycytes regulate hypophysiotropic TRH secretion via endocannabinoid release, while the TRH axons regulate tanycytes by the release of glutamate, suggesting the existence of a reciprocal microcircuit between tanycytes and TRH terminals that may be involved in the regulation of pulsatile TRH release.

MAPPING THE NEURONAL ACTIVATION OF PARENTAL BRAIN IN FEMALE ZEBRA FINCHES

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The neuronal mechanisms of parental behaviour are well studied in mammals, while in other species, especially in birds, our knowledge is limited about the neuronal regulatory network responsible for parenting. Therefore, in the present study, we examined the neuronal activation in response to feeding behaviour in females of the gregarious zebra finches (Taeniopygia guttata). To identify brain areas, which are activated during feeding, we immunohistochemically labelled the protein product of the immediate early gene c-fos in parenting and non-parenting zebra finches. The experiment was performed when the offspring reached the age of 13 days, post-hatching. During the manipulation, the offspring and the male were placed to another room, while the female stayed alone in the home cage in order to reduce activation of brain areas involved in parenting. On the following day, the offspring were separated from the males for 2 hours to facilitate begging. The separation period was followed by the reunion of the female and the offspring for 90 minutes, which was characterized with intense feeding behaviour by the females. Subsequently, the females were fixed by transcardial perfusion for fos immunohistochemistry. In the non-parenting females the reunion was not executed, so parental behaviour was not performed.
As a result of feeding the offspring, neuronal activation was found in several brain regions. The most significant increase in the number of c-fos-positive neurons was measured in the medial nucleus of the posterior hypothalamus.
This is the first study determining brain areas which are activated during offspring provisioning in birds. We found a number of different brain regions with elevated neuronal activity. These brain areas may participate in the regulation of parental behaviours as part of the social behavioural network in zebra finches.

Grant support: NKFIH OTKA K116538 and the KTIA NAP_B_13-2-2014-0004 Research Program

ROLE OF GLUTAMATE VESICULAR TRANSPORTER 3 IN LEARNING: TESTS ON KNOCKOUT MICE

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Introduction: Glutamate, as a neurotransmitter, and its receptors have been found to have an important role in learning and memory, which are crucial to adapt to the ever-changing enviroment. The glutamaterg neurons are characterized by the vesicular glutamate transporters (VGluT1-3). Among them, VGluT3 has been discovered most recently. Its occurance is rarer and often colocalises with other classical neurotransmitters. Our aim was to characterize the role of VGluT3 in the process of learning and memory formation comparing knockout (KO) and wild type (WT) mice.
Materials and methods: Three tests were conducted, each testing different aspects of the memory. On Rotarod (5 consecutive days, 3 trials per day) motor learning, on Y-maze (5 minutes) working memory, and in holeboard test spatial memory was studied. Due to the highly anxious nature of the strain, the holeboard test started with 2 days 15 minutes habituation. Then, for 5 consecutive days there were 6 trials per day, while out of the 16 holes 4 were baited, which had to be found within 3 minutes. This was followed by 2 days of reversal learning with four other rewarded holes. Throughout all these trials, extramaze cues were provided. On the final day one trial was done in dark to test whether those cues were used by the mice.
Results: On Rotarod the falling latencies of KO mice were higher than that of WT mice. Thus, the KO strain had no motoric problems. In Y-maze only the WT mice had normal spontaneous alteration, which indicates normal working memory, while KO mice did not have it. In holeboard tests the number of mice properly learning and the reference memory errors were not dependent on the genotypes. However, the working memory errors and the error of omission were higher in KO mice than in their WT littermates. Moreover, WT mice proved to be more flexible during reversal learning. Both KO and WT mice errors were significantly higher during the dark test than before, indicating that they did use the extramaze cues.
Discussion: Knocking out the VGluT3 gene did not have any effect on spatial orientation. However, it did had an impact on short-term working memory and learning flexibility.

PACAP PROTECTS AGAINST NEOVASCULARISATION IN HUMAN RETINAL PIGMENT EPITHELIAL CELLS

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Neovascularisation plays a key role in many retinal diseases, such as diabetic retinopathy and macular degeneration, some of the leading causes of blindness in the working population. Under these conditions the integrity of the pigment epithelial cells is disrupted, thus photoreceptor survival and normal vision is impossible. In addition, the retinal pigment epithelial cells are very important elements of the blood-retina barrier, and they are also known to express different angiogenic factors, such as VEGF (vascular endothelial growht factor), endothelin or angiogenin.

PACAP is known to exert retinoprotective effects, against several types of retinal injuries in vivo, including optic nerve transection, retinal ischemia, excitotoxic injuries, UV-A-induced lesion and diabetic retinopathy. Our research group previously showed that PACAP activates antiapoptotic pathways and inhibits proapoptotic signaling in retinal lesions in vitro as well. In this study we examined the possible antiangiogenic effect of PACAP on ARPE cells in sucrose induced hyperosmotic condition. Cells were treated with 200 mM sucrose for 24 hours and the expression of angiogenic markers was investigated by specific arrays, and flow cytometry. Our results showed that sucrose administration increased different proangiogenic factors like VEGF, EG-VEGF, uPA, while PACAP could decrease their levels. In accordance with several previous studies these findings also suggest, that PACAP could be a possible candidate in the treatment of diseases caused by neovascularisation.

Acknowledgement: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, GINOP-2.3.2-15- 2016-00050 “PEPSYS”, the National Research, Development and Innovation Fund K119759, MTA-TKI, PTE AOK KA Research Grant, Bolyai Scholarship, National Brain Research Program (KTIA_13_NAP-A-III/5), TAMOP 4.2.4.A/2-11-1-2012-0001 „National Excellence Program”, The UNKP-16-3-IV, UNKP-16-4-III, UNKP-16-4-IV, New National Excellence Program of the Ministry of Human Capacites, and Centre for Neuroscience, University of Pecs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

FUNCTIONAL CONNECTOME CHANGES IN AN ACUTE KETAMINE MODEL OF SCHIZOPHRENIA IN RAT.

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The connectome is the multilevel and timed connection matrix of the brain recruited for information processing. Anatomical connections form the fundamental brain network but functionally the different types of oscillatory electric activity create a temporarily connected fraction of the anatomical connectome generating an output to the motor system. There are so called connectome diseases like Schizophrenia in which the sensory input generates a schizophrenia specific temporary connectome, in turn, the signal processing became diseased showing hallucinations and adverse behavioural reactions. Our aim in this study is to demonstrate how the connectome changes are reflected in an animal model of schizophrenia in local field potential recording from the rat cortex.
Recently, a plastic foil based 32 channel electrode was developed in our laboratory and MTA EK MFA and we were able to print 32 electrode surfaces on a 3 times 7mm area. This electrode can be used to investigate functional coherence on large areas during normal physiological activities. After the control period, low dose (15 mg/kg IP.) ketamine injection was performed inducing schizophrenia like state. In control and ketamine treated animals we recorded visually evoked potentials (VEP) to measure the visual data processing. Comparing the two groups of data we were able to disclose the phase and power relationships among the oscillatory EEG activity driven connectivity changes after ketamine injection.
The data is analysed with coherence mapping combined with advanced 2 dimensional kernel current source density analysis. We revealed high stability in different EEG synchronization states and also under ketamine effect (see D. Cserpán's poszter). The pattern of high coherence cortical sites are corresponding to the anatomically identified functional brain areas of the rat brain. Multichannel coherence analysis also allowed to demonstrate the connectome changes in different vigilance states of the rat. Flash induced VEP as a stimulus driven form of connectivity controlling mechanism also showed reproducible changes under ketamine application. The plastic foil based multichannel electrode is able to detect brain waves of freely moving rats similarly to the human EEG mapping technologies. As such it is suitable for translational studies on animal models of schizophrenia and the results are comparable with the data obtained on human subjects. Our present technology provided an excellent translational tool for schizophrenia research and drug development.

THE SPECIFICATION OF A STANDARD COMPRESSION-INDUCED SPINAL CORD INJURY MODEL WITH LOW SPONTANEOUS RECOVERY OF MOTOR FUNCTION IN RAT

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Traumatic spinal cord injury represents for patients a serious clinical problem with an influence on their physical and mental health. Up to present time, there is still no effective therapy for trauma-injured spinal cord, which could recover a neurological function loss and integrate patients to the normal life. Development and application of an effective therapy to clinical practice depends on various factors. One of them is a proper selection of experimental animal model which sufficiently simulate a formation and progress of injury in human. The main aim of our experiments was to specify a standard compression model of traumatic spinal cord injury that offers reproducible results at morphological, molecular and behavioural level and causes slow spontaneous regeneration of motor hind limb function, which is extremely important for an appropriate possible therapeutic effect determination.
Adult Wistar female rats underwent a spinal cord compression at Th 9-10 level with 30g, 40g and 50g weights lasting 15 minutes. We were testing their motoric hind limb function by BBB score for 28 days. We also monitored a persistence of their bladder function loss. After 4 weeks, we were determined the extent of damage in nervous tissue with the standard histological staining - Luxol fast blue combined with Cresyl violet and quantified by ImageJ program. The quantification of main components of axons - neurofilaments (NF) and myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP) was carried out by western blot analysis and confirmed by imunofluorescent labeling. The largest tissue lost were determined in the impact site and it was reduced to rostral and caudal direction. The rostro-caudal extent of tissue damage was dependent on compression force. The locomotor testing by BBB score demonstrated the slow spontaneous regeneration of hind limb function in all experimental animal groups. The bladder function loss was persisted for 2-3 weeks after injury. Neurological function returns, both motoric and sensory also depended on compression force. The lowest amount of MBP and NF was found in the epicenter of injury, what reflect a significant damage of axons, the main structural components of white matter. The levels of both proteins gradually increase in rostro-caudal direction from lesion site. The lowest amount of GFAP was determined also in the impact region and the highest GFAP expression was detected in the regions closest to the lesion site, while it gradually decreases in rostro-caudal direction. On the basis of behavioral testing and histological and biochemical analysis as the most appropriate model was selected 40g compression lasting 15 minutes.

Supported by VEGA Grant No. 2/0160/16.

REGENERATION AND NEUROPROTECTION AFTER SPINAL CORD INJURY INDUCED BY SECRETOME-BASED THERAPY

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Spinal cord injury results in irreversible tissue damage followed by very limited recovery of function. In our earlier study we grafted neuroectodermal stem cells (NE-GFP-4C) into the spinal cord following contusion injury. The stem cell grafts have induced significant functional recovery supported by extensive axonal regeneration. The grafted cells produced GDNF, IL-6, IL-10 and MIP-1 alpha. Functional blocking experiments proved that these factors played pivotal role in the functional recovery. Based on these results, we hypothetise that intraspinal delivery of these factors into the injured spinal cord may be able to induce as good morphological and functional recovery as grafted neuroectodermal stem cells.
A thoracic (T11) contusion injury was induced by an Infinity Horizon impactor with a force of 150 kdyn, without disrupting the dura mater. The delivery of secretome factors started 7 days after contusion for 2 weeks via osmotic pumps directly into the lesion cavity. Animals in the control groups received only saline either via osmotic pumps or without pump. In the positive controls NE-GFP-4C neuroectodermal stem cells ($5x10^5$) were grafted intraspinally 7 days following the injury. Locomotor analysis of the animals was performed through the use of the BBB test and a detailed kinematic analysis system of the hind limb movement. The extent of supra- and propriospinal axonal sparing/regeneration was determined by retrograde tracing with Fast Blue from a hemisection gap produced two segments caudal to the injury cavity. Detailed morphological analysis was performed to evaluate the effects of grafted stem cells and factor treatment.
In animals treated with NE-GFP-4C stem cells consistent plantar stepping with predominantly parallel paw position and consistent forelimb and hind limb coordination could be observed. Application of osmotic pumps with all the four factors showed similarly results, but these animals rotated predominant paw position during locomotion. In contrast, the control animals showed frequent weight-supported plantar steps and frequent forelimb and hind limb coordination. Larger numbers of Fast Blue labelled propriospinal neurons was counted in the animals belonging to groups with stem cell grafting and secretome treatment than in control groups. Interestingly, the number of retrogradely labelled corticospinal neurons was highest in stem cell-treated group. Morphologically, the contusion cavity at the epicenter was significantly smaller in grafted and secretome-treated animals than in controls. The amount of spared white matter was significantly greater in treated cords compared with controls. These results suggest that the secretome-based treatment is a promising putative therapeutic approach to treat spinal cord injury.

CO-AGONISTS DIFFERENTIALLY TUNE GLUN2B-NMDA RECEPTOR TRAFFICKING AT HIPPOCAMPAL SYNAPSES

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The subunit composition of synaptic NMDA receptors (NMDAR), such as the relative content of GluN2A- and GluN2B-containing receptors, greatly influences the glutamate synaptic transmission. The receptor co-agonists, glycine and D-serine, have intriguingly emerged as potential regulators of the receptor trafficking in addition to their requirement for its activation. Using a combination of single molecule imaging, biochemistry and electrophysiology we show that glycine and D-serine relative availability at rat hippocampal glutamatergic synapses regulate the trafficking and synaptic content of NMDAR subtypes. Acute manipulations of co-agonist levels, both ex vivo and in vitro, unveil that D-serine alter the membrane dynamics and content of GluN2B-NMDAR, but not GluN2A-NMDAR, at synapses through a process requiring PDZ binding scaffold partners. In addition, using FRET-based FLIM approach, we demonstrate that D-serine rapidly induces a conformational change of the GluN1 subunit intracellular C-terminus domain. Together our data fuels the view that the extracellular microenvironment regulates synaptic NMDAR signaling.

FUNCTIONAL CONNECTIVITY NETWORKS OF CORTICO-CORTICAL EVOKED POTENTIALS

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The electrical stimulation of the cortical tissue can evoke an early (N1) and late (N2) cortico-cortical evoked potential (CCEP) components. In this study we applied functional connectivity analysis to investigate the resilience and network topology induced by the electrical stimulation. We also compared the synchronization and network topology in case of the stimulation inside and outside the epileptic zone (EZ). 16 presurgery epileptic patients were analyzed with subdural grid positions. The CCEP mapping was performed by injecting current pulses (10 mA, 0.2ms pulse width, 25 trials) into all adjacent electrode contacts. Dynamic functional connectivity (phase synchronization across trials) was applied on the N1 (10-50ms; filtered to 10-40 Hz) and N2 (50-500ms; filtered to 1-4 Hz) evoked potentials. We applied Minimum Spanning Tree analysis to the express topological parameters of the networks. N1 has a higher local synchrony and faster resilience from the stimulation point toward more distant electrodes than N2. N1 has a more star-like network topology than N2. N1 synchrony was higher and the network became more centralized, when the current was injected to the EZ. The neural mechanism of N1 based on direct cortico-cortical connections, while N2 thought to be the result of more complex network topologies, which can explain our more distributed N2 network compared to the local N1 network. The extensive synchrony and centralized topology of the N1 network in case of EZ stimulation support the idea of increased excitability of the epileptogenic zone.

TISSUE ACIDOSIS-INDUCED PAIN

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The talk will discuss the mechanism essential for detection of acidosis in humans. The occurrence of local acidosis in inflammation is known since 1930. The metabolic acidosis in not sufficiently oxygenated but metabolically active tissue plays a pathophysiological relevant role in the brain, in the heart, in rapidly growing tumors, in intermittent claudication, in the eroded gastric mucosa and, more general, in inflammation. In areas of local hypoperfusion the hypoxia leads to a pH drop, the extent of which, even without synergism with other factors, may be sufficient to sensitize and activate nociceptive neurons, and thus trigger pain. As molecular mechanisms acid sensing ion channels, and shortly thereafter the proton sensitive capsaicin receptor TRPV1 have been described. In addition many ion channels have a reduced conductivity in an acidic medium, which would add excitatory drive when blocking potassium channels which contribute to the resting membrane potential. The chemo-sensitive ion channel TRPA1 was published in 2003; the species differences described for various agonists between rodents and humans may explain why the exclusive pH sensitivity of the human TRPA1 channel was found only in 2014. The presentation will cover this background and more recent findings which seriously question the involvement of the targets mentioned above in a psychophysical study in healthy human subjects. Neither inhibition of TRPV1 by BCTC, nor inhibition of TRPA1 by A-967079 nor inhibiting acid-sensing ion channels by amiloride changed the acidosis-induced pain ratings in human skin. Once again, the nature of the principal acid sensor in humans is unclear.

DETOUR TEST AS AN INNOVATIVE TOOL FOR EXAMINING PERSEVERATIVE BEHAVIORS AND LEARNING SKILLS IN SHEEP

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Perseveration is an uncontrolled repetition of a behavior which occurs despite the absence or cessation of the stimulus that evoked it. Perseverative behaviors are key symptoms of several neuropsychiatric diseases and previous studies in this field have used the sheep as an animal model. However, there is a lack of tools to examine perseverative behaviors in sheep. The aim of this study was to examine perseverative behaviors, as well as learning and memory abilities in lambs. To this aim, 17 lambs (Sarda breed, 37-days-old) were subjected to the detour test, in which the lambs had to detour a V-shaped semitransparent obstacle to reach the dam on the opposite side. On the first day of the test, lambs were subjected to three consecutive trials and could choose any side of the obstacle to reach the aim. Two days later, lambs were first subjected to three trials the same as in day 1 to examine their memory skills. Subsequently, the side preferred by the lamb was closed for three successive trials, in order to examine perseverative behaviors and the ability to change habits. The time spent to reach the dam was calculated in all trials/days. Moreover, the frequencies of returns to the center of the "V" and to the closed side were analyzed as a measure of perseveration. On day 1, lambs completed the task in average 43.5, 13.12 and 9.18 seconds, on trial 1, 2 and 3, respectively ($P<0.0001$). On day 2, lambs completed the task in average 4.63, 7.21 and 9.57 seconds (non-significant differences), on the first three trials. Lambs solved the maze either from the right or left sides (59% - right, 41% - left) and kept their choice in all subsequent trials until the chosen side was closed. The average time spent to conclude the task was significantly increased in the first trial when the chosen side was closed (139 sec.), compared to the others, including the first trial on day 1 ($P<0.0001$). The average frequency of returns to the center of the „V" was 4.35 times on the first trial, and less than 1 on trials 2-3 of day 1 as well as on trial 1-3 of day 2. The average frequency of returns to the center was significantly increased in the first trial when the chosen side has been closed (8.94 times), compared to the others ($P<0.01$). The frequency of returns to the closed side was higher on the trial when for the first time the gate has been closed, compared to the following trials ($P<0.0001$). Obtained data show that lambs have a predisposition toward learning how to solve a win-shift spatial problem in a detour test under social motivation and are able to memorize it. The results from the day 2 indicate the occurrence of perseverative behaviors when a further obstacle interdicts the learned task. In conclusion, the detour test can be an effective tool for examining perseverative behaviors in lambs and might be used to assess behavioral outcomes in ovine models of brain disorders.

SODIUM CHANNEL BLOCK AND MODULATION BY RILUZOLE AND ITS PHOTOREACTIVE AZIDE DERIVATIVE

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The neuroprotective drug riluzole preferentially inhibits the persistent component of sodium currents, the up-modulation of which is involved in hyperexcitability-related disorders such as epilepsy, ALS, pain syndromes and arrhythmias.
In order to be therapeutically efficacious, sodium channel inhibitors must have a certain type of functional selectivity (e.g., persistent component selectivity, selective inhibition at a specific firing frequency, selective inhibition of cells with pathologically low membrane potential). Specific types of functional selectivities are caused by specific types of inhibition mechanisms. The inhibition mechanisms by sodium channel inhibitors are diverse, and significant details are still unknown, such as: i) the contribution of channel block (steric or electrostatic inhibition of ion flux through the pore) *vs.* modulation of channel gating (making non-conducting conformations energetically more favorable); ii) the contribution of state-dependent accessibility *vs.* state-dependent affinity; iii) the kinetics of drug access/egress and binding/unbinding processes.
We intended to study the mechanism of inhibition by riluzole, using the compound itself, and its photoreactive analog, azido-riluzole. When sodium currents were evoked by double depolarizing pulses with <2 ms hyperpolarizing gaps between them, post-gap currents were fully inhibited, indicating that binding sites were fully occupied by riluzole. However, with ~30-40 ms hyperpolarizing gaps ("resting block") the inhibition was minimal (~10 %). This may indicate either fast unbinding or modulation (delayed recovery from inactivation), or both. We studied the kinetics of riluzole onset and offset, and found the offset to be too slow to explain lack of inhibition after ~30 ms. The contribution of fast unbinding and modulation was also tested using azido-riluzole. In the absence of UV irradiation azido-riluzole caused similar "resting block" to riluzole, but weaker inhibition after <2 ms gaps, and both effects were fully reversible upon wash-out. After UV irradiation both "resting block" and modulation became stronger, and both persisted after wash-out. This indicates that sodium channels could conduct ions even with covalently bound inhibitor. After perfusion with 100 μM azido-riluzole, 3 min of UV irradiation, and wash-out, inhibition by block was 70 ± 5.0 %, while together with modulation the inhibition was > 95 %.

HYPOTHALAMIC CONTROL OF RAPHÉ CIRCUITS

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Originating from cell clusters in the brainstem dorsal raphé nuclei (DRN), serotonin (5-hydroxytriptamine, 5-HT) is an important neuromodulator implicated in many (patho)physiological functions. 5-HT neurons are involved in the regulation of brain states, mood, reward and sensory processing, but the origin of their activity has remained relatively unexplored. Using anterograde and retrograde viral tracing techniques in various transgenic animals we show that the lateral hypothalamus (LH), a brain area involved in the regulation of metabolic states and sleep sends massive glutamatergic and GABAergic projections to the DRN. Using a combination of local photostimulation of ChR2 expressing LH axons in the DRN, patch clamp recordings and post hoc immunohistochemistry in the recorded neurons we show that glutamatergic LH projections monosinaptically target identified 5-HT neurons via AMPA/KA receptors. Identified DRN GABAergic neurons, on the other hand, receive monosynaptic GABAA mediated inhibitory inputs from the LH. This cell type specific modulation of DRN circuits by the LH was also encountered in single unit recordings performed in anesthetized and awake head restrained animals. These results identify the LH as a potent and specific modulator of DRN circuits and could have important implications in various serotonergic functions.

CHARACTERIZATION OF SEROTONINERGIC BRAIN SYSTEM AND SEROTONIN-ASSOCIATED BEHAVIOR IN TNF DEFICIENT MICE

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Background: Both serotonin and tumor necrosis factor (TNF) are expressed in the central nervous system and can modulate the same type of behavior such as aggression and depressive-like behavior. However, there is no research studying the interaction among this type of behavior, the serotonergic system and TNF *in vivo* at the same time.
Methods: To study the TNF effect on the serotoninergic system we used adult males of TNF deficient (KO) and wild-type (WT, C57Bl/6) mice. We studied the behavior in open-field test (OF), intermale aggression and forced-swim test (FST), the functional activity of $5HT_{1A}$ and $5HT_{2A}$ receptors, the levels of serotonin and its metabolite 5-hydroxyindolacetic acid (5-HIAA) in the midbrain (MB), the frontal cortex (FC) and the hippocampus (HPC) in KO and WT mice. In addition, the levels of mRNA of *HTR1A* (gene coding $5HT_{1A}$) and *HTR2A* ($5HT_{2A}$) in these structures, *SLC6A4* (serotonin transporter) and *TPH2* (tryptophan hydroxylase 2) in MB were evaluated.
Results: KO and WT mice did not differ in motion activity measured in OF, the percentage of aggressive animals, and number of attacks, but the attack time was significantly lower in KO mice ($p<0.05$). In the FST KO mice showed decreased depressive-like behavior (evaluated as the time of immobility) as compared to WT mice ($p<0.05$). The levels of serotonin in FC ($p<0.01$) and HPC ($p<0.05$), but not in MB were higher in KO mice. There were no differences in 5-HIAA levels in all structure of both strains. The functional activity of the $5\text{-}HT1_A$ receptor did not differ between strains, but the functional activity of the $5\text{-}HT_{2A}$ receptor was higher in KO mice. There were no difference in the expression of *HTR1A* and *HTR2A* in all structure between WT and KO. In MB there were no difference in the level of *TPH2,* but the expression of *SLC6A4* was significantly higher in KO mice ($p<0.05$).
Summary: In our study for the first time we have described the effect of TNF deficiency not only on the levels of serotonin, but also on the functional activity and the mRNA levels of its receptors. We have showed that in KO mice the level of serotonin in HPC and FC as well as the functional activity of the $5HT_{2A}$ receptor are higher than in WT mice leading to the decrease in levels of aggression and depressive-like behavior. Probably in order to compensate the high level of serotonin the expression of the serotonin transporter is upregulated in KO mice. This work opens up a new perspective in studying TNF being serotonergic system interaction.

The study is supported by Russian Foundation for Basic Research (15-04-08254).

REPRESENTATION OF BINOCULAR DISPARITY IN THE HUMAN BRAIN USING FUNCTIONAL MAGNETIC RESONANCE IMAGING

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Introduction: Perception of depth based on retinal disparities derives from the horizontal separation of the eyes. Disparity can be crossed or uncrossed depending on the relative distance of the target from the fixation plane; thus disparity is an exact measure of binocular depth. Our previous study, based on reaction time (RT) measurements, suggests two distinct neural processing mechanisms for crossed and uncrossed disparities. The aim of our present study was to confirm the previous findings with functional magnetic resonance imaging (fMRI) and study the disparity tuning of cortical activation.
Methods: Data of five subjects (21 to 24 years) with normal or corrected to normal vision and normal binocular depth perception are analysed in this study. fMRI was performed in a 3T Siemens scanner. FSL 5.0.9 was used to analyse fMRI data. Stimuli were presented on NordicNeuroLab's VisualSystem with stereoscopic goggles. We used a block design, where both stimulus and interstimulus time lasted for 18 s. Stimuli were dynamic random-dot stereograms with a central square target at 2x5 different disparities (±2.3, 6.8, 15.9, 29.5, 59 arcmin) and a central fixation point. In each block the depth of the target alternated between zero and one of the tested disparities at 0.67 Hz. During the interstimulus time, zero disparity random dots were presented with the central fixation point. Each disparity condition was repeated 5 times in a session. In order to accurately identify visual areas the examination was completed with retinotopic mapping.
Results: First we calculated the average response for each disparity. The occipital pole, lateral occipital cortex, superior parietal lobe, intraparietal sulcus and temporo-occipital areas comprised significantly responding voxels to the visual stimulation. Any other additional activation in the dorsal frontal lobe was most probably non-visual (e.g. supplementary or frontal eye field). We compared the activation between crossed and uncrossed stimuli and investigated whether either the crossed or uncrossed disparities or both showed a U shaped activation trend similarly to the RTs. There was no significant observable difference between the type of disparities but we surprisingly found a decreasing linear trend with increasing disparity in the occipital pole for uncrossed disparities. For crossed disparities, no significant quadratic or linear trend was found in the responses.
Conclusion: Our experiment confirms that disparity is represented in the occipital pole and the above listed brain areas. These regions are most likely part of the dorsal visual stream. However, the precise location of the activity has to be determined by using retinotopic mapping. The blood oxygenation level dependent (BOLD) responses are yet to be determined too.

Acknowledgements: Hungarian Brain Research Program "KTIA_NAP_13120130001"; OTKA K108747
EFOP-3.6.1.-16-2016-00004
Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs
The project has been supported by the European Union, co-financed by the European Social Fund
The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

EXAMINATION OF HEARING IMPAIRMENT IN HYPOPHYSIS ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) KNOCKOUT MICE

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PACAP is a polypeptide with well-known neurotrophic and cytoprotective effects, which is also present in the inner ear and auditory pathway. We have proved its antiapoptotic effects in inner ear cell cultures against oxidative stress. We have found differences in the expression of the specific PAC1 receptor of PACAP, and of Ca^{2+}-binding proteins between the inner ear of PACAP deficient (KO) and wild type (WT) mice.
The hearing thresholds of WT and KO male mice were compared with auditory brainstem response (ABR) test. We performed immunohistochemistry of the neuron activation marker c-Fos protein in the ventral and dorsal cochlear nucleus (VCN, DCN), superior olivary complex (SOC), nuclei of the lateral lemniscus (NLL), and inferior colliculus (IC) after acoustic stimulation. Furthermore, we measured protein expression with angiogenesis array kit from cochlear duct lysates.
Hearing was significantly altered in KO mice resulting in higher hearing threshold at click and low frequency burst stimuli compared to WT mice. Morphological findings were in accordance with functional findings; after noise treatment c-Fos staining showed decreased neuron activation in the VCN and DCN in KO animals, than in WT mice. However, there was no difference in SOC, LLN, or IC between the two groups after acoustic stimulation. Several angiogenic factors differ in KO mice from WT mice. Higher IGFBP-1, acidic-FGF and lower osteopontine levels were detectable in KO mice cochlear duct lysates compared to WT mice.
We showed both functionally and morphologically that in the absence of PACAP there is impairment of hearing functions. The angiogenesis array examination showed changes at molecular level in the inner ear, which could be related to the detected changes. The exact function of PACAP in the inner ear is unknown, therefore, we plan to continue our morphological and molecular biology experiments.

Support: National Research, Development and Innovation Fund K119759; GINOP-2.3.2-15-2016-00050 „PEPSYS”; EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs; PTE AOK KA Research Grant; National Brain Research Program (KTIA_13_NAP-A-III/5); TAMOP 4.2.4.A/2-11- 1-2012- 0001; New National Excellence Program-16-4-IV; MTA TKI; Bolyai Scholarship. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

A GRAPH THEORETIC APPROACH TOWARDS MODELLING THE CONNECTIVITY OF THE CEREBRAL CORTEX AT THE MESOSCALE

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Network analysis plays significant role in understanding the structural and functional organization of the brain. In the multi-level cortical network, microscopic, i.e. single neuron based connectivity in a microscopic cortical tissue volume and macroscopic levels formed by cortical regions and areas are relatively well explored. Between these two levels at the mesoscale the cortical network is organized between neuronal populations via their modular connectivity. However, the mesoscale, or the so called columnar network architecture of the cerebral cortex is not known. To overcome this gap of knowledge, we develop a mesoscale network model of the macaque monkey cerebral cortex representing the interactions of cortical areas. The interaction network is a topologically faithful derivation of the original graph. The size and order of the derived graph is *significantly greater than* the original, while its density decreased significantly. However, only a slight increase of the diameter and the average path length *was* observed. Reciprocity is significantly reduced in the derived interaction network. Analysis of the interaction network and using the results to recalculate the indices in the original one revealed new characteristics of the areas relevant to their role in cortical signal flow. Furthermore, cluster analyses of the interaction network highlighted the diversity of functions each area of the original network is involved in. In summary, the interaction graph allows a better understanding of the role of the individual areas in cortical signal flow.

This research was supported by the grant ERA-NET FLAG-ERA, Human Brain Project, NKFIH NN-118902: CANON project.

MELANOCORTIN 4 RECEPTOR LIGANDS MODULATE ENERGY HOMEOSTASIS THROUGH UROCORTIN 1 NEURONS OF THE CENTRALLY PROJECTING EDINGER-WESTPHAL NUCLEUS

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The role of the urocortin 1 (Ucn1) expressing centrally projecting Edinger-Westphal (EWcp) nucleus in energy homeostasis and stress adaptation response has previously been investigated. Morphological and functional studies have proven that orexigenic and anorexigenic peptidergic afferents and receptors for endocrine messengers involved in the energy homeostasis are found in the EWcp. The central role of the hypothalamic melanocortin system in energy homeostasis is well known, however, no data have been published so far on possible crosstalk between melanocortins and EWcp-Ucn1. First, we hypothesized that members of the melanocortin system [i.e. alpha-melanocyte stimulating hormone (alpha-MSH), agouti-related peptide (AgRP), melanocortin 4 receptor (MC4R)] would be expressed in the EWcp. Second, we put forward, that alpha-MSH and AgRP contents as well as neuronal activity and Ucn1 peptide content of the EWcp would be affected by fasting. Third, we assumed that the intra-EWcp injections of exogenous MC4R agonists and antagonist would cause food intake-related and metabolic changes. Ucn1 neurons were found to carry MC4Rs, and they were contacted both by alpha-MSH and AgRP immunoreactive nerve fibers in the rat. The alpha-MSH immunosignal was reduced, while that of AgRP was increased upon starvation. These were associated with the elevation of FosB and Ucn1 expression. The intra-EWcp administration of MC4R blocker (i.e. HS024) had a similar, but enhanced effect on FosB and Ucn1. Furthermore, alpha-MSH injected into the EWcp had anorexigenic effect, increased oxygen consumption and caused peripheral vasodilation. We conclude that the melanocortin system influences the EWcp that contributes to energy-homeostasis.

EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, PTE ÁOK KA 2017-01. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

TAAR1 MODULATION OF D2 RECEPTOR SENSITIVITY AND DOPAMINE NEUROTRANSMISSION

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Trace Amine-Associated Receptor 1 (TAAR1) is a G protein-coupled receptor that is expressed in the mammalian brain and is known to influence monoaminergic transmission. Monoamines, such as dopamine, play an important role within the frontostriatal circuitry, which is critically involved in high order cognitive processes. TAAR1 selective ligands have shown potential antipsychotic, antidepressant and pro-cognitive effects in experimental animal models; however, the mechanisms through which TAAR1 can affect frontostriatal system-related functions remain to be clarified. In this study, we investigated how TAAR1 modulates frontostriatal signaling by analyzing dopamine- and glutamate-mediated processes and related behaviors in the striatum and the prefrontal cortex (PFC) of TAAR1 knockout (TAAR1-KO) mice. In the striatum, TAAR1-KO mice showed an up-regulation of postsynaptic D2 dopamine receptors and concomitant activation of the AKT/GSK3 pathway without changes in D1 dopamine receptor function. In the PFC, we observed deficient NMDA glutamate receptor functionality located in the pyramidal neurons of layer V, as assessed by electrophysiology, with concomitant changes in NMDA receptor subunit composition. The dysregulated frontostriatal transmission in TAAR1-KO mice was associated with aberrant behaviors in several tests, indicating a perseverative and impulsive phenotype. Conversely, pharmacological activation of TAAR1 with selective agonists reduced premature impulsive responses observed in the fixed-interval conditioning schedule in normal mice. Our study indicates that TAAR1 plays an important role in the modulation of frontostriatal function, through changes in D2 receptor-mediated dopaminergic and glutamatergic transmission. Furthermore, these data uncover novel mechanisms involved in such modulation and provide a theoretical framework to predict the therapeutic efficacy of TAAR1-based drugs in the treatment of disorders related to aberrant frontostriatal functions.

EVALUATION OF THE MOUSE BRAIN REPAIR AFTER ISCHEMIC LESION USING IN VIVO IMAGING MODALITIES AND DEDICATED TRANSGENIC MOUSE MODELS

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The brain repair after ischemic brain lesion (i.e. stroke), involves a row of interconnected events mediated by neuroinflammation and including plasticity, active removal of damaged cells by apoptosis, and possible addition of new cells through neuroregeneration. The appropriate monitoring of brain damage consequences and repair processes in the animal models is crucial in the evaluation of new therapeutic approaches. The *in vivo* small animal imaging modalities are considered as a major addition to preclinical setting as they allow the monitoring of molecular processes in the living animals. The preclinical imaging setup in our facility, Laboratory for Regenerative Neuroscience a.k.a. GlowLab, includes magnetic resonance imaging (MRI) for small animals, and optical imaging modality represented by bioluminescence imaging (BLI).
Medial cerebral artery occlusion in the mouse brain was used as an animal model of the stroke. To visualize molecular events related to the activity of Tlr2, Gap43, and Casp3 in the brain in vivo bioluminescent imaging was applied. Imaging was performed by IVIS Spectrum Pre-clinical In Vivo Imaging System (Perkin Elmer, US) in living animals genetically modified to carry luciferase reporter by recording the emitted light from the brain. The ischemic lesion was monitored by T2 MRI modality by Bruker BioSpec 70/20 USR with 7T magnetic field.
Tlr2 expression corresponding to the neuroinflammation was highly upregulated after stroke, with a peak after 2 days. Gap43 expression provided insight in axonal outgrowth and repair after stroke, reaching a peak a week after the damage. The presence of apoptosis related CASP3 in the brain was visualized by application of caged luciferin, DEVD-aminoluciferin, which was released by caspase enzymatic activity. The presence of caspase was shown in the subset of Gap43 expressing cells. In these cells, CASP3 activity increased after ischemic lesion, suggesting that CASP3 and GAP43 might be part of a common molecular pathway involved in early stress response after stroke. Monitoring inflammation, repair, and apoptosis by bioluminescent imaging in the living animals allowed to analyze brain repair after stroke. It is an important asset to monitor and evaluate innovative therapies needed for the treatment of brain diseases.

The study was supported by EU FP7 grant GlowBrain (REGPOT-2012-CT2012-316120).

INTERLEUKIN 1-BETA INDUCED CYTOKINE EXPRESSION IN PRIMARY SPINAL ASTROCYTE CULTURES

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Accumulating evidence shows that the neuron-glia crosstalk plays a key role in pain hypersensitivity. Upon activation spinal glia produce inflammatory cytokines and other factors which influence nerve cell functions leading to central sensitization and enhanced pain states. The most prominent representative of proinflammatory cytokines is interleukin-1 beta (IL-1β) which acts on its neuronal and astrocytic receptors, interleukin-1 receptor type 1 (IL-1RI) leading to cell-type specific responses. Our earlier data shows that the spinal astrocytes are the main source of IL-1β in the chronic phase of inflammatory pain and astrocytes also express the ligand binding IL-1RI unit of the IL-1 receptor.
In order to further study astrocytic activation induced by IL-1β upon different stimuli we generated primary astrocyte cultures from spinal cord of C57BL/6 and IL-1RI deficient mouse.
By using cytokine array method we investigated the responsiveness of the astrocyte cell cultures to IL-1β. Our results demonstrated a significant increase in the expressional level of a proinflammatory cytokines. One of them IL-6 is known to be involved in inflammatory pain. Therefore we tested with western blot experiments and immunohistochemistry the expressional changes of IL-6 in primary spinal astrocyte cultures upon treatment with IL-1β. Our data show that IL-1β triggers a cascade of cytokines which further increase neuron-glia and glia-glia interactions.

TEMPORAL RESPONSE FEATURES OF RETINAL GANGLION CELLS ARE MOSTLY DETERMINED IN THE INNER RETINA

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It has been established that retinal ganglion cells (RGCs) respond to light stimulation with either a singular burst of spikes or keep spiking while stimulation is maintained. Depending on this temporal response feature (transiency), both ON and OFF RGCs have been considered to be either transient or sustained. However, the defining mechanism behind transiency is still debated. Recent work has suggested that transiency is determined by the kinetics of the postsynaptic glutamate receptors (mGluR6, AMPA, Kainate) at the photoreceptor-to-bipolar cell synapse. In this work, however, we utilize two independent methods to determine RGC response transiency and present a number of evidence indicating that it is, in fact, determined by inner retinal mechanisms and/or RGC intrinsic properties. These include: (i) existence of both transient and sustained ON cell responses, contrary the fact that ON responses are all initiated by the same postsynaptic mGluR6 receptor; (ii) existence of both transient and sustained ON and OFF cell responses under low-scotopic light levels, in which condition the primary rod pathway provides the sole signalling pathway; (iii) RGC transiency values did not change significantly when responses were recorded at different light intensity levels through different signalling pathways; (iv) OFF ganglion cell responses retained their original transiency values following dl-2-amino-4-phosphonobutyric acid (APB; mGluR6 agonist) incubation contrary to this pharmacological rerouting of signal flow to OFF RGCs. Therefore, contrary to present hypotheses, converging evidence of this study indicate that RGC response transiency is not defined in the outer retina, but is partially or entirely determined by inner retinal interactions and/or by RGC intrinsic membrane properties.

Support: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

THE THREE HIT CONCEPT OF DEPRESSION. WHAT DOES MATERNAL DEPRIVATION AND SUPERIMPOSED CHRONIC MILD STRESS DO IN PACAP MUTANT MICE?

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Major depression is a common cause of chronic disability. Despite decades of efforts, no equivocally accepted animal model is available for studying depression. Numerous genetically modified animal models were developed to assess the depression-like phenotype. Early life stress and other chronic environmental factors such as chronic variable mild stress were used with promising, but somewhat limited results.

Since depression is considered as a multifactorial disease with genetic, epigenetic and environmental background, our main aim was to test the validity of a new model based on the three-hit concept of vulnerability and resilience. Genetic predisposition (hit 1, mutation of pituitary adenylate cyclase-activating polypeptide, PACAP gene), early-life adversity (hit 2, 180-min maternal deprivation, MD180) and chronic variable mild stress (hit 3, CVMS) were combined. The reversibility of the expected changes were tested by selective serotonin reuptake inhibitor (i.e. fluoxetine) treatment. Physical (i.e. body and adrenal weight), endocrinological (corticosterone titer), behavioral (forced swim test, tail suspension test, marble burying test, light dark box test) and functional morphological tools (assessment of chronic neuronal activity by FosB immunolabeling) in limbic and brainstem stress centers were used to validate the model.

Body- and adrenal weight changes as well as corticosterone titers proved that CVMS was effective. Forced swim test indicated increased depression in CVMS PACAP heterozygous mice with MD180 history, accompanied by elevated anxiety level in marble burying test. Corticotropin-releasing factor immunoreactive neurons in the oval division of the bed nucleus of the stria terminalis showed increased FosB expression, which was refractive to CVMS exposure in wildtype and heterozygous mice. Urocortin1 neurons became over-active in CMVS-exposed PACAP knock out mice with MD180 history, suggesting the contribution of centrally projecting Edinger-Westphal nucleus to the reduced depression and anxiety level of stressed knockout mice. Serotoninergic neurons of the dorsal raphe nucleus lost their adaptation ability to CVMS in MD180 mice. Fluoxetine treatment did not reverse all changes found in this model.

In conclusion, the construct and face validity criteria suggest that MD180 PACAP heterozygous mice on CD1 background upon CVMS may be used as a reliable model for the three-hit theory. Taking the common therapy resistance in major depression in consideration, the partial ineffectiveness of anti-depressant therapy further supports the predictive validity of this model.

Financial support: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, Research grant of the University Pécs, PTE ÁOK KA 2017-01, Hungarian Research Grant No.: OTKA PD 100706, MTA-TKI, GINOP-2.3.2-15-2016-00050 "PEPSYS". The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARS) IN ANTICONVULSANT EFFECT OF DOCOSAHEXAENOIC ACID (DHA) AGAINST SEIZURES INDUCED BY PENTYLENETETRAZOLE

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Aim: DHA as polyunsaturated fatty acids (PUFAs) has anticonvulsant effect. PUFAs selective ligands for peroxisome proliferator-activated receptors (PPARs). PPARs agonists have anticonvulsant effect. The aim of this study is evaluation of interaction between DHA and PPARs against seizures induced by pentylenetetrazole (PTZ).
Method: Eight groups of 10 male mice (20-30g) were used in this study. Groups 1, 2, 3, and 4 received PTZ alone (60mg/kg, i.p), DHA 300μM (intracerebroventricular (i.c.v., 15min before PTZ injection), PPARϒ antagonist (GW9662, 2 mg/kg, i.p., 4 h before PTZ injection) and PPARα antagonist (GW6471, 1 mg/kg, i.p., 4 h before PTZ injection), respectively. Four other groups of mice were pretreated with GW9662 (2 mg/kg) or GW6471 (at doses 1, 0.3 and 0.1 mg/kg) and after 4 h, DHA (300Mmol) was injected to the animals. After 15 min, all animals received PTZ (60 mg/Kg, i.p.). The incidence of clonic seizures and latency of clonic seizures were recorded and analyzed by Fisher's exact probability test and One Way ANOVA .
Results: The percentages of clonic seizure incidence were 100, 38.9, 100 and 90%, respectively. The pretreatment of GW6471 could completely reverse DHA anticonvulsant effect (90.9%, $P \leq 0.01$ v.s DHA), but GW9662 could not (63.6%). The suppressing effect of GW6471 on DHA anticonvulsant activity decreased dose-dependently at doses of 0.3 and 0.1 mg/kg (58.3 and 50%, respectively). There was no difference in seizure latencies between groups.
Conclusion: These results indicate an interaction between DHA and PPARα receptor on suppression of seizures induced by PTZ.

OPTOGENETIC MANIPULATION OF LATERAL HYPOTHALAMIC GABAERGIC ACTIVITY AFFECTS APPETITE DURING SUBSEQUENT WAKEFULNESS.

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The lateral hypothalamus (LH) integrates homeostatic needs into coordinated behaviors including sleep and feeding. We have recently shown that GABAergic (Vgat-positive) cells in the LH have multi-tasking capabilities, as the same cells are active during both REM sleep and during food-directed behaviors. Therefore, here we tested whether manipulations of LH Vgat neuronal activity during sleep can affect food intake behaviors.
To this end, we targeted channel-rhodopsin 2 (ChR2), a stabilized step-function opsin (SSFO) or archaerhodopsin (ArchT3.0) to the LH (bilateral) of Tg(vgat)::Cre mice. Sleep-state-dependent optogenetic stimulation of LH Vgat cells was performed using chronically implanted optic fibers for four hours at the end of the light period.
Optogenetic stimulation of LH Vgat neurons caused rapid arousal, which was followed by an immediate feeding initiation. Repeated stimulation over several days increased the amount of consumed food. Conversely, optogenetic inhibition of LH Vgat cells prolonged sleep and did not cause direct effects on food intake. Interestingly, the inhibition of Vgat cells specifically throughout all REM sleep episodes at the end of the light period significantly reduced food intake in the following dark period.
Our results show that the level of LH Vgat neuronal activity during sleep affects food intake during the subsequent arousal. Enhanced activity of LH Vgat cells appears to increase energetic demands and induces feeding at the expense of sleep. Our study provides a possible mechanistic link between the etiology of overweight/obesity and chronic sleep curtailment.

THE EFFECT OF TNF-α PRETREATMENT ON EMBRYONIC SPINAL CORD GRAFTS AFTER LUMBAR 4 VENTRAL ROOT AVULSION AND REIMPLANTATION

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Several diseases or physical damage to the spinal cord cause irreversible motoneurone loss. A feasible way to rescue and/or replace these motoneurons is transplantation of embryonic spinal cord tissue into the injured cord. Our aim was to observe the integration of the embryonic motoneurons into the host spinal cord with functional and morphological methods.
Embryonic spinal cord pieces derived from 13-day-old SD-GFP rat embryos. The embryonic tissue was transplanted into the adult Sprague-Dawley (SD) rat spinal cord after lumbar 4 (L4) ventral root avulsion and reimplantation. Three experimental groups have been set up. In the first group the ventral root was avulsed and reimplanted without any treatment. Animals in the second group received embryonic motoneurons after L4 ventral root avulsion and reimplantation. In the third group the embryonic cells were treated with TNF-α prior to the transplantation.
Six months later electromyography (EMG) was performed to record the electrical activity of selected ankle flexors and extensors of the hindlimbs on the intact and injured sides. In the grafted animals rhytmic locomotor limb movement was observed with EMG. The motor units in the extensor digitorum longus (EDL) and soleus muscles activated rhytmically producing a coordinated hindlimb movement in the transplanted animals. In the EDL two populations of the motor unit action potentials (MUAPs) were distinguished based on the firing period, frequency, duration and amplitude. In the transplanted animals the number of the MUAPs was higher and some MUAPs demonstrated bimodal distribution.
The morphological investigations confirmed the functional results. Retrograde labelling explored the contribution of the grafted neurons to the reinnervation of the target muscles. Reciprocal connections between transplanted cells and the host tissue have been revealed by anterograde tracing with Phaseolus vulgaris leucoagglutinin. Further differencies were found between the two groups. TNF-α pretreatment increased the number of reinnervating neurons. This explains the improved rhytmical locomotor activity and coordination of movement in the grafted animals with TNF-α pretreatment.
Our results confirmed the strong interaction between host and graft tissue and provides evidence that TNF-α exerts a positive effect on the embryonic grafts.

EXAMINATION OF MANGANESE TOXICITY FOR BEHAVIOR ASSOCIATED MEMRI IN THE RAT

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The manganese-enhanced magnetic resonance imaging (MEMRI) is an in vivo technique to map brain activities in rodents. The main disadvantage of this method is the toxicity of manganese. In the MEMRI experiments, the appropriate systemic doses for rodents are in the range of 16 - 180 mg/kg. The aim of the present experiment was to find the non-toxic dose of manganese for behavior associated MEMRI in rats. The administration was repeated and separated by 24 h to reach the dose of 40 mg/kg or 60 mg/kg, respectively Hepatotoxicity of the $MnCl_2$ was evaluated by determining serum aspartate aminotransferase, alanine aminotransferase, total bilirubin, albumin and protein levels. Neurological examination was also carried out. The animals were tested in visual cue discriminated operant task. The different doses of $MnCl_2$ as contrast agent were tested for MEMRI in a 3T clinical MR scanner. T1 values were determined before and after $MnCl_2$ administrations. Manganese-enhanced images of each animal were subtracted from their baseline images to calculate decrease in the T1 value (ΔT1) voxel by voxel. Non-toxic dose of $MnCl_2$ was tested for operant behavior associated MEMRI too. The subtracted T1 maps of trained animals performing visual cue discriminated operant task, and those of naive rats were compared. The dose of 60 mg/kg $MnCl_2$ showed hepatotoxic effect, but even these animals did not exhibit neurological symptoms. The dose of 20 and 40 mg/kg $MnCl_2$ increased the number of omissions and did not affect the accuracy of performing the visual cue discriminated operant task. Using the accumulated dose of 40 mg/kg, voxels with a significant enhanced ΔT1 value were detected in the following brain areas of the visual cue discriminated operant behavior performed animals compared to those in the controls: the visual, somatosensory, motor and premotor cortices, the insula, cingulate, ectorhinal, entorhinal, perirhinal and piriform cortices, hippocampus, amygdala with amygdalohippocampal areas, dorsal striatum, nucleus accumbens core, substantia nigra, and retrorubral field. In conclusion, the non-toxic dose of $MnCl_2$ is low for behavior associated MEMRI. The dose of 40 mg/kg $MnCl_2$ was capable to produce measurable differences in the T1 relaxation time in a 3 T magnetic field strength and able to map brain activity in correlation with the operant behavior of freely moving rodents.

Support: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

REPEATED SEIZURES MODIFY BEHAVIOUR AND MEMORY PROCESSES IN RATS

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Epilepsy is one of the most frequent neuronal disorders. Severe epileptic discharges may cause modifications in neuronal activity both on synaptic and network level, which often lead to cognitive disorders like learning and memory deficits. Seizure activity may have both short- and long term effects. If the short-term changes are not followed by complete recovery, this may lead to permanent neuronal alterations. In the present experiments 4-AP, a potassium channel blocker was used to provoke epileptic seizures. Our aim was to investigate the effect of epilepsy-related acute and chronic changes on general behaviour, learning- and memory processes. Different behavioural tests, electrophysiological and immunohistoblot investigations were performed to examine the effect of seizure activity on synaptic plasticity and learning activity.
Rats were treated with 4-AP for twelve consecutive days to provoke epileptic seizures. One group of them was examined immediately after the end of treatment ("acute group"), while the other 3 month later ("chronic group"). To examine learning and memory processes, eight-arm radial maze and novel object recognition tests were used. Besides this, locomotor activity and anxiety were also investigated in open field test. Electrophysiological measurements were performed on surviving hippocampal brain slices. Extracellular field potentials in the CA1 region were recorded to check alterations in the synaptic efficacy. To evoke LTP, Schaffer-collaterals were stimulated with high-frequency stimulation. Parallel with the electrophysiological experiments, expression levels of NMDA and kainate receptors were investigated in the hippocampus using the immunohistoblot technique.
Open field tests showed that acute 4-AP treated rats were less anxious and were more active than rats of the acute and chronic control group. However, in the novel object recognition test, control rats spent significantly more time with the novel object than 4-AP treated animals. Also in the radial maze test, the treated animals had impaired performance. LTP studies showed that in both 4-AP treated groups the efficacy of LTP induction was moderately higher than in the corresponding control group, although the basic excitability was similar in all of the groups. Immunohistoblot measurements revealed that receptor expression levels were significantly lower in the treated animals than the controls.
Altogether we can say that repetitive 4-AP treatment caused not only short-term changes, but also evoked long-term alterations both on electrophysiological, immunohistological and behavioural level too. Although in the synapses of the hippocampus slice, LTP developed more readily than in controls, the treated animals had lower performance in the applied learning tests, both immediately after the end of treatment and also 3 month later.

DISSECTING THE ROLE OF 5-HT IN MATERNAL BEHAVIOUR WITH GENETIC TOOLS

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In mammals, maternal care has an essential role to ensure the survival of the offspring. Triggering the repertoire of behavioural responses involved in adequate maternal care implies functional changes in brain circuits and involves numerous physiological, cellular and molecular changes. Serotonin (5-HT) neurotransmission plays an important role in this plasticity. In genetic models with constitutive lack of brain 5-HT, maternal behaviour is perturbed and survival of litters is compromised ($Pet1^{-/-}$; Lerch-Haner et al. 2008, $Tph2^{-/-}$ Alenina et al., 2009, $VMAT2^{SERT\text{-}Cre}$ Trowbridge et al., 2011). However the mechanism involved are not known. We use conditional KO and pharmacogenetic strategies to approach this question. Cre-recombinase delivery in Vmat2 flox mice allowed to reduce 5-HT transmission selectively in dorsal/median raphe circuits and viral delivery of the inhibitory pharmacogenetic tools DREADDs (Designer Receptor Exclusively Activated by Designer Drugs) allowed acute transient inactivation of the 5-HT raphe neurons of interest (Fernandez et al., 2017). We demonstrate that 5-HT transmission is required at the time of parturition for the onset of mother/pup interactions during lactation. The possible circuits involved will be discussed at the time of the meeting.

THE PARATHYROID HORMONE 2 RECEPTOR PARTICIPATES IN PHYSIOLOGICAL AND BEHAVIORAL ALTERATIONS OF MOTHER MICE

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The neuromodulator system consisting of parathyroid hormone 2 receptor (PTH2R) and its ligand, tuberoinfundibular peptide of 39 residues (TIP39) regulates many physiological and behavioral functions. For example it has a role in thermoadaptation, and evokes antidepression- and anxiolytic-like effects in behavioral tests. Both receptor and ligand are abundant in preoptic and hypothalamic regions important in maternal behavior and thermoregulation, and TIP39 is also highly activated in mother rodents. Thus we addressed if the PTH2R contributes to the lactational hyperthermia and altered behaviors during motherhood.
The core body temperature (T_c) of PTH2R knockout mice (KO) and their wild type (WT) littermates was continuously recorded via telemetric device in virgin, pregnant and lactating stages. During lactation we also conducted the following behavioral tests: pup retrieval to the nest, observation of undisturbed maternal behavior, forced swimming, and open field test.
T_c showed circadian rhythms in both the KO and WT at all stages. In virgin mice, we found no significant difference between genotypes in temperature or locomotion. Except for the virgin stage, we found significant differences between the T_c of KO and WT animals, WT temperature being higher despite the increased locomotor activity in KO mice. Pregnant and mother mice had significantly increased T_c in both KO and WT animals compared to virgins (albeit to a lesser extent in KO). KO mothers spent more time out of the nest in a one hour undisturbed observation, and showed higher locomotor activity in the open field test, otherwise the maternal behaviors of the two groups did not differ. We found that in the forced swimming test, KO mothers engaged significantly more time with floating and less with struggling than WT animals.
We conclude that the PTHR2R is an important contributor of elevated core body temperature during the lactation period. This finding is consistent with previous literature on the heat-inducing role of PTH2R in male mice and also with the marked induction of TIP39 in the postpartum period in mothers. Furthermore, mice lacking the PTH2R demonstrate depression-like behaviors without major alteration in other behavioral tests suggesting the selective involvement of the PTH2R in postpartum depression.

The work was supported by OTKA K116538 and the KTIA_NAP_B_13-2-2014-0004 Program.

SENSORY-MOTOR CORTEX CONNECTIVITY ALTERATIONS DURING DYSTONIC MOTOR BEHAVIOR INDUCED BY INTRACEREBELLAR KAINIC ACID ADMINISTRATION

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Dystonia is a very disabling neurological motor syndrome. Although recent studies sustain the role of basal ganglia and cerebellar dysfunction in the pathogenesis of dystonia, the mechanisms are not yet fully understood. Our study offers novel insights in the sensory-motor cortex connectivity during dystonic postures and movements. We performed electrocorticogram recordings both in the left sensory cortex and left and right motor cortices on freely moving wild type albino mice before and after induction of dystonic motor attacks by intracerebellar application of kainic acid. We investigated the power spectral density and coherences between motor cortex and sensory cortex before and during dystonic attacks. We found low coherence between motor and sensitive cortices in beta and gamma band during dystonic movements. Moreover, low coherence between motor and sensorial cortices beta band during dystonic postures were observed. Our study offers novel insights in the sensory-motor cortex connectivity after cerebellar administration of kainic acid that induces dystonic postures and movements.

This work was supported by the PN-II-RU-TE-2014-4 project/UEFISCDI to Daniela Popa

COMMON GENETIC VARIANTS OF THE ABCA1 GENE ARE ASSOCIATED WITH ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a genetically complex neurodegenerative disorder. The ATP-binding cassette, sub-family A, member 1 gene (ABCA1) is a positional and functional candidate gene for AD. This gene has an important role in cellular cholesterol efflux and it is located close to an AD linkage peak on chromosome 9 at position q22. In the present case-control association study, we examined the possible association between three single nucleotide polymorphisms of the ABCA1 gene (rs2230805: G474A, rs2230806: G656A and rs2066718: G2311A) and AD risk.
DNA samples were collected from 431 AD probands and from 302 elderly, cognitively intact, ethnically matched controls in Hungary. A consensual clinical diagnosis of AD was established according to the NINCDS-ADRDA criteria. Mini-Mental State Examination was used as a measure of global cognitive performance. Genomic DNA was extracted from peripheral blood leukocytes. The genetic analyses were performed by TaqMan real-time PCR method.
No statistically significant difference was found in the distribution of genders or in mean age between the AD and control groups ($p>0.1$), while APOE ε4 allele frequencies were shown to differ significantly ($p<0.05$). The investigated genotype frequencies were in Hardy-Weinberg equilibrium for both cases and controls ($p>0.1$). Haplotype analysis revealed that rs2230805, rs2230806 and rs2066718 polymorphisms created a linkage disequilibrium (LD) block with a strong LD between rs2230805 and rs2230806 polymorphisms. The genotype frequencies of the rs2066718 polymorphism did not differ significantly between the AD and control groups ($\chi2=2.358$; $p=0.308$). Significant associations were founding the case of the rs2230805 and the rs2230806 polymorphisms in the recessive model. Compared to the controls (C), the rs2230805 G/G genotype was over-represented in the AD group (G/G: AD:56.8%, C:47.0%; $\chi2=7.787$; $p=0.020$), and the rs2230806 G/G genotype was also more frequent in the AD group (G/G: AD:53.6%, C:45.0%; $\chi2=5.245$; $p=0.073$). Our results support the hypothesis that ABCA1 rs2230805 and rs2230806 polymorphisms are associated with late-onset AD; however, further confirmations with independent samples may be required.

This project was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and by a grant from (M. P. & J. K.) the Hungarian Research and Technology Innovation Fund through the Hungarian Brain Research Program (KTIA_13_NAP-A-II/16).

NANOSCALE BAYESIAN CLUSTERING-BASED CHARACTERIZATION OF THE PRESYNAPTIC MOLECULAR ARCHITECTURE AT GABAERGIC SYNAPSES

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While the molecular composition of the neurotransmitter release machinery has already been catalogued, our knowledge on the nanoscale blueprint of the presynaptic active zone remains rather limited. Super-resolution imaging offers unique possibility to address how the distinct molecular players are precisely arranged within the active zone to support vesicle release and how their relative nanoscale abundance determines neurotransmitter release probability. It is conceivable to hypothesize that these biological parameters exhibit large cell-type- and synapse-specific variability to most optimally shape synaptic transmission. Therefore, we recently exploited combined confocal and STORM super-resolution microscopy and developed the VividSTORM analysis software to facilitate quantitative nanoscale molecular imaging at identified synapses, which belong to predefined cell types. In the present study, we used a novel clustering method based on Bayesian statistics (Rubin-Delanchy et al., 2015, Nature Methods, 12:1072-6) to uncover the nanoscale architecture of the active zone at hippocampal interneuron terminals. We first incorporated the Bayesian clustering algorithm in the freely available VividSTORM software and experimentally validated its robustness for unbiased quantification of synaptic protein distribution. We next performed immunostaining for the active zone cytomatrix protein bassoon and utilized correlated confocal/STORM imaging in order to investigate its nanoscale distribution in identified hippocampal interneuron boutons. Bassoon showed a highly clustered distribution pattern at CB_1 cannabinoid receptor-positive GABAergic axon terminals targeting the cell body of CA1 pyramidal neurons. Interestingly, we found no difference in the overall abundance of bassoon, in the number of bassoon clusters, and in the size of bassoon clusters in the perisomatic axon terminals belonging to the two CB_1-positive basket cell types classified by the presence or absence of the vesicular glutamate receptor type 3 (vGluT3). In contrast, STORM imaging revealed considerably higher density of presynaptic CB_1 receptors on vGluT3-containing perisomatic axon terminals compared to vGluT3-negative boutons in the stratum pyramidale. Therefore, in the final set of experiments, we measured CB_1/bassoon ratio in identified axon terminals and found that more CB_1 receptors are present to control a single release machinery unit at vGluT3-expressing interneuron synapses. These findings demonstrate that the presynaptic receptor/effector ratio is different between hippocampal interneuron types and further illustrate that super-resolution imaging supported by analysis tools such as unbiased Bayesian clustering can substantially advance our understanding of how nanoscale differences in the presynaptic molecular architecture may contribute to the regulation of synaptic function in a cell-type-specific manner.

RAPID EFFECT OF ESTRADIOL ON DIFFUSION DYNAMICS OF GLUTAMATE RECEPTORS

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Changes in diffusion properties of glutamate receptors (AMPAR and mGluR1) play pivotal role in synaptic plasticity. Among many different factors, controlling the synaptic plasticity, gonadal steroid estradiol (E2) is an essential factor. In spite of the well established genomic effect of E2 on synaptic plasticity little if any attention has been given to the rapid action of E2 glutamate receptor diffusion. Using single molecule live cell imaging we examined the effects of E2 on the lateral diffusion of AMPAR and mGluR1 molecules in the plasma-membrane. Single AMPAR or mGluR1 molecule trajectories were individually tracked and analyzed both on the membrane of soma and neurits and mean square displacement as well diffusion coefficient (D) was determined.. Movement parameters were calculated every 5 min, and compared statistically to the corresponding vehicle control. Both AMPAR and mGuR1 molecules showed restrictive and area specific movements along the neurits and somas with multimodal trajectories. Administration of 100 nM E2 resulted in a rapid (<5min) decrease in D of AMPAR molecules. The administration of 100pM and 100 nM of E2 evoked a clear dose-dependent effect on the D of AMPAR molecules with limited or no effect on the D of mGluR1 molecules. Our findings provide first evidence that E2 rapidly alter membrane diffusion of glutamate receptors in living neurons. These data suggest that E2 rapidly alters the synaptic plasticity via altering the surface movement of AMPAR and mGluR1 receptors.

Acknowledgement: Hungarian Brain Research Program (KTIA_NAP_13-2014-0001), OTKA (112807), EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

SIGNALS USED IN MODELING "UNPREDICTABLE STRESS" AND THEIR SPECIFIC EFFECTS ON THE LONG-TERM PLASTICITY OF SYNAPSES

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We have studied alterations in the properties of CA1 long-term potentiation (LTP) induced by high frequency stimulation of Schafer collaterals (HFS, 100 Hz, 1 s,) in hippocampal slices of juvenile rats induced by the exposure of animals to different individual stressors usually used in batteries of chronic unpredictable stress (CUS), a widely used model of depression. The animals were divided into 6 groups. The rats of experimental groups were isolated from other rats the night before the experiment and subjected to a certain stressor for 16 hours (food or water deprivation, a wet bedding in the cage, and stroboscopic illumination with a frequency of 120 light bursts per minute). The group of socially isolated animals was considered as the "active control" for other experimental groups. The animals of the passive control group were maintained in their home cages. Social isolation for 16 h did substantially affect neither the magnitude and nor the development of LTP. The effects of stroboscopic illumination and water deprivation appeared most severe, though opposite: the first stressor had activating effect, whereas the second one inhibited the development of LTP. In addition to the effects of these factors on the LTP magnitude, they also affected the patterns of LTP development. In this study weak tetanization with different probability of maintenance was used, and most of stressors, in spite of the similar LTP magnitude, influenced significantly on the process of consolidation. In hippocampal slices from rats maintained on wet bedding for 16 h, LTP was more variable as compared to the slices from rats from other groups. In this group, the time course of LTP development significantly differed from that observed in the control or socially isolated rats. The weakest effect on LTP was observed in hippocampal slices of the rats exposed to food deprivation. In these animals, only some differences were observed in the development of LTP as compared to socially isolated rats. These data allow ranging stressors used in CUS paradigms according to the severity of their potential effects on neuronal function and animal behavior.

This work was supported by the Russian Science Foundation (grant #14-25-00136).

THE KEY ROLE OF NUCLEUS INCERTUS IN ALLOWING THE FORMATION OF CONTEXTUAL MEMORY.

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Contextual fear strongly depends on hippocampal memory formation. During contextual fear conditioning (CFC) the subject has to establish a new episodic memory by associating a context (conditioned stimulus, CS) with an aversive event (unconditioned stimulus, US). After successful CFC, mice show fear that is detectable by observing their freezing behavior, when they need to face the same context. The septo-hippocampal connections also play an essential role in episodic memory encoding and promote synchronous hippocampal oscillations, the so-called theta rhythm, which remains prominent during exploration and new memory formation. As it is shown in a parallel poster presentation, the pontine nucleus incertus (NI) sends a highly specific GABAergic projection to theta rhythm-related forebrain areas. NI targets somatostatin-positive interneurons in the hippocampus, the direct inhibition of which is known to veto CFC memory formation. In addition, NI also targets several cell types in the medial septum that could also promote hippocampal theta rhythm generation and memory formation. Certain NI neurons fire phase-locked to hippocampal theta, and NI was also shown to play a role in the regulation of spatial working memory. However, the exact role of NI in hippocampal theta rhythm modulation and episodic memory formation is still poorly understood.

To examine the role of NI in hippocampal theta generation, we injected Cre-dependent adeno-associated viruses expressing channelrhodopsin into the NI of vesicular GABA transporter (vGAT)-Cre mice and optogenetically stimulated either the GABAergic cell bodies of NI directly or their terminals in medial septum. In parallel, we recorded the hippocampal network activity *in vivo* in freely moving animals. We found that both of these optogenetic stimulations caused a decrease in hippocampal theta power during exploration and paradoxical sleep.

Furthermore, in behavioral experiments, we found that mice were not able to form CFC memories, if GABAergic cells of the NI were optogenetically stimulated during repeated presentation of the 2-second-US. However, they showed significant freezing behavior if stimulation was regularly shifted by 15 seconds compared to repeated 2-second-US presentation. In addition, mice stimulated during US presentation showed lower anxiety levels in an elevated plus maze test compared to control subjects injected only with tracer viruses, further confirming their apparent lack of memories.

Our results show that NI seems to play an essential role in regulating hippocampal episodic memory formation by inhibiting septo-hippocampal theta-rhythm generation and also by directly inhibiting medial septum and hippocampal somatostatin-positive interneurons. These findings may help us to better understand certain neuropsychiatric disorders like posttraumatic stress or chronic anxiety disorders.

ANATOMICAL ANALYSIS OF THE ROD PATHWAY IN HUMAN RETINA

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Vision in darkness (scotopic vision) is mediated by a specific high-sensitivity pathway in the retina. Rod photoreceptors feed their signals into rod bipolar cells (RBCs), which transfer the signal to AII amacrine cells. The AII amacrine cells are a crucial interneurone in the rod pathway because they contact both on-type bipolar cells (through gap junctions) and off-type bipolar cells (through glycinergic synapses), thus giving the rod signals access to on– and off-pathways in downstream visual circuits. This connectivity makes AII amacrine cells a target for optogenetic induction of light sensitivity to restore vision in retinal diseases, but their distribution and sampling density in human retina is currently unknown. Here, we mapped AII cells and other rod pathway elements in post-mortem human donor eyes. Our results indicate rod pathway spatial resolution would be limited by the peak density of AII cells peaking at 1.5 mm outside the fovea.

TRAUMATIC BRAIN INJURY IN A RAT LATERAL FLUID PERCUSSION MODEL: ELECTROPHYSIOLOGICAL, MORPHOLOGICAL AND BEHAVIORAL MANIFESTATIONS

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Post-traumatic epilepsy (PTE) is a major concern for patients with traumatic brain injury (TBI). The risk of developing PTE after TBI peaks within the first year after TBI, however, it is sustained for >10years. PTE accounts for 10-20% of epilepsy cases in the general population. Immediate and early seizures after TBI are acute symptomatic events resulting in secondary brain damage which induces further complications and being significant risk factor for PTE development.
There are virtually no rodent studies of TBI-induced PTE linking early seizures with PTE and exploring early epileptogenesis. Lateral fluid percussion brain injury is a widely used model of TBI in rats reproducing clinical signs and presumably PTE pathogenesis during the late period. To study early consequences of severe TBI we used this model (3-4 atm) in adult male Sprague-Dawley rats. To estimate the time course of epileptiform discharges during wake-sleep cycle, global brain function video-electrocorticograms were recorded during a week prior and a week after TBI. To monitor epileptiform discharges we developed an automatic detection algorithm of EEG events with high value of power spectral density based on wavelet transform. Symptoms of depression and anxiety were assessed in light-dark box and elevated plus-maze tests. Histological analysis of brain tissue was performed 1 week after TBI.
Immediately after TBI tonic-clonic seizures occurred. High voltage rhythmic spikes (HVRS) were detected in background records and after TBI, particularly during the early stage of NREM sleep. After TBI, the number of HVRS was 8-fold higher in about 50% of animals as compared to sham operated controls suggesting that HVRS represent a subclinical epileptiform activity in acute period of TBI. Histological studies revealed cortical damage to the ipsilateral hemisphere (neurodegeneration, gliosis, microglial activation, accumulation of IgG) as well as remote ischemic-like damage to the hippocampus. During acute posttraumatic period rats demonstrated symptoms of anxiety and depression associated with sleep disturbances.
Thus, we have shown for the first time behavioral, electrophysiological and morphological consequences of TBI during the early period. HVRS may represent epileptiform activity potentially involved in PTE development and be one of early markers/events of commencing epileptogenesis.

Supported by RSF grant # 16-11-10258 (electrophysiological analyses) and RFBR grant # 16-04-00855 (behavior, histology).

ANALYZING ACCEPTABILITY OF QUALITY BY APPLYING USER CENTERED DESIGN APPROACH OF HUMAN COMPUTER INTERACTION

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Keywords: Interaction, Quality, Engineering, Interface

Software process research concerns with the methods and technologies used to assess, support, and improve software development activities. The field has expanded up to address the increasing complexity and criticality of software development activities with respect to major quality issues. Problems in designing of interface unit to improve interaction between human and computer faces problems related to quality. This is one of the extreme challenges encountered by Human Computer interaction professionals and software professionals. In turn, this means initiating a rigorous quality regime, which is both defined and measurable alike is to get the process right first time.

Our study shows quality is one of the crucial parameter due to User-centered design process (UCD), Efficiency, Integrity, Reliability, Usability, Accuracy, Review, Ergonomics, Risk Assessment, Maintenance, Software Metrics which causes a range of factors to come into play. Project management and analysis of quality difficulties continually cause problems for designers and project managers while the implementation of efficient data quality issues in software processes can be used to improve the quality of the product, published software process models do not cater explicitly for the recent growth in global software engineering.

This study is able to answer the type of design decisions and what are the impacts (good and bad) in the Human computer interaction and software engineering processes. The future work of this study make able whether this approach effectively promotes collaboration between SE and human computer interaction and, if so, how this collaboration may be further propagated to other models, both in human computer interaction and in software engineering.

BENEFITS OF LIFESTYLE-CHANGES IN PSYCHO-IMMUNO- AND PHYSICAL FUNCTIONS OF UNIVERSITY STUDENTS

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Introduction: Strong evidence shows that physical inactivity increases the risk of many adverse health conditions, and shortens life expectancy so healthy way of life is a priority. Because much of the world's population is inactive. Sedentary lifestyle shortens lifespan and may contribute to the process of immunosenescence and age-linked atrophy of the thymus. Physical inactivity-related immune alterations and the reversal of this process is not well described yet.
Health and wellness industry are among the top boosting businesses today. It is hard to find an individual solution among the several diets and exercise-plans.
Our study aimed to quantify the impact of physical inactivity and regular physical activity on numerous psychological, physiological and immunological parameters. Previously physically inactive subjects took part in a six-month lifestyle change program, including workouts three times a week and diet suggestions. Subjects were university students (LSG) compared to the control group (CG). During the study1 hour exercise 3 x a week (strength training and cardiovascular/aerobic exercises and customized individual diet suggestions were designed). The intensity of exercise (max. 65%) was adjusted according to the actual fitness of the participants. The study lasts 6 months with 3 measurements (initial data/T0, third month data/T1 and closing data /T2). Questionnaries were filled (SF-36, QOL), anthropometric parameters, cardio-respiratory measurements (pulse/HR, blood-pressure/BP, O_2-saturation), physical endurance with the 6 minute walking test (6MWT), and handgrip strength test were carried out. Blood and immunological parameters (sjTREC, cytokines) were also measured.
Results: Currently the T0 and T1 results of the two groups (LSG, CG) are analyzed. The majority of LSG parameters changed, while there are no measured significant differences in the CG. According to the anthropometric data BMI of the LSG has not changed, although their body fat percentage dicreaserd (T0: 31,5±6,3%, T1: 29,4±6,8%), we found significantly decreased skin-folds (Biceps: p= 0,001; Triceps: p= 0,027; Pectoralis: p= 0,002; Abdomen: p= 0,02; Mid-axilla: p= 0,005; Suprailiacalis: p=: 0,0006; Thigh: p= 0,024; Calf: p= 0,015). Muscle volume increased from 28,6±3,7% to 30,0±5,5% in average. The resting HR is 5bpm lower; the systolicBP is 6Hgmm lower on average compared toT0 in LSG. At T0 657,5±46m, at T1 675,4±46,3m achievement was registered (p= 0,043) during the 6MWT, this is 1,4 ml/kg/min improvement in average of VO_{2max}. In the CG group improvements were not detected.
Conclusion: Data at T1 and T2 results show significant improvement in LSG, while in the CG no significant changes were found showing that a lifestyle-change program is beneficial in physical and molecular/immunological terms in adulthood as well.

Keywords: life-style changed, university students, stamina, blood markers

LOCAL APOPTOTIC MECHANISMS UNDERLIE COMPLEMENT-MEDIATED SYNAPTIC PRUNING

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C1q, a member of the immune complement cascade, is implicated in selective pruning of synapses by microglial phagocytosis. C1q-mediated synapse elimination is obligatory in brain development and in maintaining synaptic plasticity, while its pathological activation is observed in neurodegenerative diseases. However, molecular mechanisms underlying C1q-controlled synaptic pruning are mostly unknown. This study addresses molecular composition distortions in the synaptic proteome leading to synapses tagged with C1q. C1q has been revealed to be attached to murine cerebral cortical synaptosomes, moreover, our data demonstrated the preferential localization of C1q to the presynapse. Fluorescence-activated synaptosome sorting of C1q-tagged and untagged synaptosomes and gel-based proteomic comparison of these two synaptic populations were conducted. Proteomic analysis of C1q-tagged synaptosomes revealed that C1q selectively recognizes synapses characterized by impairments in energy metabolism, synaptic transmission, and signal transduction. Pathway analysis of proteomic alterations indicated the presence of apoptotic processes in C1q-tagged synapses, which hypothesis was confirmed experimentally using flow cytometry and immunohistochemistry techniques. Our results unveil that the C1q label-based synaptic pruning is triggered by and directly linked to the apoptotic degradation of synapses.

NOVEL, GUANYLATE CYCLASE C INDEPENDENT, SIGNALING PATHWAY FOR UROGUANYLIN IN THE BRAIN

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Guanylate cyclase C (GC-C) is a membrane receptor first discovered in intestine as receptor for heat-stable enterotoxin of *E. coli*. Upon activation by uroguanylin (UGN), which belongs to guanylin peptides family, GC-C leads to an increase in intracellular production of cGMP. Since GC-C is expressed in dopaminergic neurons of *Substantia nigra compacta* and *Ventral tegmental area*, GC-C knockout (KO) mice develop ADHD-like behavior. However, GC-C expressed in hypothalamus in *Arcuate nucleus* has controversial roll on feeding behavior and satiety. In this study, we report existence of novel GC-C independent signaling pathway for UGN in the mouse brain. Electrophysiological recordings of astrocytes (99% pure primary astrocytes culture isolated by magnetic activated cell separation) showed that UGN hyperpolarized cell membrane. Since membrane permeable cGMP depolarized cell membrane, GC-C is not receptor for UGN in astrocytes which is not surprising since mRNA for GC-C was not present in those cells. However, we report for the first time that GC-C is present in neurons of other parts of the brain, in addition to midbrain and hypothalamus, like cerebral cortex and cerebellar Purkinje cells and deep nuclei. Therefore, we propose astrocytes as cell model for research of this novel, GC-C independent signaling pathway for guanylin peptides. Astrocytes play major role in regulation of extracellular fluid pH that can change neural activity. We showed that UGN via this novel signaling pathway can modulate astrocyte pH. Application of UGN during ammonic pulse increased post-acidification recovery slope showing that UGN activates Na^+/H^+ exchanger. Furthermore, other mechanism to control astrocyte pH is HCO_3^- transport. UGN increased alkalization slope after astrocyte exposure to CO_2/HCO_3^- showing increase in HCO_3^- transport. To determine which GC-C independent signaling pathway is activated, we performed Ca^{2+} imaging in cerebral and cerebellar cortex on brain slices of wild type and GC-C KO mice and astrocytes where UGN increased intracellular Ca^{2+} concentration.
Here, we showed for the first time existence of new, GC-C independent, but Ca^{2+} dependent signaling pathway for UGN in the brain. All this results are showing that cGMP is not second messenger for UGN in astrocytes and also correspond to previous research that showed that cGMP inhibits Ca^{2+} signaling and does not increase intracellular Ca^{2+} concentration. We propose astrocytes as cell model for this novel signaling pathway. UGN via this novel signaling pathway can change cell membrane potential and intracellular pH.

CANNABIDIOL INHIBITS METHAMPHETAMINE-INDUCED REINSTATEMENT

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Methamphetamine (METH) is a widely abused, highly potent and severely addictive psychostimulant. The most problematic issue to treat addiction is relapse even long time after abstinence. Previous studies showed that there is a tight connection between sleep impairment and relapse. Also, it was reported that Cannabidiol (CBD) might be a potential treatment for drug craving and relapse. In this study, we used a drug-induce conditioned place preference (CPP) to investigate whether cannabidiol (CBD), a phytocannabinoid, can prevent METH-induced reinstatement. In order to induce CPP, animals were given METH (1mg/kg; sc) for five days. CPP induced with METH lasted for 10 days after cessation of METH treatment and priming dose of METH (0.5 mg/kg, sc) reinstated the extinguished METH-induced CPP. our results showed CBD could suppress the METH-induced reinstatement. In conclusion, CBD prevent METH-induce CPP effectively. What we reported on the effect of CBD, might have been related to the interaction between CBD and different neurotransmitters such as dopamine that involve in drug reinstatement. Finally, CBD can be considered as the agent to reduce the risk of the relapse, but it needs more investigation.

WHITE-MATTER CHANGES CORRELATE WITH PERIPHERAL NEUROINFLAMMATORY PROCESSES IN PARKINSON'S DISEASE

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The evolving paradigm shift in the role of neuroinflammation in neurodegenerative disorders has led to observations that proved neuroinflammation to be a pivotal part of pathogenesis in Parkinson disease (PD).
Neutrophil-lymphocyte ratio (NLR) has been a popular marker to measure peripheral inflammatory response. As CNS inflammation can only be proved through biopsy, studies have addressed NLR to differentiate between PD patients and controls or different subtypes of PD. Connectometry is a statistical approach based on diffusion tensor imaging with the ability to reveal white matter tracts with statistical significance to a variable of interest. Herein we implemented connectometry to find tracts in with decreased/increased "quantitative anisotropy" in patients with early Parkinson disease compared to controls.
Method: Participants involved in this research were recruited from Parkinson's Progression Markers Initiative (PPMI) (www.ppmi-info.org/data/). The diffusion data were reconstructed in the MNI space using q-space diffeomorphic reconstruction to obtain the spin distribution function. Diffusion MRI connectometry was used to study the effect of NLR. A multiple regression model was used to consider sex, age, H&Y, and NLR in a total of 39 subjects. The analysis was conducted using DSI Studio (http://dsi-studio.labsolver.org).
Neutrophil and lymphocyte counts were determined using autoanalyser device on fresh whole blood samples of patients, preserved with EDTA.
Result: The connectometry analysis identified splenium of corpus callosum, bilateral cingulum, bilateral inferior longitudinal fasciculi (ILF), bilateral fornixes and right uncinate fasciculus with decreased connectivity related to NLR (false discovery rate=0.0554238).
Discussion: Neuroinflammatory processes are proposed to contribute to PD. Activated microglia accumulate in areas of neurodegeneration, months before the onset of motor symptoms and CD4+ T-cells infiltrate the substantia nigra and cytokine levels increase in the affected areas in the substantia nigra. Neuroinflammation is now believed to be the common pathway by which mitochondrial dysfunction, environmental toxins and perhaps infections, i.e. peripheral inflammation, culminate to result in dopaminergic specific neural death.
Our study revealed reduced white matter integrity in areas previously reported to be affected in PD, with NLR as a marker of peripheral inflammation. Cingulum is implicated in cognitive functions and is known to be implicated in PD loss of executive function and dementia. The ILF has an integrative function is visuospatial tasks and is disturbed in PD patients with visual hallucinations and fornix is mandatory for preservation of memory and attention is these patients. Our observations suggest that white matter degeneration is early PD might have pathological instems in peripheral inflammation.

THE LOCAL NEURONAL NETWORK AND SINGLE UNIT CORRELATES OF ELECTRICALLY EVOKED POTENTIALS IN THE HUMAN NEOCORTEX

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Introduction: Cortical electrical stimulation (CES) can be reliably used both in the investigation of cortical excitability and in the localization of pathological cortical areas. It is a widely used technique also in the mapping of functional connections between distant cortical areas. The local cortical activation-inhibition sequence underlying electrically evoked cortico-cortical potentials (CCEPs) is little known in humans. Our aim was to study these physiological mechanisms in drug-resistant epileptic patients implanted with subdural (grid or strip) electrodes.
Materials and methods: In order to reveal the effect of CES on the level of cortical layers we used intracortical laminar multielectrodes (24 contacts, inter-contact distance: 200μm) inserted into the cortex perpendicularly to its surface. We studied the effect of single (n=7) and paired-pulse (n=10) CES applied on the contacts of grid or strip electrodes. We applied brief single (10mA, 0.5Hz, train of 25) and paired (ISI: 6.6, 10, 20, 30, 40, 50, 100, 200, 500, 1000ms, on the best single response electrode-pair) pulses on adjacent contacts of the subdural electrodes, while recorded the responses both on the superficial and intracortical electrodes. We analyzed the cortical depth distribution of local field potentials, current source density (CSD), changes in spectral power (0-200Hz), and in multiple and single unit activity (MUA and SUA, respectively) in the time window of CCEPs.
Results: We identified P1, N1 (early), P2, N2 (middle) and P3 (late) components with mean peak latencies of 10, 30, 60, 160, 400ms, respectively. Based on the CSD analysis, the laminar profile of the CCEPs showed the following patterns. For the early components, a surface current source and middle layer sink (P1) followed by surface sink and a layer IV source (N1) associated with an increase in MUA and SUA. For middle latency components: a surface source and a layer IV sink (P2) subsequently a cortical wide source in the middle layers (N2) accompanied by MUA and SUA decrease. For the late component (P3): a middle layer sink and increase in MUA and SUA. In the paired pulse setting, the surface potential amplitude differences of N1-P2, P2-N2 were correlated with ISI. We found similar N1-P2 curve characteristics in 8 out of the 10 patients characterized by an excitation at ISI 7 and 10ms, inhibition at ISI 20-50ms and a long interval excitation at ISI 200 and 500ms.
Conclusions: We identified both excitatory (P1, N1, P3) and inhibitory (P2, N2) CCEP components. In our paired pulse stimulation setting we demonstrated these inhibitory and excitatory effects on the second CCEP response. Our research may shed light on, previously unknown, local neuronal network reactions to electrical and magnetic stimulation of the human neocortex. Based on these data better neuromodulatory therapies and diagnostic tools can be developed in the future.

Supported by KTIA_NAP_13-1-2013-0001

BYPASSING SWEETS FOR ALCOHOL: ALTERED REWARD FUNCTIONS IN RAT MODELS OF GASTRIC BYPASS SURGERY

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Roux-en-Y gastric bypass (RYGB) is a very effective and common treatment for morbid obesity and its comorbidities including type-2 diabetes. Similar to human studies, rodent models of obesity have shown that RYGB produces reduced preference and motivation for sweet and fatty foods and thus may reduce both palatability and rewarding effects of 'junk foods'. RYGB also has been shown to dramatically reduce food cravings and food addiction. In contrast, concerns have been raised by clinical reports of an increased risk for alcohol/substance use disorder (AUD/SUD) following RYGB surgery. This presentation will focus on our recent studies in dietary obese rats that provide evidence that RYGB increases the rewarding effects of alcohol independently from postoperative changes in altered absorption from the gastrointestinal tract. Recently, we found a similar, increased, self-administration of morphine in dietary obese RYGB rats compared to surgical controls. We propose that the effect of surgery on reward-guided behaviors is due to alleviated obesity-related deficits in dopamine signaling, which is beneficial to eating behaviors and in turn, for long-term weight maintenance following RYGB while also posing a risk for "reward transfer", i.e., substituting drugs for food. As potential underlying mechanisms upstream from the gut to the brain reward system, our recent studies investigated the role of altered gastrointestinal hormones (e.g., ghrelin, GLP-1) as well as changes to vagal functions following RYGB surgery. Our initial findings points to functional improvements in hindbrain neuronal functions and suggests that altered ghrelin signaling is contributory. Future studies are warranted to identify underlying mechanisms responsible for the differences in alcohol effects following different surgical protocols, including metabolic, pharmacokinetic, neural and hormonal factors. Additionally, investigating the potential mechanisms through which bariatric surgery may result in AUD/SUD is important not only to identify patients that may be potentially at risk of increased risk for AUD/SUD after surgery, but also to identify plausible pathways that may represent novel pharmacological targets for the treatment of AUD/SUD in general.

This research is supported by National Institutes of Health grants AA024490, DC013904, and by a CURE grant from the Pennsylvania Department of Health.

PREDICTING OPTIMAL STIMULATION SITES IN PARKINSON'S DISEASE WITH PROBABILISTIC TRACTOGRAPHY

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Introduction: Deep brain stimulation (DBS) in medically intractable Parkinson's disease, accompanied by severe fluctuations and ON-OFF periods, became a gold standard therapy in the last 3 decades. The subthalamic nucleus, the surgical target in Parkinson's DBS connects to several cortical areas in the human brain. Visualizing these connections might reveal an ideal target for stimulation.
Methods: 34 patients, who underwent STN DBS between 2013-2015 at the Department of Functional Neurosurgery and Centre for Neuromodulation at the National Institute of Clinical Neurosciences in Budapest and at the Department of Neurosurgery of the University of Szeged, were enrolled in this study. T1- (1 mm, isotropic) and T2-weighted (2 mm, isotropic), DTI (2 mm, isotropic) sequences were acquired at most 3 months before surgery. The subthalamic nuclei were identified by two independent researchers on T2 images. Postoperative CT scans were acquired at least 6 weeks after DBS implantation to exclude pneumocephalus related artifacts and brain shift. DTI datasets have been analyzed and probabilistic estimation of white matter fibres was carried out with Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques (BEDPOSTX) available in the FMRIB Software Library (FSL 5.0.9). Estimation of cortical surfaces was carried out using Freesurfer 5.3. Secondary motor ares were identified using the Human Anatomical Motor Template in each patient. DBS lead contacts were identified on postoperative CT scans co-registered to preoperative T1 anatomical images. Preoperative and postoperative UPDRS III scores were evaluated by a neurologists specializing in movement disorders.
Results: Probabilistic tractography revealed 7 connectivity regions within the STN projecting to the limbic areas, the prefrontal cortex, the pre-supplementary area, the supplementary motor area, the premotor cortex, the primary motor cortex, and the primary sensory cortex. Distance between the highest probability regions in the aforementioned connections and the optimal stimulating contacts were correlated with the postoperative UPDRS III scores revealing significant correlations between the SMA related STN subregions and the clinical outcome in both centers.
Conclusion: Our result indicate that the best clinical outcome can be achieved in Parkinson DBS when the stimulating contact is placed into the subregion of the STN projecting to the SMA. We think that our study does not only provide valuable data during surgery, but can also aid neurologist during the programing of more advanced multi contact segmented leads.

KTIA_NAP_13-1-2013-0001

SECRETAGOGIN POSITIVE CELLS IN THE DEVELOPING HUMAN FOREBRAIN

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Cell migration is a principal process in central nervous system development which is shaped by different signaling molecules and factors. We have recently identified secretagogin, a calcium-sensor protein, in neurons of the rodent rostral migratory stream and its human foetal equivalent where it regulates enzyme externalization to digest the extracellular matrix, thereby promoting forward neuroblast migration. Along this line we posed the question if secretagogin-containing cells occur in other forebrain regions to facilitate brain development. To explore this, we took advantage of human brain samples from fetal, perinatal and postnatal stages (altogether 28 case) and performed immunohistochemistry. Secretagogin-containing cells densely populated the subventricular zone of pallial and subpallial anlages. In the pallium, including cortex, cells migrate in radial or tangential orientations in the different layers of the cortical plate. Secretagogin-containing cells appeared in both types of migratory orientation, with their bipolar shape arranged accordingly. They were numerous during midgestation but their density further increased at the early third trimester to robustly decrease before birth. In the diencephalon, dorsal thalamus harboured a large number of secretagogin-containing cells with their axons prominently labelled and turning around the ventral caudate to enter the developing cortex. In the hypothalamus immunoreactive cells occurred in its different subdivisions. Subpallial structures, such as caudate and putamen were typically spared from immunoreactive cells. In the adult cortex and thalamus, secretagogin-containing cells occur only sporadically. Considering the large number of secretagogin-containing cells, their typical orientation especially in the pallial anlage and its striking difference to the adult pallial pattern we suggest that secretagogin plays a regulatory role in neuroblast migration in the developing human brain.

MODELING SCHIZOPHRENIA USING INDUCED PLURIPOTENT STEM CELLS DERIVED FROM A PATIENT HARBOURING A DE NOVO MUTATION IN KH-TYPE SPLICING REGULATORY PROTEIN (KHSRP)

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Schizophrenia is a chronic, debilitating psychiatric disorder characterized by hallucinations, delusions, negative symptoms, and cognitive dysfunction that represents a major public health challenge. The currently available pharmacological and nonpharmacological treatment options alleviate the clinical symptoms, however one third of patients remain resistant to treatment and demonstrate a poor prognosis. Therefore, research targeting molecular disease pathways in schizophrenia can improve our understanding of its complex etiology and may open new windows of opportunity for pharmaceutical drug development. To this end, we present an approach based on next generation sequencing and induced pluripotent stem cell (IPSC) based in vitro disease modeling. IPSCs are self-renewable and pluripotent cell lines, therefore they can be differentiated into neuronal lineages for the investigation of functional and molecular phenotypes in vitro. Moreover IPSCs can be derived from both healthy and diseased individuals by means of somatic cell reprogramming.
De novo mutations (DNMs) have been implicated in the etiology of schizophrenia and show enrichment in critical biological pathways in the disorder; however in most cases their exact biological significance remains inconclusive. IPSCs are also a plausible model system to demonstrate the effects of DNMs in progenies of interest, particularly in forebrain glutamatergic neurons.
We have generated IPSCs from a case-parent trio, where the schizophrenia patient has been identified as a DNM carrier by exome sequencing. In the investigated trio the proband is a carrier of 3 nonsynonymous DNMs in genes leucine rich repeat containing 7 (LRRC7), KH-Type Splicing Regulatory Protein (KHSRP), and Killer Cell Immunoglobulin-Like Receptor, Two Domains, Long Cytoplasmic Tail, 1 (KIR2DL1). LRRC7 encodes densin-180, a postsynaptic density protein in glutamatergic synapses, while KIR2DL1 encodes killer cell immunoglobulin-like receptors (KIRs) that are transmembrane glycoproteins. KHSRP is a highly conserved RNA-binding protein implicated in miRNA maturation, axonal growth, and dendritic spine development. Based on bioinformatics prediction tools we hypothesized that the KHSRP mutation may have deleterious effects that give rise to the phenotype seen in the patient. The Chr19:6416869C>A mutation results in a Gly to Cys change at AA position 403 that lies between the third and fourth KH domain and may affect the functional properties of the protein.
IPSC lines were generated from peripheral blood mononuclear cells using Sendai virus based reprogramming. IPSCs were characterized using alkaline phosphatase staining, qPCR and immunocytochemistry. The DNMs were validated in the IPSC lines by Sanger sequencing. After characterization the IPSCs were used to differentiate neuronal progenitors and mature dentate gyrus neurons. These are used to investigate neuronal phenotypes including morphology, neurite outgrowth, synaptic connectivity and electrophysiological activity. We explored the molecular effects of the KHSRP mutation using established molecular biology approaches.
The role of KHSRP as a putative schizophrenia candidate gene is discussed in light of our results.

This study is funded by the National Brain Research Program (NAP) of Hungary (Grant NAP-B KTIA_NAP_13-2014-0011 to JR and NZ).

BASAL FOREBRAIN NEURONS RESPOND TO REINFORCEMENT IN PAVLOVIAN CONDITIONING

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The basal forebrain (BF) has widespread cholinergic, GABAergic and glutamatergic projections which are thought to mediate multiple cognitive functions including learning and attention. We recently demonstrated that BF cholinergic neurons respond to reward and punishment and this response is scaled by the unexpectedness of the reinforcer (Hangya et al, 2015). Additionally, putative GABAergic neurons of the BF also display activation proportional to outcome expectations (Lin et al., 2008). These studies raise the possibility that the BF may be involved in broadcasting signals related to reward prediction error or behavioral salience.
To directly test this hypothesis, we trained mice on an auditory pavlovian cued outcome task, in which two pure tones of different pitch predicted likely reward (water) and unlikely punishment (air puff) or vica versa. Mice indicated to have learned the task by showing stronger anticipatory licking after the tone predicting likely reward. Next, we recorded single neurons from the frontally projecting portion of the BF (horizontal limb of the diagonal band of Broca, HDB) while mice were performing the task.
Around 80% of the neurons responded to different behaviorally relevant events (LED signaling a no-lick period, auditory cue, reinforcement) either with increased or decreased firing rate.
We found BF neurons activated both by reward and reward predicting cues, consistent with possible reward prediction error coding in the BF. Interestingly, we found a significant population of cells inhibited by reinforcement delivery during the task. This can be either the result of local inhibition mediated by GABAergic interneurons or the response of the basal forebrain glutamatergic cells.
Characterizing the activity of BF neurons in a task where outcome contingencies are carefully controlled will help us determine whether reward prediction error or salience coding is present in the basal forebrain. Understanding the nature of the behavioral signal broadcasted by the basal forebrain may clarify how the BF participates in associative learning and facilitate translational research aiming to reveal the roles the BF plays in diseases characterized by cognitive decline.

NEURAL BASIS OF DISTRACTOR INTERFERENCE DURING VISUAL WORKING MEMORY MAINTENANCE

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Filtering out distracting objects appearing in the focus of attention during working memory (WM) maintenance is incomplete, resulting in distractor interference effects on WM performance. However, neural processes determining the efficacy of distractor exclusion remains poorly understood. Here we tested the hypothesis that the magnitude of ventral attentional network activation - signalling saliency/bottom-up attentional capture - in response to distractor stimuli predicts the strength of the distractor interference using functional magnetic resonance imaging (fMRI). The results revealed that task-irrelevant WM delay distractor face stimuli strongly activate the face-selective visual cortical areas as well as the regions of the ventral attentional network, including the superior colliculus, pulvinar, and the anterior insula. Furthermore, there was a close association between within-subject alterations in face identity WM performance and distractor-evoked fMRI responses in both the face-selective fusiform face area (FFA) and the right anterior insula. These results imply, that the strength of distractor interference will be determined by its ability to engage the neural resources within the ventral attentional network.

A NOVEL NEUROPLASTIN-TRAF6-DEPENDENT CASCADE INDUCES HIPPOCAMPAL SPINOGENESIS

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Synaptic adhesion systems are intrinsic mechanisms essential in synapse formation. In human and rodent synapses, the cell adhesion molecules Neuroplastins 55/65 (Np55, Np65) are abundantly present (Herrera-Molina et al., 2017; Beesley et al., 2014; Sarto-Jackson et al., 2012). While polymorphisms in the Np gene promotor are linked to cortical thickness and intellectual ability (Desrivieres et al., 2015), we have shown deficits in hippocampus-dependent learning, retrograde amnesia for hippocampal associative memories as well as reduced synapse plasticity and less excitatory synapses in the DG and CA1 hippocampus in Np-deficient mice (Bhattacharya et al., 2017; Herrera-Molina et al., 2017; Herrera-Molina et al., 2014). Indeed, our initial finding that Nps regulate the number of excitatory contacts in CA1 pyramidal neurons in vivo and in vitro (Herrera-Molina et al., 2014) has been recently confirmed (Amuti et al., 2016). Because Np cytoplasmic domain contains a tumor necrosis factor receptor-associated factor 6 (TRAF6) binding motif, we focus on the role of Np-TRAF6 interaction in synapse formation and stabilization. Nptn-/- hippocampal neurons form less and shorter dendritic protrusions during spinogenesis. Rescue of dendritic protrusion formation by Np expression did not occur in mutant Nptn-/- hippocampal neurons co-transfected with TRAF6 siRNA. Also, different Np mutants in the TRAF6 binding site failed to promote dendritic protrusion formation in wild type and CA1 and DG Nptn-/- neurons. Np-TRAF6 direct interaction was confirmed using molecule docking modelling in silico, surface plasmon resonance, pulldown and immunoprecipitation assays and a series of negative dominant and mutant constructs. In HEK cells over-expressing different Nps tagged with fluorescent proteins, we observed multimerization of Nps (FRET experiments), recruitment of cytosolic TRAF6 and robust Np-TRAF6 co-localization (confocal imaging) in newly formed actin-based filopodia. All these effects were reduced by TRAF6 siRNA transfection. Examination of downstream signaling cascades lead us to identify a role for NF-κB and PI3K/Akt/WASP pathways in Np-TRAF6-induced formation of dendritic protrusions and filopodia in neurons and HEK cells respectively. Our data support the existence of a new interaction between Np and TRAF6 resulting in cascade of intrinsic mechanisms able to coordinate gene transcription and actin cytoskeleton organization during early spine formation and stabilization.

RH-M thanks SFB854-B8; SKV thanks GRK1167

PARKINSON'S DISEASE: 200 YEARS STUDYING DEGENERATION AND PROGRESSION.

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Parkinson's disease (PD) is the second neurodegenerative and progressive clinical disease in which age is the single most common risk factor. As aging is increasing in the planet we expected between 8.7-9.3 million of Parkinsonian patients in the worl by 2030. At the moment, PD is affecting 1 in 1000 people in general population but 2-3 % of people over 75. PD was named by French neurologist, Jean-Martin Charcot, 50 years after the first description of the disease made in 1817 by James Parkinson, in London. We are now celebrating the 200 years of the first clinical description made in only 6 people but there is documentation of the disorder in chinese writings as well as others from the ancient Egypt and India. We do not still know the cause of neurodegenration and since the description made by James Parkinson, the etiology has been atributed either to intoxication or to genetics (nurture *versus* nature). During these 200 years many ideas and findings and progresses have been done. In the XXI century we are facing a new disease as the new treatments give new opportunities to the patients leaving longer and better, but many challenges are still open. We are facing non-motor symptoms, the fact of progression of the disease and new clinical features as dementia and side effects of medication. One of the main fields of interest is to get biomarkers, early diagnosis/risk and develop neuroprotective therapies in order to prevent the clinical features; but first of all we need to understand when the disease starts as the disease has a long prodromal-premotor non-dopaminergic stage before the classical motor features appear (due to dopaminergic disfunction). Another endeavour consist in understanding the mechanisms of the disease progression (as the role of either misfolding and trafficking of abnormal proteins or inflammation) in order to develop new therapeutical multi-targeted strategies in a personalized way to slow-down clinical features and improve the quality of life of the patients.

FEAR CONDITIONING IN THE RODENT AMYGDALA IS SECRETAGOGIN-DEPENDENT

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Cell rely on strict maintenance of intracellular calcium homeostasis. Protein interactions typically rely on cell-state specific signaling events that are powerfully regulated by calcium sensor proteins. A recently explored member of this family is secretagogin, which has been first identified in pancreatic beta cells, but is also expressed in neurons of distinct brain regions, such as amygdala. This complex brain domain is necessary to control emotion, learning and memory. Here, we show the exact diagram of secretagogin-expressing neurons in different subdivisions of the mammalian amygdala, including human. In rats, immunoreactive neurons were especially condensed in the extended and centromedial amygdaloid nuclei, as well as in the anterior division of the basolateral amygdala. *In vivo* retrograde tracing from known amygdalar targets allowed us to identify secretagogin-immunoreactive projection neurons only sporadically. Multiple immunolabeling with the typical neuronal calcium binding proteins calbindin, calretinin and parvalbumin suggested that secretagogin is rather promiscuous since it could co-localize with all these proteins, nevertheless, with considerable differences in type and frequency to brain regions. Ultrastructural analysis showed that secretagogin is present in all neuronal compartments, but typically in postsynaptic domains. Actually, we could immunoprecipitate N-methyl-D-aspartate receptor (GluN2A/B subunit) with secretagogin. Secretagogin appeared in the synaptosomal fraction and in the postsynaptic density of micropunched amygdala samples. Further, secretagogin and GluN2A/B co-localized in neuronal somata and proximal neurites in primary amygdala cell culture. Immunoprecipitation showed that fear conditioning increased secretagogin-linked GluN2A/B which was paralleled with ERK1/2 phosphorylation. Conversely, knock-down of secretagogin expression *in vitro* decreased ERK1/2 phosphorylation in a cell line with endogenous secretagogin expression. *In vivo*, fear conditioning did not induce ERK 1/2 phosphorylation in secretagogin knock-out mice unlike in wild types. We suggest that secretagogin-expressing cells represent a hitherto unexplored interneuron population in the mammalian amygdala where secretagogin reshapes synaptic function in fear-conditioned behaviour.

EXTENSIVE ASTROCYTE SYNCHRONIZATION ADVANCES NEURONAL COUPLING IN SLOW WAVE ACTIVITY IN VIVO

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Slow wave activity (SWA) is a characteristic brain oscillation in sleep and quiet wakefulness. Although the cell types contributing to SWA genesis are not yet identified, the principal role of neurons in the emergence of this essential cognitive mechanism has not been questioned. To address the possibility of astrocytic involvement in SWA, we used a transgenic rat line expressing a calcium sensitive fluorescent protein in both astrocytes and interneurons and simultaneously imaged astrocytic and neuronal activity in vivo. Here we demonstrate, for the first time, that the astrocyte network display synchronized recurrent activity in vivo coupled to UP states measured by field recording and neuronal calcium imaging. Furthermore, we present evidence that extensive synchronization of the astrocytic network precedes the spatial build-up of neuronal synchronization. The earlier extensive recruitment of astrocytes in the synchronized activity is reinforced by the observation that neurons surrounded by active astrocytes are more likely to join SWA, suggesting causality. Further supporting this notion, we demonstrate that blockade of astrocytic gap junctional communication or inhibition of astrocytic Ca2+ transients reduces the ratio of both astrocytes and neurons involved in SWA. These in vivo findings conclusively suggest a causal role of the astrocytic syncytium in SWA generation.

This work was supported by grants ERA-Chemistry OTKA 102166, TECH-09-AI-2009-0117 NKFP NANOSEN9 and KMR_12-1-2012-0112 TRANSRAT.

HETEROGENEOUS NEURONAL FIRING PATTERNS DURING HUMAN NEOCORTICAL POPULATION ACTIVITY IN VITRO

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Background: Epilepsy is a disorder associated with neuronal hyperactivity. Surgical tissue removal can be a solution when pharmacological treatment is ineffective. Human brain tissue obtained this way generates spontaneous population activity (SPA), which has previously been attributed to epileptic processes.
Aims: We compared neocortical tissue of pharmacoresistant epileptic patients to neocortical tissue obtained during brain tumor surgery from non-epileptic patients and patients with pharmacologically controlled epilepsy. Our aim was to describe the population activity occurring in all three of these patient groups and investigate differences between them.
Methods: For this purpose, the local field potential gradient was recorded from neocortical brain tissue slices using a laminar multielectrode. The population activity and clustered neuronal activity were detected, cross-correlated and analyzed. The resulting variables were analyzed for associations with epilepsy diagnosis group, patient age and gender, neocortical location and others using multiple regression analysis.
Results: Two types of population activity could be differentiated by their recurrence frequency and current source density amplitude. Interictal-like discharges (IID), characterized by large amplitude were only observed in epileptic patients, while synchronous population activity (SPA), having a lower amplitude and a higher recurrence frequency, was found in all patient groups. Moreover, it only showed minor differences across the various epilepsy diagnosis groups. In certain cases, both types of population activity and/or multiple types of SPA could be observed simultaneously within the same recording. Clustered neurons showed very heterogeneous firing patterns during SPA across all groups. Differences in firing behavior could be associated with differences in patient age, cell type, neocortical lobe of origin, epilepsy diagnosis group and others.
Conclusions: As the observed population activity could be observed in patients without any indications of epilepsy and was very similar across different epilepsy diagnosis groups, it cannot be directly related to epileptic processes. Investigating neuronal activities in epileptic and non-epileptic human tissue might help to elucidate the subtle border between physiological and pathological neuronal population activity.

FEEDING AND METABOLIC ATTRIBUTES OF THE GLUCOSE MONITORING-NEURONS IN THE CINGULATE CORTEX

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Our research group previously identified glucose-monitoring (GM) neurons in the cingulate cortex (cctx), and these cells were proved to be influenced by catecholamines already known to participate in feeding-associated learning, and memory mechanisms in other parts of the brain.
In the present experiments, in order to further examine their involvement in feeding and metabolism, we aimed to determine 1) the complex functional characteristics and 2) the consequences of selective destruction of these local GM neurons in the cingulate cortex.
To do so, in adult male Wistar rats, extracellular single neuron activity was recorded by means of multibarreled glass microelectrodes during a) microelectrophoretic application of neurochemicals, b) intraoral gustatory stimulations, and c) intragastric infusions. After bilateral microinjection of streptozotocin (STZ) into the cctx, a) acute (20 min) and subacute (2 weeks) glucose tolerance tests (GTTs) were performed, b) the concentration of plasma metabolites (total cholesterol, HDL, LDH, triglycerides, uric acid) was determined and c) taste responses of the animals were tested by means of the taste reactivity test as well.
Approximately, 15 % of cingulate cortex neurons responded to microelectrophoretic administration of D-glucose, furthermore, these cells were found to be twice as much responsive to microiontophoretically administered catecholamines, than the glucose-insensitive neurons were. The GM neural cells displayed responsiveness to more taste qualities than the GIS units did. One fifth of the tested neurons responded to intragastric infusion of MSG and the lower concentration of NaCl. The dynamics of the blood glucose curves of STZ treated animals during GTT has changed significantly, furthermore, predominantly positive responses were seen to the unpleasant tastes after the GM neuron destroying microinjection.
Our findings suggest the involvement GM neurons of the cctx in adaptive regulatory mechanisms of the homeostasis. Destruction of these chemosensory neurons appears to cause various metabolic and gustatory alterations as well, thus, directly jeopardize the well being of the organism.

Supported by: PTE ÁOK KA 2013/34039/1; EFOP-3.6.1-16-2016-00004. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

ANALGESIC EFFECTS OF THE NOVEL SEMICARBAZIDE-SENSITIVE AMINE OXIDASE INHIBITOR SZV-1287 IN MOUSE PAIN MODELS WITH NEUROPATHIC MECHANISMS: INVOLVEMENT OF TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 AND ANKYRIN 1 RECEPTOR

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Semicarbazide-sensitive amine oxidase (SSAO) produces tissue irritants by deamination of primary amines, which activate Transient Receptor Potential Ankyrin 1 (TRPA1) and Vanilloid 1 (TRPV1) receptors expressed predominantly on nociceptors. Since there are no data about its functions in pain, we studied the effects and mechanisms of action of our novel SSAO inhibitor and dual TRPA1/TRPV1 antagonist multi-target drug SzV-1287 in different pain models.
Acute chemonociception was induced by TRPV1 and TRPA1 activation (resiniferatoxin and formalin, respectively), chronic arthritis by K/BxN serum transfer, traumatic mononeuropathy by sciatic nerve ligation. SzV-1287 (20 mg/kg i.p.) was investigated in C57Bl/6J wildtype (WT), TRPA1- ($TRPA1^{-/-}$) and TRPV1-deficient ($TRPV1^{-/-}$) mice. Paw mechanonociception was measured by aesthesiometry, thermonociception by hot plate, nocifensive behavior by licking duration, volume by plethysmometry, myeloperoxidase activity by luminescence and plasma extravasation by fluorescence imaging, glia activation in pain-related brain regions by immunohistochemistry.
SzV-1287 significantly inhibited both TRPA1 and TRPV1 activation-induced acute chemonociception and hyperalgesia. In K/BxN arthritis, daily SzV-1287 injections significantly decreased hyperalgesia, L4-L6 spinal dorsal horn microgliosis, edema and myeloperoxidase activity. SzV-1287-evoked anti-hyperalgesic and anti-edema effects were absent in $TRPV1^{-/-}$, and remarkably reduced in $TRPA1^{-/-}$ mice. In contrast, myeloperoxidase-inhibitory effect was absent in $TRPA1^{-/-,}$ but not in $TRPV1^{-/-}$ animals. Acute SzV-1287 administration resulted in approximately 50% significant reduction of neuropathic hyperalgesia 7 days after nerve ligation, which was not observed in either $TRPA1^{-/-}$ or $TRPV1^{-/-}$ mice.
SzV-1287 inhibits chronic inflammatory and neuropathic pain via TRPV1 and TRPV1/TRPA1 activation, respectively, highlighting its drug developmental potential.

Support: NAP B KTIA_NAP_13-2014-0022 (MTA-PTE NAP B Pain Research Group, identification number: 888819), GINOP-2.2.1-15-2016-00020, GINOP-2.3.3.-15-2016-00050, EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

MULTIMODAL NEUROIMAGING MICROTOOL FOR INFRARED OPTICAL STIMULATION, THERMAL MEASUREMENTS AND RECORDING OF NEURONAL ACTIVITY IN THE DEEP TISSUE

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Summary: Infrared neural stimulation (INS) uses pulsed near-infrared light to generate highly controlled temperature transients in neurons, leading them to fire action potentials. Stimulation of the superficial layer of the intact brain has been presented, however, the stimulation of the deep neural tissue has larger potential in view of therapeutic use. To reveal the underlying mechanism of deep tissue stimulation properly, we present the design, the fabrication scheme and functional testing of a novel, multimodal microelectrode for future INS experiments in primates. Three modalities - electrophysiological recording, thermal measurements and infrared wave guiding ability - were integrated based on silicon MEMS technology. Due to the advanced functionalities, a single probe is sufficient to determine safe stimulation parameters in vivo. As far as we know, this is the first multimodal microelectrode designed for INS studies in deep neural tissue.
Motivation and results: Recent technology of silicon microelectrodes facilitates the integration of several functionalities to address unique questions in experimental neuroscience. In this work, we present the development of a multimodal, single crystalline silicon-based neural microelectrode for infrared neural stimulation in the deep tissue. In our system, silicon has both mechanical and optical functionality provided by post-processing steps based on the combined use of wet chemicals. Integrated coupling lens and a low-loss wave-guiding shaft delivers the infrared light (1310 nm) at an overall average efficiency of 25.4 %. The electrodes also hold electrophysiological recordings sites (4-16 channels) and a platinum filament for monitoring the evoked temperature response and accumulated heat (between 34-39 °C) in the stimulated neural tissue, respectively. The infrared light is easy to deliver via a standard LC connector, neural signals and temperature information can be read out through a Preci-Dip connector. The electrophysiological recording was tested by electrochemical impedance spectroscopy. The thermometer's characteristic was derived by a calibration procedure with a medical Negative Temperature Coefficient thermistor used as reference. The infrared wave guiding behaviour was simulated and the efficiency results are validated by relative beam power measurements on blunt tip optical dummies. Loss mechanisms and geometric optimization are investigated by ray tracing analysis in Zemax code. Besides the calibration of the basic functionalities, the longevity of the passivation layers were also investigated in a long term soaking test.

NEURAL RESPONSES EVOKED BY AUDITORY STIMULATION DURING THE SLEEP-WAKEFULNESS CYCLE IN THE CAT

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Changes of auditory cortical evoked potentials depending on vigilance are well known. However, the underlying cellular and circuit level processes are still unclear. In the present study, we implanted chronically linear array metal multielectrodes into auditory cortex (AC) of cats. We recorded spontaneous local field potential (LFP), multiunit (MUA) and single unit activity (SUA) and the same signals evoked by single or pairs of clicks of freely moving animals. We analyzed and compared the properties of spontaneous activity and evoked responses during the different phases of the sleep-wakefulness cycle.
In our previous studies, we found that auditory stimulation during slow-wave sleep (SWS) evoked a down-state-like pattern in the AC that is very similar to spontaneous down states. To characterize the activity of the AC neurons in the evoked down states, we presented single clicks as well as pairs of clicks with different delays within pairs. We found that the LFP response to the second stimulus of the pair is modulated by the phase of evoked potential elicited by the first stimulus. During SWS, late positive component of the first evoked potential (evoked down-state) effectively attenuated the amplitude of evoked potential elicited by the second stimulus. The strength of attenuation depended on the phase of the evoked potential component elicited by the first stimulus. However, on the cellular level, effects of stimulation vary among different types neurons. We found that putative pyramidal cells' firing in response to the second click in the pair was more reduced compared to interneurons.
To describe the firing properties of single units related to the sleep slow waves, we detected the phase of the slow waves and analyzed the preferred phase for firing of each single unit using circular statistics. We found that the different neuron types prefer different phase of the slow wave to fire. During the spontaneous sleep oscillation, both putative principal cells and interneurons prefer to fire at the initial phase of the up state. Circular statistics revealed that interneurons are sharply locked to the beginning of the up-state phase, while firing of the principal cells is less synchronized.

Supported by: Hungarian Brain Research Program - Grant No. KTIA_13_NAP-A-IV/1,2,3,4,6, EU FP7 Grant No. 600925 NeuroSeeker

EFFECT OF EXOGENOUS AND ENDOGENOUS PACAP ON HUMAN PROXIMAL TUBULE CELLS IN VITRO

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Pituitary adenylate cyclase activating polypeptide (PACAP) is an endogenous neuropeptide first isolated in ovine hypothalamus based on its adenylate cyclase activating effect. It has been shown to be present throughout the entire body including the urinary system. Renoprotective effects of PACAP have been proven in various in vivo and in vitro experiments, but its effects in HK-2 human proximal tubule epithelial cells have not been elucidated yet.
The aim of the present study was to investigate whether exogenous PACAP could influence the survival rate of HK-2 cells exposed to gentamicin treatment. For investigating its effect on cell proliferation and survival after gentamicin treatment, cell viability was evaluated using MTT assay. PACAP did not influence the proliferation activity of HK-2 cells. Its protective effect could be detected against gentamicin-induced decrease in cell viability. Obtaining further insight into the background mechanism of gentamicin exposition and PACAP's effect, numerous kidney-related proteins were detected using kidney biomarker array, which revealed significant changes in levels of DPP IV and VEGF.
Moreover, actions of PACAP on LPS-evoked cytokine expression changes were also investigated. Our data showed that PACAP could counteract the LPS-induced expression changes of the following cytokines: GM-CSF, GROα, I309, IL-1ra, IL-6, IL-13, serpin E1.
Additionally, the possible role of endogenous PACAP was also examined using ADCYAP1 small interfering RNA transfection. We detected no difference between cell survival of cells undergoing silencing and cells without transfection suggesting that either there is no endogenous PACAP in HK-2 cells or there might not be any protective role of endogenously present PACAP.
In summary, the present study showed no protective role of endogenous PACAP, but it could alleviate gentamicin-induced nephrotoxicity and could counteract LPS-evoked changes of expression of certain cytokines.

Supported by: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pecs, PTE AOK Research Grant KA-2016-03, KTIA_13_NAP-A-III/5, Bolyai Janos Scholarship, GINOP-2.3.2-15-2016-00050 "PEPSYS", LP2011-08/2011, MTA-TKI. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

ACUTE AND CHRONIC PAIN THRESHOLD CHANGES IN WISKET RATS

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Introduction: Schizophrenia is a complex chronic neuropsychiatric disorder, affecting approximately 1% of the population. It is characterized primarily by positive and negative symptoms and cognitive disturbances. Positive symptoms include hallucinations, voices that converse with or about the patient, and delusions that are often paranoid. Negative symptoms include flattened affect, loss of a sense of pleasure, loss of drive, and social withdrawal. Clinical studies revealed that schizophrenic patients are often show abnormal pain sensitivity, however, only a few schizophrenic animal models investigated this phenomenon. A new substrain of Wistar rats (WISKET) was developed by selective breeding after social isolation and subchronic ketamine treatment. The WISKET rats display several schizophrenia-related deficits, including disturbed sensory gating, motor activity, cognitive function, thermoregulation, cortical electrophysiological abnormalities, opioid and cannabinoid receptor activation in different brain structures, and decreased acute heat pain sensitivity. The aim of the present study was to determine the degree of mechanical allodynia during chronic inflammation, and the potency of opioid ligands applied systemically or intrathecally.
Methods: Monosodium iodoacetate (MIA) was injected into one of the ankle joints of the adult, male WISKET and control Wistar rats to induce an experimental osteoarthritis. Two weeks after MIA injection, the degree of edema and the mechanical pain threshold were detected at both hind paws, and the antinociceptive effects of morphine (1mg/kg subcutaneously), or endomorphin-1 (5 µg intrathecally) were assessed.
Results: MIA caused significant degree of edema in both the control and WISKET animals, but none of the treatments influenced it. The MIA-induced allodynia was blunted in the WISKET rats, while the mechanical sensitivity did not change on the non-inflamed side. Morphine produced significant antinociceptive effect in both groups, but it appeared earlier and was more prolonged in the WISKET compared to the control rats. In contrast, the intrathecally administered endomorphin-1 caused similar degree of antinociception in both groups.
Conclusion: Our study proved that decreased level of inflammatory pain was developed in WISKET rats with enhanced sensitivity to systemically administered morphine. In contrast, endomorphin-1 resulted in equivalent efficacy in both groups, suggesting that primarily the supraspinal processes altered in this schizophrenia animal model.

SUPRAGRANULAR REGULAR SPIKING INTERNEURONS FIRE WITH VARIABLE SLEEP SPINDLE AND THETA PHASE PREFERENCE DURING SLEEP IN FREELY BEHAVING RATS

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Interneurons show spatiotemporally structured firing relative to rhythmic population activity in the hippocampus, but our understanding of neocortical interneurons in relation to coherent network behaviour is less clear. We applied a drug free installation of a pipette microdrive assembly for juxtacellular recording and labelling of supragranular regular spiking interneurons (RSIs) in the parietal cortex during natural sleep in Wistar rats. RSIs had wider action potentials compared to fast spiking interneurons (FSIs) and lower overall firing frequency in non-REM sleep. Fourteen out of 19 RSIs showed rhythmic activity during spindle episodes with firing locked to a particular phase of spindle cycles: ten cells increased firing near the trough, two cells near the peak, one cell at the descending phase, and one cell at the ascending phase, respectively. In addition, millisecond precision temporal analysis of firing revealed high gamma components nested in the spindle rhythm in spindle trough and spindle peak coupled RSIs. Fifteen out of 19 RSIs were recorded during REM sleep and showed elevated firing in comparison to non-REM episodes without significant phase preference (n=8 cells), or sustained phase locked activity near the peak (n=3 cells), near the trough (n=3 cells) and during the ascending phase (n=1 cell) of the ongoing theta oscillation. Three cells exhibited similar phase preference in theta oscillations as in spindle oscillations. In conclusion, half of the recorded RSIs showed phase locked firing near the spindle trough, as we found for FSIs, suggesting cell class specific crosstalk at the trough of spindle oscillations during natural sleep.

MORPHOLOGICAL BASIS OF POSSIBLE SENSO-MOTOR INTERACTIONS IN THE PERIPHERY OF THE POND SNAIL, LYMNAEA STAGNALIS L

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Because of its relative simple nervous system, the pond snail, Lymnaea stagnalis has become one of the favourite experimental models for analysing basic principles of neural integration related to the perception of the environment. Previous studies dealing with sensory processes have focused on centrally located neurons, whereas peripheral sensory neurons (PSN), which are also fundamental elements in controlling behavior, are less studied, especially regarding their possible intercellular connections. Applying histo- and immunohistochemical methods, different PSN, including catecholaminergic, nitrergic and glutamatergic cells, have been described in gastropod species, although their possible functional relationship to each other, or other neural and non-neural elements remained unknown. Therefore, our aim was to explore the innervation pattern of three peripheral organs (lip, tentacle, foot) of adult Lymnaea, using antibodies raised against the biogenic amines serotonin (5-HT) and histamine (HA), tyrosine hydroxylase (TH), glutamate (Glu), and the endogenous molluscan neuropeptides, FMRFamide (Fa) and Mytilus inhibitory peptide (MIP), all proven key members of signaling systems of the gastropod nervous system. Special attention was paid to the distribution, functional morphology and possible interconnections of the immunolabeled elements. A dense network of 5-HT-immunoreactive (IR) varicose fibers of extrinsic origin was found in the sub-epithelial layer of the three organs, positioned parallel with the surface. Series of HA-, TH-, Fa- and MIP-IR sensory cells were located under the epithelium both in the lips and tentacles; their axon processes were intermingled with the 5-HT-IR network. In the tentacle, Glu-IR neurons as well as HA-IR and Fa-IR sub-epithelial fibers also occurred. Densely packed Glu-IR and a few HA-IR and Fa-IR sensory cells, accompanied by an extremely heavy TH-, Fa-, and MIP-IR sub-epithelial innervation system, were present in the foot. Double-labeling experiments revealed mostly a distinctly separated but close localization of the axon processes containing the different signal molecules. Only occasional co-localization of 5-HT-IR with Fa- and MIP-IR, respectively, was seen the lip, and 5-HT-IR with HA-IR in the foot. Based on our findings a complex organization of afferent and efferent elements is suggested in the periphery of Lymnaea, in the course of which interactions between the neuronal elements containing different signal molecules may occur. The interactions may involve simultaneous/parallel actions and/or sequential modulation of the signaling events, including possible local decision making. The results also provide a firm basis for further studies to reveal the synaptic organization and functioning of the peripheral senso-motor system, using correlative light- and electron microscopy and behavioral assays.

Supported by an OTKA grant, No. 111990.

PERINATAL EXPOSURE TO 5-HYDROXYTRYPTOPHAN REDUCES BARREL SIZE IN THE SOMATOSENSORY CORTEX AND INCREASES EXPLORATORY BEHAVIOR IN ADULT RATS

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The region in rodent somatosensory cortex called the barrel field (BF) contains distinct features (barrels) consisting of clustered presynaptic thalamocortical axons (TCA) surrounded by postsynaptic layer IV neurons and separated from each other by cell-free areas (septa). The largest barrels with pronounced topographical organization are contained within the posteromedial barrel subfield (PMBSF) and represent the major facial whiskers, which rodents use to actively explore their environment. Growing evidence suggests that serotonin (5-hydroxytryptamine, 5HT) may play an important role in modulation of BF formation. Drugs targeting the 5HT system are widely used in treatments of affective and anxiety disorders. Although selective serotonin reuptake inhibitors represent a common choice of 5HT enhancers during pregnancy, the immediate 5HT precursor, 5-hydroxytryptophan (5HTP), is being increasingly offered as a natural and safer alternative to antidepressant medication. However, consequences of developmental exposure to increased 5HTP concentrations on brain development and behavior have not been studied in animal models or humans. In the present study, we examined the possible influence of perinatal treatment of rats with 5HTP (25mg/kg) on the PMBSF barrels organization, delineation and size. Wistar rats were treated with either 5HTP or saline, from gestational day 13 until birth by subcutaneous injections to pregnant females, and from postnatal day (PND) 1 until PND 21 by receiving subcutaneous injections themselves. Serum 5HT levels were measured by ELISA at the end of treatment. Brain samples were collected from animal sacrificed on PNDs 22 and 70. Cytoarchitecture of the barrel field and barrel size were analyzed after the respective Nissl or cytochrome oxidase staining of tangentially oriented serial sections across the dorsolateral telencephalic wall. The size of the barrels was measured on the scanned slices using NIH ImageJ software. Chronic 5HTP treatment significantly raised serum 5HT concentrations. In both, pups and adults, barrels of 5HTP-treated animals appeared organized and well-defined but were significantly smaller, compared to those in control rats. Since TCA lack 5HT-sinthesyzing machinery, it is possible that excessive extracellular 5HT levels, originating primarily from the increased peripheral 5HT concentrations, prenatally affected TCA branching, while leaving the postnatal TCA pattering and organization of layer IV neurons into barrel walls intact. As adults, these animals displayed significantly increased exploratory activity, which might indicate somatic sensation impairment, possibly as a consequence of the reduced barrel size. Our results obtained on an animal model suggest a need for thorough examination of the potential effect of 5HTP treatment on the developing human brain and its possible neurological/behavioral consequences.

GLIOTIC REMODELING IN LONG-TERM ORGANOTYPIC CULTURE OF THE HUMAN RETINA

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Purpose: Injuries of the nerve tissue result in a gliotic reaction. The pathomechanism of this process is not entirely described, and most of our knowledge is collected from animal experiments. In our recently developed organotypic culture system the post mortem adult human retina can survive for more than 10 weeks, while preserving its main anatomical architecture. This new technique allows us to study gliotic reactions in adult human tissue over a long period of time. In this experiment we examine gliotic reaction in detail and describe the temporal changes of Müller cells, astrocytes and microglia.
Methods: Retinae from adult human organ donors with very short post mortem intervals (<2 hours) were used in the study. Approximately 5x5 mm big pieces of freshly isolated retina were placed on polycarbonate membrane and were cultured in serum-free chemically defined medium for up to 10 weeks. The cultures were fixed at different time points and were analyzed by immunohistochemistry using glia-specific markers. Expression levels of glia-specific proteins were measured by Western Blot.
Results: The overall retinal morphology was well preserved, all retinal layers were maintained, and every major cell types survived. As a sign of retinal edema, the retinal thickness was increased variably. Müller cells became hypertrophic and showed an increased expression of vimentin and GFAP, while the expression of glutamin synthetase decreased significantly. At the outer surface the endfeet of Müller cells developed tiny processes extending beyond the outer limiting membrane. With antibody against S100 beta we were able to distinguish between astrocytes and Müller cells in gliotic samples. Reactive astrocytes with swollen cytoplasm were found in the inner retina, where their processes formed two main horizontal layers. In non-cultured controls the microglial cells were restricted to the inner retina, while in cultures a fraction of the microglia invaded the outer retina as well. In some cases, close morphological relation between microglia and degenerating photoreceptors was visible.
Conclusions: To our knowledge our model provides the first experimental tool that allows long-term investigation of retinal gliosis in three-dimensional human retina. Our results underlie functional differences of the different glial cell subtypes and can help to understand the pathomechanism of retinal gliosis. More to that, our culture system could be used as a reliable tool for testing effects of chemical compounds on retinal gliosis.

REGIONAL DIFFERENCES IN THE EXPRESSION OF EXTRACELLULAR MATRIX MOLECULES IN THE OLFACTORY BULB

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In the central nervous system (CNS) the macromolecules of the extracellular matrix (ECM) fill heterogeneously the extracellular space. The molecules can form loosely arranged network among the cells and can appear in several condensed forms. They surround perikarya and proximal dendrites as perineuronal net, they can form nodal ECM at the Ranvier nodes and they can appear around the synapses as axonal coat. Smells are detected by primary sensory neurons of olfactory epithelium. Due to the turnover of the neurons, the newly formed axons supposedly establish new synaptic contacts in the olfactory bulb. As the molecules of ECM are involved in the plasticity of nerves system, the role of these molecules are supposed in the background of neural regeneration having a permissive and non-permissive features. The aim of present work was to map the distribution of various chondroitinsulfate proteoglycans (CSPG) such as aggrecan, brevican, neurocan and versican in the olfactory bulb. Examinations were performed on adult Wistar rats. Olfactory bulbs were removed and fixed in St. Marie's fixative. Immunohistochemical reactions were made on cross sections. To determine the localization of these molecules we have used immunohistochemical reaction for the detection of axons. According to our results, the expression of CSPG molecules shows a special staining pattern in the layers of the olfactory bulb. Aggrecan shows similar distribution pattern to that we found in the previous examinations after WFA staining. Expression of brevican and versican appears along the axons in dot-like form. In case of neurocan we found a strong staining zone in the external plexiform layer. Ring-like pattern of perineuronal net (PNN) was not detectable in either case. The organization of these molecules may be related with the high degree synaptic plasticity of the olfactory bulb.

Support: OTKA K115 471 and MTA TKI 355

THE EFFECT OF SOMATOSTATIN 4 RECEPTOR AGONISTS IN MOUSE MODELS OF NEUROPATHIC PAIN, ANXIETY AND DEPRESSION-LIKE BEHAVIOR

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Chronic neuropathic pain caused by nerve damage of different etiological factors is often coupled with anxiety and depression, deteriorating quality of life for patients. The current medical treatment is often ineffective or even if partially effective, the severe side-effects limit their long-term use. Therefore, identification of the key mediators and their targets for the developement of an analgesic drug with a completely novel mechanism is necessary. Somatostatin has been proven to have analgesic, anti-inflammatory and antidepressant effects mediated by the somatostatin 4 receptor (sst_4) without influencing endocrine functions. We examined the *in vivo* actions of two compounds of our patented small molecule non-peptide sst_4 agonists (VCC158015, VCC885587; Vichem Kft.) that had been shown to have the best sst_4 receptor activation abilities on transfected CHO cells. Traumatic mononeuropathy was induced in male NMRI mice by the partial ligation of the sciatic nerve. The mechanonociceptive threshold of the hind paw was measured with a dynamic plantar esthesiometer. Depression-like behavior was examined by tail suspension test (TST) and forced swim test (FST), which represent different neuronal activation mechanisms of depression. Anxiety was investigated by elevated plus-maze (EPM) and spontaneous locomotor activity by open field test. The agonist or the methylcellulose vehicle was administered orally an hour before the examinations. In the neuropathy model the mechanonociceptive threshold of the 7th postoperative day was remeasured after treatment.
About 35-40% drop of the mechanonociceptive threshold (hyperalgesia) developed 7 days after the nerve ligation. Both agonists significantly and dose-dependently alleviated this mechanical neuropathic pain response (20, 100 and 500 µg/kg orally). The largest dose of both compounds had 55-70% antihyperalgesic effect, but one of them decreased the spontaneous locomotor activity. The 100 µg/kg dose with a 35-50% antihyperalgesic effect decreased the time spent immobile (which indicates depression-like behavior) in the TST, but had no effect in the FST. Neither agonist had anxiolytic effects in the EPM test.
Our novel orally administered sst_4 agonists efficiently alleviated neuropathic mechanic hyperalgesia and depression-like behavior, hence providing a promising tool for an interesting and valuable combined treatment of neuropthic pain and depression.

Support: KTIA_NAP_13-2014-0022, GINOP-2.3.2-15-2016-00048, GINOP-2.3.2-15-2016-00050, EFOP-3.6.1.-16-2016-00004
Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs
The project has been supported by the European Union, co-financed by the European Social Fund
The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

INVOLVEMENT OF CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS OF SUBTYPE 2 AND OPIOID RECEPTORS, LOCATED WITHIN PERIAQUEDUCTAL GRAY MATTER, IN CENTRAL AND PERIPHERAL CRF-INDUCED ANALGESIC EFFECT ON SOMATIC PAIN SENSITIVITY IN RATS

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Corticotropin-releasing factor (CRF) is involved in the regulation of somatic pain sensitivity under stress. Analgesia is one of the characteristics of stress reaction and CRF is involved in providing of stress-induced analgesia. Exogenous CRF may produce analgesic effect and mimic stress-induced analgesia.CRF action is mediated by CRF receptors of subtype 1 and 2 (CRF-R1 and CRF-R2 receptors). Periaqueductal gray matter (PAGM) of the midbrain is one of key structures of antinociceptive system. It plays a role in the descending modulation of pain. PAGM is involved in stress-induced analgesia and somatic pain regulation. However, a role of the PAGM in CRF-induced analgesia remains unclear. The aim of the study was to investigate the involvement of CRF-R2 receptors and opioid receptors localized in PAGM in analgesic effect caused by central or peripheral CRF in conscious rats. CRF was administered in PAGM (0.7 mkg/rat) or intraperitoneally (40 mkg/kg). The involvement of CRF-R2 receptors and opioid receptors was studied by administration of their antagonists astressin2 B and naltrexone, respectively. Naltrexone or astressin2 B were injected centrally (intra-PAGM) or peripherally before CRF administration. Somatic pain sensitivity was tested by tail flick latency (tail flick latency was induced by tail's thermal stimulation). Both peripheral and central CRF administration caused an increase in tail flick latencies (analgesic effect). CRF-induced analgesia was accompanied by an elevation of plasma corticosterone levels. Intra-PAGM administration of naltrexone (1 mkg/rat) or astressin 2B (1 mkg/rat) attenuated the central as well as peripheral CRF-induced analgesia. Peripheral administration of naltrexone (1 mg/kg, i.p.) also reduced central as well as peripheral CRF-induced analgesic effect. Peripheral administration of astressin2 B (200 mkg/kg, s.c.) resulted in inhibition of peripheral CRF-induced analgesia. The results obtained suggest that opioid and CRF-R2 receptors are involved in central as well as peripheral CRF-induced analgesia and one of the mechanisms of CRF-induced analgesic effect on somatic pain sensitivity may be mediated through opioid and CRF-R2 receptors localized in PAGM.

The study was supported by grant of Russian Science Foundation (RSF) - 14-15-00790.

KISSPEPTIN-13 EVOKES HYPERALGESIA IN FEMALE MICE

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Introduction: Sex differences in pain sensitivity and pain tolerance over the menstrual cycle as well as the overrepresentation of characteristic pain syndromes in women point toward the involvement of the reproductive system in pain modulation. Kisspeptin, a hypothalamic neuropeptide, well-recognized for its role in the regulation of the reproductive axis, is also a member of the anti-opioid RF-amide family. Following the report on the hyperalgesic action of kisspeptin, the aim of the present study was to investigate the effect of kisspeptin-13, an endogenous derivative of kisspeptin, on nociception in adult female C57BL6 mice.
Methods: Different doses (0.5-2 µg/2 µl) of kisspeptin-13 were injected intracerebroventricularly to both male and female mice, after of which the pain sensitivity was assessed by the heat-radiant tail flick test. The test was performed 30, 60 and 120 min after peptide treatment. To assess the changes of nociceptive phenotype over the estrous cycle methylene blue-stained vaginal smears were prepared of female mice and then evaluated. As interaction between RF-amides and the opioid system has been linked before the relative gene expression levels of opioid receptor family members (OPRD1, OPRK1 and OPRM1) were determined by quantitative real-time PCR 2 hours after kisspeptin-13 treatment from amygdala, anterior cingulate cortex (ACC) and dorsal root ganglia (DRG) samples.
Results: Our results showed that kisspeptin-13 dose-dependently decreased the pain threshold of female mice. Furthermore, kisspeptin-13 treatment resulted in significant relative gene expression downregulation in case of OPRM1 in amygdala, and DRG samples of mice in estrous and upregulation in amygdala and DRG of mice in diestrous stage. Increased OPRM1 expression were found in ACC of proestrous. The relative gene expression of OPRK1 was significantly downregulated after kisspeptin-13 challenge in amygdala in metestrous and upregulation in DRG of estrus and metestrous. Kisspeptin-13 resulted in marked relative gene expression upregulation of OPRD1 in amygdala uniformly in all stages of the estrous cycle, however in samples from ACC and DRG significant relative gene expression decline was detected in diestrous and proestrous.
Conclusion: These data underlie the hyperalgesic action of kisspeptin-13 in female mice and suggest kisspeptin exerts its action at least partially through altering the expression pattern of opioid receptors in a region- and estrous cycle-dependent manner.

This work was supported by the Hungarian Brain Research Program-KTIA_13_NAP-A- III/8.

THE IMPACT OF INTRACELLULAR CHLORIDE MODULATION ON THE CELLULAR VIABILITY IN RAT PRIMARY HIPPOCAMPAL NEURONAL CULTURE SUBJECTED TO OXYGEN-GLUCOSE DEPRIVATION

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Cerebral ischemia in vivo results in neurons' deprivation of oxygen and glucose and consecutive inability to conduct an aerobic metabolism on which neuronal functioning strictly depends. This condition is mimicked in vitro by oxygen-glucose deprivation (OGD) and is characterized by important alterations in neuronal ionic homeostasis. Recently, considerable attention has been focused on the intracellular Cl- concentration and its modulation by cation-chloride cotransporters Na-K-Cl cotransporter (NKCC-1) and K-Cl cotransporter (KCC2), as the transmembrane chloride gradient is critical for the GABAergic inhibition and subsequent response to ischemic damage. In the present study we explored the effect of bumetanide, a specific inhibitor for NKCC-1 and DIOA, a KCC2 inhibitor, on OGD-exposed primary hippocampal cultures.
Primary cultures of hippocampal neurons were obtained from postnatal day 0 Wistar rat pups. After 7 days in vitro, cell cultures were exposed to OGD conditions of increasing duration (1 h, 1.5 h and 2h) and the 2 hour OGD attained a severity threshold that triggered significant metabolic deprivation when compared to control normoxic cultures ($p<0.001$). Further, cell cultures exposed to 2 hour OGD or control conditions received treatment with either bumetanide (10μM) or DIOA (20μM). Assessment of cellular metabolism and viability was performed using resazurin assay, after 3-hours of reoxygenation in a normoglycemic/normoxic medium.
Our results showed that the decreased cellular viability triggered by the 2-hour exposure to OGD was further decreased by DIOA ($p<0.05$), with lack of significant effect from bumetanide treatment ($p>0.05$).
In conclusion, DIOA may be detrimental to mature hippocampal neurons in culture when subjected to OGD by increasing intracellular chloride with impact on GABAergic inhibition. Our data support the KCC2 involvement in mature hippocampal neuronal excitability. Future studies could further exploit the chloride cotransporters modulation as a potential tool in neuroprotection studies.

RIN1 REGULATES SYNAPTIC PLASTICITY BY COORDINATING ACTIN DYNAMICS AND AMPA RECEPTOR ENDOCYTOSIS IN DENDRITIC SPINES

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The ability to form and recall memories relies on the activity-dependent modulation of the synaptic strength between neurons. Synaptic strength is modulated by altering actin remodelling in dendritic spines, thus changing their morphology, or by regulating neurotransmitter receptor trafficking at the postsynaptic area. Ras and Rab interactor 1 (RIN1) is a protein mostly expressed in forebrain neurons. In non-neuronal cells, RIN1 is known to promote Abl-mediated changes in actin remodelling and Rab5-mediated receptor endocytosis. As the lack of RIN1 enhances the acquisition and persistence of fear-associated memory in transgenic mice, we aimed to elucidate how RIN1 regulates plasticity-related neuronal mechanisms.
We prepared embryonic hippocampal cell cultures from RIN1 knock-out (RIN1ko) mice and transfected them with fluorescently labelled wild type and point mutant RIN1 constructs. Time-lapse recordings of living neurons revealed that the motility of the dendritic filopodia, which are precursors of mature dendritic spines, is increased by RIN1 and is dependent on the interaction between RIN1 and Abl kinase. In accordance with this, measuring the recovery of actin fluorescence after photobleaching (actin-FRAP) proved that RIN1 increases actin remodelling in filopodia via regulating Abl kinases.
Whole-cell voltage clamp experiments proved that RIN1ko neurons possess elevated amplitudes of miniature excitatory postsynaptic currents (mEPSCs) evoked by spontaneous glutamate release without any significant change in the frequency of mEPSCs. Quantitative confocal microscopy in transfected RIN1ko neurons showed that re-introducing RIN1 decreases the ratio of mushroom shaped spines as well as the amount of GluA1 AMPA receptor (AMPAR) subunits within the plasma membrane regions of Shank2 immunopositive postsynaptic areas. In RIN1ko neurons, overexpression of wild type RIN1 exerted its effects on GluA1 endocytosis via its Rab5 GEF activity. Upon chemically induced LTD (cLTD), postsynaptic GluA1 levels and the density of dendritic spines were reduced significantly in wild type neurons. On the other hand, lack of RIN1 impaired cLTD-induced changes in the cultivated neurons, indicating that RIN1 is an important regulator of GluA1 endocytosis and morphological redistribution during cLTD.
Taken together, we show that RIN1 downregulates surface AMPARs and increases actin dynamics within dendritic protrusions. These results indicate that RIN1 destabilizes synapses, reduces synaptic efficacy and prevents the formation of new connections, highlighting RIN1 as a key regulator of extincting fear and aversive memories.

Research was supported by the KTIA_NAP_13-2014-0018 grant to KS, by the PF247/13-1 DFG project to AH and by the MÖB-DAAD travel exchange program.

BDNF AND PROBDNF IN THE REGULATION OF AGGRESSIVE BEHAVIOR

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Brain-derived neurotrophic factor (BDNF) plays crucial role in neuronal survival, development, differentiation and plasticity. It is known that mice with decreased BDNF expression including knockout and conditional knockout showed increased intermale aggression. In our study we focused on BDNF expression in Norway rats selectively bred for 85 generations for high level of aggression towards man or its absence.
Considerable differences between highly aggressive and nonaggressive rats were shown both in BDNF mRNA and protein levels. Significantly increased BDNF mRNA level was found in the frontal cortex (FC), raphe nuclei area of midbrain (RN), nucleus accumbens (NAcc), amigdala (Am) and hypothalamus (Ht) of aggressive rats compared to nonaggressive rats. BDNF mRNA levels in the hippocampus (Hc) and substantia nigra (SN) were unaltered. At the same time, significantly increased BDNF level as well as proBDNF level was found in the Hc, Am and NA of aggressive rats compared to nonaggressive rats. We have also found that BDNF protein level was increased in the RN and striatum. In the FC of nonaggressive rats only proBDNF level was decreased.
It can be assumed that BDNF-mediated regulation of neuronal function could be a general mechanism for different types of aggressive behavior. We have found that rats genetically predisposed to a high level of defensive aggression showed decreased social behavior and increased pathological aggressive behavior towards juvenile males. Also the highly aggressive rats demonstrated increased predatory aggression – latency time of muricide was shorter in highly aggressive than in tame animals. At the same time, both lines of rats did not differ significantly in intermale aggression. The obtained data allow to suggest a close relation between defensive, predatory and pathological aggressive behavior that indicate similar genetic mechanisms underlie these types of aggressive behavior. The observed increase in the BDNF expression in the number of structures which play the key role in the regulation of fear memory (Hc-Am) as well as modulation of aggressive behavior (NAcc-Ht) confirms our hypothesis.
Thus, for the first time it was shown that BDNF and proBDNF contribute to the genetically defined aggressiveness in rats.

The work supported by the Russian Scientific Foundation (#17-15-01021).

LIPID RAFT ISOLATION FROM MOUSE BRAIN TISSUE UNDER CONDITIONS THAT RETAIN SUBMEMBRANE DISTRIBUTION OF GANGLIOSIDES AND PROTEINS

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Cell membranes are not uniform in their protein and lipid composition but organized in microdomains termed lipid rafts with higher concentration of (glyco)sphingolipids, cholesterol, and specific membrane proteins. The investigation of lipid rafts is thriving since the disturbed lipid microenvironment is recognized as highly important in pathogenesis of numerous human disorders. It is challenging to isolate lipid rafts from the bulk membrane in a way that would accurately reflect their composition and organization in living cells. Most often, detergent-resistant membranes enriched in cholesterol and sphingomyelin are extracted using the non-ionic detergent Triton X-100 (Tx-100). However, Tx-100 causes a redistribution of gangliosides and glycosylphosphatidylinositol-anchored proteins, which makes it an unacceptable choice for investigating the exact relationship between membrane gangliosides and proteins.
The aim of this work was to develop a method for lipid raft isolation that would retain ganglioside and protein distribution for the purposes of a larger study investigating the crosstalk between gangliosides and specific membrane proteins. Since no-detergent methods of lipid raft isolation were not sufficiently effective or reproducible in our hands, the detergent Brij O20 was used in extraction followed by optimized sucrose density ultracentrifugation. Successful raft isolation was confirmed by Western blotting of non-lipid raft membrane protein transferrin receptor and lipid raft markers protein flotilin and ganglioside GM1 detected by cholera toxin subunit B immuno-overlay. The distribution of these markers, as well as gangliosides GT1b, GD1a, and specific membrane proteins, was compared in lipid rafts and the bulk membrane isolated with Tx-100 and Brij O20. Ganglioside distribution was found to be different as a result of Tx-100 vs. Brij020 isolation. Using Tx-100, the majority of ganglioside GD1a appears in raft fractions with almost no staining in bulk membrane, while Brij O20 isolation reveals a higher proportion of GD1a in the bulk membrane. Striking evidence for protein redistribution in Tx-100 isolation was found for transmembrane protein neuroplastin (Np) which is known to be affected by ganglioside environment. In Tx-100 isolation, the vast majority of Np is segregated in non-raft fractions (90% of all Np), compared to Brij O20 isolation where around 70% of Np is distributed in non-raft fractions.
We show that isolating lipid rafts using Tx-100 leads to redistribution of gangliosides and specific proteins. This work will enable more accurate lipid raft analysis in respect to ganglioside and membrane proteins composition and lead to improved resolution of lipid-protein relations within lipid rafts.

COMPOSITIONAL CHANGES OF BRAIN GANGLIOSIDES LEAD TO DISPERSAL OF CELL ADHESION MOLECULE NEUROPLASTIN FROM THE LIPID RAFT FRACTIONS

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Lipid rafts are membrane microdomains characterized by high content of cholesterol, sphingomyelin and glycosphingolipids, particularly gangliosides. Gangliosides are especially important for modulating various cellular events in mammalian brain, such as signal transduction, adhesion, and cellular recognition. There is strong evidence that ganglioside composition ensures correct positioning and functions of specific membrane proteins through interactions of gangliosides and proteins within membrane subdomains. In addition, any disturbance of ganglioside composition leads to changes in lipid raft integrity. In our previous work, we have reported altered expression and distribution of transmembrane glycoprotein neuroplastin (Np) in hippocampus of mice lacking complex gangliosides. Np is a cell adhesion molecule involved in promoting neurite outgrowth, regulation of structure and function of synapses and synaptic plasticity. The aim of this study was to analyze membrane positioning of Np within specific membrane subdomains - lipid rafts and non-rafts, in cortical tissue of mice with aberrant ganglioside synthesis. Cortical tissue was dissected from brains of B4galnt1 knock-out (KO) and St3gal2/3 double knock-out (DKO) mice and their corresponding wild-type (WT) controls. The phenotype of KO mice includes demyelination and motor deficits, and is characterized by complete absence of all complex brain gangliosides. DKO mice have a distinctly different brain ganglioside composition with overexpression of complex gangliosides GM1 and GD1b and lack of GD1a and GT1b, which leads to more severe phenotype including neurological and motor deficits. In our study membrane proteins were isolated from brain tissue homogenates and segregated in lipid raft and non-raft domains using a procedure for lipid raft isolation modified in our laboratory. Neuroplastin distribution within the specific membrane fractions was analyzed using Western blotting and quantification by ImageJ. We report changed total cortical Np expression in both mouse models with aberrant ganglioside composition as compared to WT mice, more prominently in KO mice. Also, we show different positioning of Np within membrane subdomains depending on ganglioside composition. Specifically, Np is redistributed and dispersed from lipid raft to non-raft domains in analyzed cortical tissue of both KO and DKO mice when compared to WT animals. Observed alteration of Np expression and positioning within the membrane is related to documented specific changes in ganglioside composition in brain tissue of mouse models with disrupted ganglioside biosynthesis. Our results clearly demonstrate that Np expression and submembrane distribution depends on specific ganglioside milieu. Further investigation is needed in order to clarify the exact interaction of Np and gangliosides as well as functional consequences of Np redistribution caused by changed ganglioside environment.

INSULIN AND MEMORY IN THE POND SNAIL

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Insulin is well known as a hormone regulating glucose homeostasis across phyla. The finding of insulin and its receptors in the mammalian brain revolutionized the concept of insulin signaling. However, insulin's role in brain functions, such as cognition, attention and memory, remains unknown. Studies using invertebrates with their open blood-vascular system have the promise of promoting a better understanding of the role played by insulin in mediating/modulating cognitive functions.

In my presentation, the relationship between insulin and its impact on long-term memory (LTM) is discussed in the pond snail *Lymnaea stagnalis* (Linnaeus 1758). *Lymnaea* has the ability to undergo conditioned taste aversion (CTA); that is, it associatively learns and forms LTM not to respond with a feeding response to a food that normally elicits a robust feeding response. Previous studies showed that some molluscan insulin-related peptides (MIPs) were up-regulated in snails exhibiting CTA. We thus hypothesized that MIPs play an important role in neurons underlying the CTA-LTM consolidation process. To examine this hypothesis, we first observed the distribution of MIP II, a major peptide of MIPs, and MIP receptor and determined the amounts of their mRNAs in the central nervous system (CNS). MIP II was only observed in the light green cells in the cerebral ganglia, but the MIP receptor was distributed throughout the entire CNS including the buccal ganglia. Next, when we applied exogenous mammalian insulin or partially purified MIPs to the isolated CNS, we observed a long-term change in synaptic efficacy (i.e., enhancement) of the synaptic connection between the cerebral giant cell (a key interneuron for CTA) and the B1 motor neuron (a buccal motor neuron). This synaptic enhancement was blocked by application of an insulin receptor antibody to the isolated CNS.

Further, we know that the best CTA is achieved, if snails are food-deprived for 1 day before training commences. With a longer period of food deprivation (5 days), learning and memory formation does not occur. It has been hypothesized that the levels of insulin in the CNS are very important for CTA to occur. To test his hypothesis, we injected insulin directly into 5-day food-deprived snails. The injection of insulin, as expected, resulted in a decrease in hemolymph glucose concentration. Consistent with our hypothesis with insulin injection, learning and memory formation of CTA occurred. That is, the 'insulin spike' is more important than an increase in hemolymph glucose concentration for CTA-LTM. If we injected an insulin receptor antibody into the snails before the insulin injection, learning was formed but memory formation was not, which is consistent with our previous study. Therefore, a rise in the insulin concentration (i.e., insulin spike) in the CNS is considered to be a key determining factor in the process of CTA-LTM.

FAST GENERATION OF SYNCHRONOUSLY ACTIVE CORTICAL NETWORKS IN 3D NEURAL AGGREGATES DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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The complexity of the human brain and inapproachability for direct studies as well as species-specific differences between animals and humans in neurophysiology and pharmacology make human cell culture-based modeling necessary and increasingly valuable. *In vitro* cultivation of human induced pluripotent stem cell (hiPSC)-derived neural stem cells recapitulates *in vivo* processes of human corticogenesis. Although different protocols have been employed to direct differentiation of hPSC derived neuronal progenies, electrophysiological studies reveal high variations in the time course and degree of maturation. Thus, the currently used *in vitro* culture conditions might be lacking the proper environmental stimuli to adequately support the potential of electrophysiological maturation, synaptogenesis and synchronously oscillating neural network formation of human iPSC-derived neurons.
We developed a novel fast cultivation procedure to generate person specific hiPSC-derived mature neuronal assemblies *in vitro*. We used immunocytochemistry and confocal laser microscopy to describe the cellular composition and characterized the electrophysiological development on single cell as well as on network level applying Patch-clamp and microelectrode array (MEA) technology, respectively.
We will present that hiPSC neurons display functional electrophysiological properties at single cell level within few days and give rise to synchronously active networks within 10-20 days in culture. In detail, patch-clamp recordings confirmed the functional electrophysiological properties of the neurons within the aggregates indicated by spontaneous action potentials and bursting activity, excitatory and inhibitory spontaneous synaptic activity. The presented MEA-recordings will provide insights into the spatiotemporal properties of synchronous and oscillatory network activity. The autonomously active hiPSC-derived cortical neurons are localized within 3D-neural aggregates, are embedded in a glial network composed of endogenously developed S100 positive human astrocytes and interconnected by vGlut1 and PSD-95 positive mature synapses.
Cultivation of human iPSC-derived cortical neurons in 3D aggregates enhances the electrophysiological neuronal maturation on single cell and neuronal network level. We propose that our functional 3D neural aggregates are a suitable research tool to model person specific neuronal networks *in vitro*.

MENTHOL – ESSENTIAL OIL COMPONENT INCREASES BURST OF ACTION POTENTIALS EVOKED BY CARBAMATE INSECTICIDE

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Carbamate insecticides are temporary inhibitors of AChE in insects. In case of using high doses, poisoning is probable. There is a great need to lower utilization of chemical insecticides. One of the current strategies is to combine chemicals with naturally occurring bio-insecticides that can increase the activity of the first ones. In our study we propose to combine bendiocarb (carbamate insecticide) and menthol - the component of essential oil from *Mentha sp*. Menthol has confirmed insecticidal property, although its exact mode of action at insects is not well investigated. Available data suggest that menthol can act as weak AChE inhibitor, GABA receptor positive modulator or as agonist of octopamine receptor. The aim of our study was to examine if menthol increases the effectiveness of bendiocarb insecticide.
In our study we have used extracellular recordings of the total activity of the nerves of *Periplaneta americana*. We have recorded two parameters: 1) the response to mechanical stimulation of cerci, 2) the spontaneous activity of the nerve. We have evaluated those parameters after menthol application, bendiocarb application and after combination of the two chemicals. To confirm the effect on whole animals we have performed behavioral tests - we evaluated the ability of the insect to turn back from the dorsal to the ventral side.
In behavioral tests bendiocarb in concentrations 10^{-6}M and 10^{-8}M slightly increases the time of turning back of the animal from the dorsal to the ventral side. This can indicate a soft paralyzing effect on *P. americana*. Menthol 10^{-6}M applied alone also increases the time of turning back from the dorsal to the ventral side. The effect of those two chemicals separately was not significant. On the contrary, when we combined menthol 10^{-6}M and bendiocarb 10^{-6}M we observed a great increase in the time of turning back of the cockroaches. This indicates high paralytical potential of the combination of menthol and bendiocarb.
In electrophysiological experiments bendiocarb causes uncontrolled burst of action potentials, which are independent of stimulation. In comparison to the control spontaneous activity, the activity in "bursts" was 3,3 times higher in the presence of bendiocarb 10^{-6}M. Bendiocarb does not change the response to stimulation. On the contrary, in the presence of menthol spontaneous activity of the nerve cord does not change, although menthol causes fast decrease of size of response to. When we combined different concentrations of bendiocarb with menthol 10^{-6}M we observed more bursts of action potential caused by bendiocarb and its higher frequency. Menthol 10^{-6}M move the curve of effectiveness of bendiocarb to lower concentrations direction. Our results indicate the combination of menthol and bendiocarb as a high active preparation while low activity of chemicals alone.

Project was supported by National Science Center, Poland under grant: NCN 014/15/N/NZ9/03868.

MUTATION AT THE GABAA RECEPTOR BINDING SITE B2E155 RESIDUE AFFECTS MECHANISM OF GABAA RECEPTOR ACTIVATION

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$GABA_A$ receptor is the primary mediator of inhibitory neurotransmission in the adult brain. Glutamate 155 of the GABAA receptor β2 subunit is located at the binding site and has been implicated in direct interactions with the neurotransmitter. Moreover, β2E155 is likely to be an initial trigger for ion channel opening as movements of amino acids at GABA-binding site region is involved in coupling GABA binding to channel gating. In the present study we investigate the impact of β2E155 mutation on GABAA receptor kinetics. To address this issue we combined ultrafast solution exchange with patch-clamp electrophysiology to record macroscopic currents mediated by wild-type and β2E155C mutated α1β2γ2 and α1β2 receptors. Cysteine substitution of β2E155 right-shifted the dose-response curves for GABA-elicited currents. Moreover, it also slowed down macroscopic desensitization, accelerated deactivation kinetics and enhanced spontaneous activity (in α1β2γ2 receptors). Non-stationary-variance analysis performed for α1β2E155Cγ2 receptors showed a reduction of maximal open probability (compared to wild-type receptors) without affecting single-channel conductance. In addition, mutations in both types of receptor make muscimol a superagonist in comparison to GABA. We extended this macroscopic approach by microscopic recordings. Our preliminary single-channel recordings analysis suggests that mutation affected shut time distributions. Taking all together, β2E155 residue has strong impact on both binding and gating. Our analysis and model simulations indicate that the most likely mechanism of gating modulation could be acceleration of preactivation/flipping transitions.

Supported by NCN grant DEC-2015/18/A/NZ1/00395 and GR-808/NCN/2014.

EPILEPTIFORM AND SLEEP SPINDLE ACTIVITY IN ANTERIOR NUCLEUS OF THE THALAMUS IN TEMPORAL LOBE EPILEPSY PATIENTS

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Introduction*:* Deep brain stimulation of anterior nucleus of the thalamus (ANT-DBS) is an approved therapeutic approach for drug resistant epilepsy. Little is known about the participation of ANT in epileptic and sleep EEG processes. The appearance of the sleep spindles (SS) is related to interictal discharges (IID), and thalamus in general is considered as the generator of SSs. The ANT, however, is excluded from this process according to animal data.

Methods: In this present study we included 10 ANT-DBS bitemporal epilepsy patients with externalized leads and surface electrodes undergoing 2 days video-EEG monitoring (VEM). We investigated IIDs, ictal events (IE) and SSs in ANT and also in surface EEG recordings. IIDs and IEs were analysed by visual inspection, SSs were detected according to the patient own special spindle frequency, separately on the scalp (Fp1-2, F3-4, F7-8) and thalamus both sides. SS spectral peak frequency and synchronization between scalp and ANT was measured.

Results*:* IIDs appeared in the thalamus and on the scalp both in a dependent and independent manner. Three patients had seizures during VEM. Seizures did not start but spread to the ANT electrodes in all cases. The slow, frontal type of SSs were prevailing in the ANT.

Conclusion: ANT participates in both sleep and epileptogenic processes. It seems that IIDs and IE spread to the ANT. Frontal, slow frequency SSs has local sources in the ANT indicating its participation in sleep processes. Further evaluation for seizure outcome of ANT-DBS therapy and correlation with ANT recordings are underway.

Supported by KTIA_NAP_13-1-2013-0001

ATP-HYDROLYZING MEMBRANE ENZYME CD39L1/NTPDASE2 – A NOVEL PLAYER IN NEUROINFLAMMATION?

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Purine nucleotides, omnipresent in extracellular environment, act as signaling molecules. They assert plethora of physiological and pathophysiological roles through a complex network of purinergic receptors. During pathological conditions of inflammation, high millimolar concentration of ATP in extracellular milleux acts as a danger proinflammatory signal through P2 receptor families. In contrast, adenosine exerts namely anti-inflammatory role via P1 receptors. Extracellular concentration of purine nucleotides is determined by interplay of ectonucleotidases, enzymes widely distributed on cell membranes in central nervous system (CNS), as well as immune system. Ectonucleoside triphosphate diphosphohydrolase (NTPdase) 1, 2 and 3 limit ATP concentration by hydrolyzing it to ADP and AMP. Latter can be hydrolyzed further to adenosine by CD73. Unlike NTPDase1 (CD39), NTPDase2 has a high preference for ATP over ADP, which results in efficient removal of proinflammatory ATP and simultaneous accumulation of ADP, accountable for its effects as an agonist of P2Y1, P2Y12 and P2Y13 receptors. This paradigm rises a question of ectonucleotidases, especially NTPDase2, as buffers of inflammation and plausible terapeutic targets. Significance of CD39 and CD73 in immune cell regulation and activity is well documented, especially in the immunosupressive and anti-inflammatory role of Treg cells. Data on the role of CD39 and CD73 in the pathology of multiple sclerosis is accumulating, alongside with their role in its best characterized *in vivo* model - experimental autoimmune encephalomyelitis (EAE). However, data on the role of NTPdase2 in neuroinflammation, in both CNS and immune system, remains scarce. Our previous results have provided an insight into regulation of NTPDase2 gene expression and ATP hydrolysis in the spinal cords of EAE rats in a disease phase specific manner. Here, we investigated regulation of NTPDase2 in detail in both CNS and immune system on gene, protein and cellular level. Eight-week old female Dark Agouti rats were immunized with rat spinal cord tissue homogenate in complete Freund's adjuvant. Lumbar sections of rat spinal cords were isolated at three time points representing three disease stages: onset, peak and recovery. Tissue was used for NTPDase2 gene (real-time PCR) and protein expression studies (Western Blot, immunohistochemistry and immunofluorescence). In addition, NTPDase2 regulation was also investigated in lymphocytes isolated from draining lymph node by means of real-time PCR and flow cytometry.
Our results have shown that NTPDase2 expression is altered dramatically and differentially in CNS and immune system in EAE. Its expression is dominant in cells that mediate disease progression and regulated in a disease phase specific manner. In conclusion, data implies that NTPDase2 is a novel player in neuroinflammation and its role in the disease resolution needs to be further investigated.

CALLUS FORMATION IS DISTURBED IN PACAP (PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE) KO MICE

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Pituitary Adenylate Cyclase Activating Peptide (PACAP) is a naturally secreted signaling peptide which has important regulatory roles in the differentiation of the central nervous system and several peripheral tissues. However, little is known about the connection of PACAP signaling pathways to osteogenesis or bone regeneration. We investigated the morphology of callus formation in tibia of PACAP knockout (KO) and wild type (WT) mice and studied the signaling pathways regulating osteogenesis.
We performed tibia fracture with special scalpel of WT and PACAP KO mice. Fracturing was 5 mm distal from the proximal articular surface of the tibia and the depth was 0.5 mm. We investigated the mice with CT on the third day to confirm the fracture and on the 7. and 21. days after the operation to examine bone healing and callus formation. Expression of collagen type I increased in callus formation of WT mice, but lower expression was detected in callus of PACAP KO mice compared with the respective controls. As sign of enhanced bone formation increased protein expression of ALP was detected with Western blot in both genotypes. Elements of the BMP signaling pathway were also investigated and increased BMP2, BMP4 and 6 were detected in callus formation of WT mice, while decreased BMP expressions were shown on days 7 and 21 in PACAP KO mice. Moreover, elevated Smad1 expression was demonstrated in PACAP KO mice.
Our results indicate that PACAP KO mice show various signs of disturbed osteogenesis and bone healing. The clarification of whether the absence of PACAP itself or the activation of any compensatory mechanisms is the causative factor in this phenomenon requires further experiments.

Acknowledgments: Supported by grants from GINOP-2.3.2-15- 2016-00050 “PEPSYS”, the National Research, Development and Innovation Fund K119759, MTA-TKI, PTE AOK KA Research Grant, Bolyai Scholarship, National Brain Research Program (KTIA_13_NAP-A-III/5), TAMOP 4.2.4.A/2-11-1-2012-0001 „National Excellence Program”, EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

MULTIMORBIDITY MAP IN THE RESEARCH OF DEPRESSION PATHOPHYSIOLOGY

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Depressive illness is common and costly to the individual and society. Genetic makeup accounts for about 1/3rd of the risk of depression and environmental factors for about 2/3rds. Despite the heterogeneous etiology the symptoms of depression in general are very similar. As a consequence, it is extremely difficult to identify biologically distinct causal pathways. Instead, accumulating evidence suggests that overlapping or frequently comorbid disorders uniquely contribute to the pathomechanisms of depression. It has been demonstrated that many health conditions, such as other psychiatric disorders, syndromes accompanied with pain, or metabolic disorders are associated with an increased risk of subsequent depression and are more common in those with previous depression. This suggests that these disorders and depression may have some similar biological pathways of risk. For example, there is a bidirectional relationship between anxiety and depression, migraine and depression and accumulating evidence suggest comorbidity with depression in case of obesity, diabetes and cardiovascular disorders. However, it is not well understood how vulnerability to depression is increased in these disorders. Network studies investigated different diseases and their symptoms suggests that overlapping symptoms between diagnostic categories determine the number of shared genetic risk factors and the extent to which their associated proteins interact. Thus, these common biological pathways in the face of environmental adversities, such as stress, sedentary lifestyle or unhealthy diet, may contribute to the development of comorbid disorders. Using the large cohort of the UK Biobank study we investigated which disorders are directly comorbid with depression and thus likely to share common biological mechanism. In addition, we explored the network of disorders that do not show direct comorbidity but are relevant for depression through other mediating medical conditions. Combining these disease networks with environmental factors, genetic and other biomarker data is a powerful tool to further our understanding of the pathophysiology of depression.

This research has been conducted using the UK Biobank Resource (Application Number: 1602). This work has been supported by the National Development Agency (KTIA_NAP_13-1-2013-0001), Hungarian Brain Research Program - Grant No. KTIA_13_NAP-A-II/14, by the Hungarian Academy of Sciences and the Hungarian Brain Research Program - Grant No. KTIA_NAP_13-2-2015-0001 (MTA-SE-NAP B Genetic Brain Imaging Migraine Research Group), by the MTA-SE Neuropsychopharmacology and Neurochemistry Research Group, Hungarian Academy of Sciences, Semmelweis University, and by the Manchester Academic Health Science Centre, University of Manchester.

DIFFERENCES IN INVOLUNTARY ATTENTION BY AGE AND GENDER: AN ERP STUDY

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Involuntary attention allows the active processing of stimuli that could be potentially relevant when they are out of the voluntary attention focus. The Event related potential (ERP) technique has described three stages of auditory involuntary attention represented by three corresponding waves: Mismatch Negativity (MMN), P3a and Reorientation Negativity (RON).
Involuntary attention is mainly associated with frontal cortical areas that show structural and functional changes along normal aging. These same areas participate in other cognitive functions that decline in efficiency in both male and female older adults. Nevertheless, there is a lack of information about a possible decline in involuntary attention along adulthood for each gender.
The purpose of this study was to explore whether there are differences in involuntary attention along adulthood in both men and women assessed by behavioral outcomes and ERP (MMN, P3a and RON).
The sample included 80 healthy adults from 20 to 60 years old divided in 4 groups per decade. MMN, P3a and RON were obtained after subtracting the ERP of deviant stimuli from the ERP of frequent stimuli.
Reaction times and percentage of correct responses were similar in all participants regardless of age ($p>0.05$). MMN, P3a, and RON didn´t show differences either for latency or amplitude among the age groups. Nevertheless, while younger participants (20-40) showed fronto-central distribution of P3a, older participants showed a less specific topographic distribution ($F =3.83$, $p<0.01$). This results suggests a functional reorganization of the brain along adulthood in terms for novelty detection that might reflect a transition from more modular activation during youth to wider neural recruitment during older age.
The gender showed an effect in topographical distribution of P3a. While men showed a stronger frontal contribution for P3a, woman had more centroparietal contribution for this ERP. No significant interaction between gender and age was found. Sex-related differences in the topographical distribution of P3a, possibly indicate a less automatic orientation of attention in men compared to women. These differences were independent of age and did not reach significance at behavioral level. Cortical distribution of novelty-related activity adds to the dynamic changes of age along normal aging and between men and women.

Keywords: MMN, P3a, RON, involuntary attention, distraction, normal aging, topographical distribution, sex differences, compensation effect.

EFFECTS OF POSTNATAL ENRICHED ENVIRONMENT IN A MODEL OF PARKINSON'S DISEASE IN ADULT RATS

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Environmental enrichment is a widespread neuroprotective strategy during development and also in the mature nervous system. Several research groups have described that enriched environment in adult rats has an impact on the progression of Parkinson's disease (PD). The aim of our present study was to examine the effects of early, postnatal environmental enrichment after 6-OHDA-induced lesion of the substantia nigra in adulthood.
Newborn Wistar rats (n=29) were divided into control (n=16) and enriched (n=13) groups according to their environmental conditions. For environmental enrichment, during the first five postnatal weeks animals were placed in larger cages and exposed to intensive complex stimuli such as toys, objects, running tunnels and rotating rods of different shape, size and material. In 3-month-old rats dopaminergic cell loss, postoperative hypokinetic and asymmetrical motor signs were evaluated after inducing PD with unilateral injections of 6-OHDA (2 µl 6-OHDA,5 µg/µl) into the left substantia nigra. Treatment with 6-OHDA led to a significant cell loss in the substantia nigra of control animals, however, postnatal enriched circumstances could rescue the dopaminergic cells. Although there was no significant difference in the percentage of surviving cells between 6-OHDA-treated control and enriched groups, the slightly less dopaminergic cell loss in the enriched group resulted in less severe hypokinesia. In case of the number of free rearings we found reduced rearing activity after 6-OHDA injections in both groups. However, enriched animals showed a recovery on the tenth postoperative day after the acute decrease on the first day. Regarding the distance taken, enriched animals performed better, as there was no significant impairment in their movement.
In summary our present results are the first to provide evidence for the neuroprotective effect of early, postnatal enriched environment in Parkinson's disease in adult rats.

This work was supported by: National Scientific Research Fund (OTKA K104984); the National Research, Development and Innovation Fund K119759; GINOP-2.3.2-15-2016-00050; PTE AOK KA Research Grant; National Brain Research Program (KTIA_13_NAP-A-III/5), TAMOP 4.2.4.A/2-11-1-2012-0001 National Excellence Program; UNKP-16-4-IV, New National Excellence Program of the Ministry of Human Capacities, MTA TKI 14016 Program, Bolyai Scolarship, EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

PRESYNAPTIC MODULATION OF GLYCINERGIC TRANSMISSION IN THE FROG SPINAL MOTONEURONS BY 5-HT1B RECEPTORS

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An endogenous monoamine serotonin (5-HT) phylogenetically and ontogenetically is one of the most ancient neurotransmitters in vertebrates [Nakamura, 2014]. Presynaptically, 5-HT receptors modulate the release of the major neurotransmitters, such as glutamate, GABA, acetylcholine, dopamine, and noradrenaline (Fink, Gőthert, 2007). Several 5-HT receptors are expressed in the membrane of spinal motoneurons including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, 5-$HT_{2C,}$ and 5-HT_{5A}. The presynaptic effect of serotonin in frog spinal motoneurons is practically unexplored. Our previous study (Kalinina et al., 2016) showed that 5-HT decreases the frequency of glycinergic mIPSPs. In the present study, using intracellular recordings from frog spinal motoneurons, the effects of specific 5-$HT_{1,2}$ agonists and antagonist on inhibitory transmission were investigated to identify the receptor subtypes responsible for inhibition of release of glycine. The results showed that 5-HT modulated the glycinergic transmission in motoneurons by the activation of 5-HT_{1B} receptors. An agonist for 5-HT_{1A}, 5-$HT_{1B/D}$ receptors sumatriptan (10 μM) in the presence of TTX (1 μM) decreased the frequency (but not peak amplitude) of glycinergic mIPSP by 55.2±7.6 % from 3.9 ± 1.1 events/s to 1.8 ± 0.7 events/s, n=7, $p < 0.05$ and antagonist 5-$HT_{1, 2}$ receptors methysergide (10 μM) eliminated effect of sumatriptan. ?n agonist for 5-HT_{1A} and 5-HT_{7}receptors, 8-OH-DPAT as well as an agonist for 5-HT_2 receptor α-Me-5-HT (10 μM) did not show any significant effect on the inhibitory transmission, indicating the 5-HT_{1A}, 5-$HT_{7,}$and 5-HT_2 receptors do not regulate the glycine releases in the adult frog spinal motoneurons. All tested drugs had no effect on the frequency and amplitude of GABAergic mIPSPs. Kinetic parameters of glycinergic mIPSPs did not change. The obtained results suggest that 5-HT_{1B} receptors involved in the mechanism of presynaptic modulation of spontaneous vesicular release of glycine and reveal the role of serotonin in controlling the motor output of the frog spinal cord.

Supported by the Program of the Presidium of the Russian Academy of Sciences no. 19 and the RFBR grant no. 15-04-05782.

ELECTROPHYSIOLOGICAL ANALYSIS OF SYNCHRONIZATION APPLYING GABAA RECEPTOR ANTAGONIST BICUCULLINE IN HUMAN NEOCORTEX IN VITRO

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Background: The gamma-aminobutyric acid A ($GABA_A$) receptor antagonist bicuculline is largely used to induce epileptiform (interictal-like) activity in animal cortical tissue.
Aims. The aim of the studies reported here was to investigate the effect of the blockade of GABAergic inhibition in the human neocortex.
Method: Tissue slices were prepared from postoperative neocortical tissue of epileptic patients and tumor patients without epilepsy. Local field potential gradient (LFPg) recordings were obtained using a 24 channel laminar microelectrode during physiological conditions and during bicuculline baths. Current source density (CSD) and multiple unit activity (MUA) were calculated, as well as population bursts and the activity of single cells were detected. The clustered cells were classified as excitatory principal cells and inhibitory interneurons.
Results: Analysis showed that the blockade of GABAergic inhibition results in interictal-like activity in vitro in human neocortical tissue resected from both patients with epilepsy and patients with tumor but without epilepsy. Bicuculline induced activities are similar in epileptic and non-epileptic tissue, but the chance of occurrence and the recurrence frequency were higher in epileptic slices, while the amplitudes of MUA and LFPg were higher in tumor slices. Analysis showed that the application of bicuculline caused an increase in the activity of inhibitory neurons, while it decreased the activity of principal cells in the epileptic neocortical tissue. This effect seems to be less conspicuous in tumor tissue.
Conclusions: Our results show that the network properties of synchronous activity induced by bicuculline showed only slight differences in epileptic and non-epileptic patients. Both excitatory and inhibitory neuronal populations were found to participate in the generation of interictal-like activity induced by the blockade of GABAergic inhibition in the human neocortex.

A ROLE OF CB1R IN INDUCING θ-RHYTHM COORDINATION BETWEEN THE GUSTATORY AND GASTROINTESTINAL INSULA

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Anandamide (AEA) and N-oleoylethanolamine (OEA) are produced in the intestine and brain during fasting and satiety, respectively. Subsequently, AEA facilitates food intake via activation of cannabinoid type-1 receptors (CB1Rs) while OEA decreases food intake via activation of peroxisome proliferatoractivated receptor-α (PPARα) and/or G-protein-coupled receptor 119 (GPR119). Neuronal activity in the gastrointestinal region of the autonomic insula (GI-Au-I) that rostrally adjoins the gustatory insula (Gu-I) increases during fasting, enhancing appetite while umami and sweet taste sensations in Gu-I enhances appetite in GI-Au-I, strongly suggesting the presence of a neural interaction between the Gu-I and GI-Au-I which changes depending on the concentrations of AEA and OEA. However, this possibility has never been investigated. In rat slice preparations, we demonstrate with voltage-sensitive dye imaging that activation of CB1Rs by AEA induces θ-rhythm oscillatory synchronization in the Gu-I which propagates into the GI-Au-I but stops at its caudal end, displaying an oscillatory coordination. The AEA-induced oscillation was abolished by a CB1R antagonist or OEA through activation of GPR119. Our results demonstrate that the neural coordination between the Gu-I and GI-Au-I is generated or suppressed by the opposing activities between CB1R and GPR119. This mechanism may be involved in the feeding behavior based on taste recognition.

DIFFERENCES OF THE CORTICAL AND HIPPOCAMPAL SLEEP IN THE RAT MODEL OF CHOLINERGIC DENERVATION

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The aim of this study was to follow the impact of bilateral pedunculopontine tegmental nucleus (PPT) lesion on the cortical and hippocampal sleep/wake states architectures, their sleep state-related EEG microstructures, and their high voltage sleep spindles (HVSs).
Adult male Wistar rats were chronically implanted for sleep recording. EEG signals were recorded using the bilateral screw electrodes for the motor cortex (A/P: +1.0; R/L: 2.0; D/V: 1.0), and the teflon coated wire electrodes for the hippocampal CA1 region (A/P: - 3.6; R/L: 2.5; D/V: 2.5), and for the assessment of the EMG from the dorsal nuchal muscles. During the operative procedure for implantation of electrodes the bilateral PPT lesions were performed by the stereotaxically guided microinfusion of 100 nl 0.1 M ibotenic acid into the PPT of each brain side, and later verified by NADPH-diaphorase histochemistry. We recorded sleep 14, 42 and 91 days after operative procedure. After conventional amplification and filtering, the analog data were digitized (256/s) and recorded for 6 h, using DataWave SciWorks Experimenter Version 8.0. We applied Fourier analysis to signals acquired throughout 6 h, and each 10 s epoch was differentiated, on the base of EEG and EMG, as the Wake, NREM or REM state. We particularly extracted the simultaneous cortical and hippocampal Wake/NREM/REM epochs, and for each state-related EEG microstructure we used the Probability Density Estimate (PDE) routine supplied with MATLAB 6.5. In addition, we analyzed the high voltage sleep spindles (HVSs), simultaneously recorded, filtered (4.1-10 Hz band pass), and visually extracted from the motor cortex and hippocampus during REM sleep. All statistical analyses were done using Kruskal Wallis ANOVA with post hoc Mann Whitney U two tailed test.
The bilateral PPT lesion did not change the sleep/wake states architecture of the motor cortex and hippocampus during the overall follow-up period ($z \geq -2.01$, $p \geq 0.14$), but there was the severe and long-term EEG microstructure disorder during NREM sleep, expressed earlier and only within the hippocampus from 14 - 42 days, and simultaneously with the motor cortex 91 days after the PPT lesion. Although the onset of NREM sleep disorder was earlier and longer within the hippocampus, it was mainly expressed as the augmented delta amplitude and attenuated beta amplitude both at the cortical ($z \geq -2.61$, $p \leq 0.04$), and hippocampal level ($z \geq -3.55$, $p \leq 0.003$). In addition, whereas the PPT lesion permanently increased the HVS density/6 h ($z \geq -2.59$, $p \leq 0.05$), it decreased their intrinsic frequency in both structures ($z \geq -4.22$, $p \leq 0.05$) during REM sleep. Moreover, the PPT lesion permanently prolonged the HVSs within the motor cortex while it shortened them within the hippocampus ($z \geq -2.89$, $p \leq 0.04$) during REM sleep.
Sleep EEG microstructure and HVSs are different at the cortical and hippocampal level during the cholinergic denervation from the PPT.

GENERAL BEHAVIOR OF THE MAM-E17 TREATED RATS THROUGHOUT DIFFERENT AGE-PERIODS

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MAM-E17 model is a widely accepted schizophrenia rat model based on the neurodevelopmental theory of the disease. It represents numerous symptoms, which appear in a diachronic pattern parallel to schizophrenia. The purpose of this study was to monitor the behavioral characteristics of MAM-E17 rats throughout three different age-periods i.e. in prepuberty, puberty and adulthood.
For monitoring the overall locomotor activity, open field test (OPF) was used. During this test behavioral patterns of the rats (rearing, sniffing, and grooming) were also registered. Anxiety state was investigated in elevated plus maze test (EPM), moreover in OPF. To investigate morphological alterations of the brain histological analysis was carried out.
In OPF MAM-E17 rats displayed increased locomotor activity, elevated sniffing frequency and, as tendency, enhanced rearing activity. The elevated activity turned up in puberty and remained there in adulthood, too. In grooming behavior there was no difference to controls. In EPM MAM-treated rats spent more time in the open arms and visited it more times then control rats in prepuberty and in adulthood. In OPF MAM-E17 rats spent more time in the central zone and less time in the peripheral zone in prepuberty. Histological analysis revealed decreased brain lenght, reduced volume of the dorsal hippocampus and cell disarray in the CA1-CA3 region. In case of the prefrontal cortex (PFC) some of the MAM-E17 brains displayed diminished thickness, however in total, there was no significant alteration in comparison to the control group.
Locomotor activity of MAM-E17 rats was enhanced in puberty and adulthood, but not before puberty. Hyperlocomotion corresponds to positive symptoms of the disease, which similarly first appear in puberty. The significant increase of sniffing and tendenciously enhanced rearing activity, with the similar pattern, can also represent hyperactivity. This elevated activity can reflect the enhanced responsiveness to the environmental stimuli. In EPM and OPF task diminished anxiety of MAM-E17 rats was to observe with the same temporal pattern: anxiolytic effect was present in prepuberty, disappeared in puberty and returned in some extent in adulthood. This phenomenon is likely due to the pubertal maturational processes. There are contradictory results about anxiety state of MAM-E17 rats in the literature. Nevertheless our results are strongly supported by the fact that in two different paradigms consistently the same behavior was to observe with the same temporal pattern.
Our experiments provided new data representing the first comprehensive study about general characteristics of MAM-E17 rats carried out in prepuberty, puberty and adulthood on the same rats. The present findings confer basic information to accomplish the schizophrenia related animal research.

The project has been supported by the European Union, co-financed by the European Social Fund and EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

TRANSIENT O-GLCNAC ELEVATION AND TAU DEPHOSPHORYLATION INDUCED BY OXIDATIVE STRESS IN SH-SY5Y CELLS

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O-linked β-N-acetlyglucosamine or O-GlcNAc modification is a dynamic, reversible post-translational modification occurring on the Ser/Thr moieties of cytosolic and nuclear proteins. This modification impacts more than 1000 cytoplasmic, nuclear and mitochondrial proteins. Recent studies suggest that O-GlcNAc is also abundant in neuronal tissue and that chronic imbalance between O-GlcNAc and phosphorylation on tau protein is a main contributor to Alzheimer´s disease. Interestingly, previous studies also showed that upon stress, O-GlcNAc elevation occurs which seems to have a protective role in cell survival.
The aim of this study was to investigate the dynamic change of O-GlcNAc in the neuronal cell line SH-SY5Y after treatment with 0.5 mM H2O2 for 30 min. We found that intracellular O-GlcNAc rapidly increased, reached the peak level at around 2 hours post-stress and then returned to the basal level. We have also found that tau protein phosphorylation at site S262 showed parallel, whereas at S199 and PHF1 sites showed inverse dynamic to O-GlcNAc.
In conclusion, our results demonstrated that transient oxidative stress causes a rapid, temporary increase in O-GlcNAc in SH-SY5Y neuroblastoma cells. Furthermore, this short-term oxidative stress changes the dynamic balance between O-GlcNAc and phosphorylation on tau proteins. These findings suggest that the pathophysiology of neurodegenerative diseases might be connected to cellular stress adaptation mechanisms.

INVESTIGATION OF HEMOKININ-1 GENE EXPRESSION PROFILE IN THE MOUSE BRAIN

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Hemokinin-1 (HK-1) encoded by *TAC4/Tac4* gene is the newest member of the tachykinin family. It was originally discovered in B lymphocytes, but later widespread expression was revealed in several human and mouse tissues. Due to its highly abundant distribution, it has been involved in different pathophysiological conditions, including hematopoietic and smooth muscle malignances, lung and joint inflammation, pruritic and pain processing. Compared to the prominent peripheral expression of HK-1, significantly weaker level was detected both in the human and mouse central nervous systems (CNS) using real-time quantitative PCR (RT-qPCR).
Since limited expression data is available about hemokinin-1 in adult brain, our goal was to elucidate its neural localization and gene expression pattern. Due to lack of specific commercially available HK-1 antibody, we focused on its detection at the mRNA level using (i) RT-qPCR in distinct mouse brain regions obtained by micro-punching and (ii) tyramide signal amplification (TSA) -based *in situ* hybridization (ISH) method on vibratome-sectioned brain slices. The TSA method is a widely used technique to increase signal sensitivity even 200 times compared to standard ISH colorimetric detection methods. In order to investigate the localization of *Tac4* mRNA in C57BL/6 mice, we generated a *Tac4* mRNA specific antisense probe. Samples collected from *Tac4-/-* mice and sense probe against *Tac4* mRNA were used as negative controls, respectively.
We detected low expression levels of *Tac4* mRNA using specific primers in the RT-qPCR in various mouse brain regions including the basal forebrain, hippocampus, medial preoptic nucleus, locus ceurelus and periaqueductal gray matter, bed nucleus of the stria terminalis and amygdala. No expression was found in samples collected from *Tac4-/-* mice confirming the specificity of these signal. Using *Tac4* antisense probe, we detected *Tac4* specific signals possibly in glia cells of the anterior commissure, optic tract, corpus callosum and fimbria of the hippocampus. There were no signals with the sense probe.
These results indicate that HK-1 has a low basal expression in the normal, healthy mouse brain. In order to clarify the *Tac4* specific cell types, co-localization studies will be performed using different glial and neuronal markers. Furthermore, to determine potential expression changes, brain samples of pathological conditions obtained from disease models (neuroinflammation, neurodegeneration, pain and depression) will be investigated.

Supported by KTIA_NAP_13-2014-0022 (888819), EFOP-3.6.1-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs
The project has been supported by the European Union, co-financed by the European Social Fund TAMOP-4.2.4.A/2-11/1-2012-0001 'Apaczai Scholarship' for J. K., GINOP-2.3.2-15-2016-00050 - PEPSYS, GINOP-2.3.2-15-2016-00048 - STAY ALIVE. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

CALCIUM-BINDING PROTEIN PROFILE OF THE CB1 CANNABINOID RECEPTOR - POSITIVE HIPPOCAMPAL INTERNEURONS

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Hippocampal GABAergic interneurons can be classified into numerous types based on their different molecular, morphological and electrophysiological properties. It has been known for a long time that several EF-hand calcium-binding proteins such as parvalbumin, calbindin and calretinin display cell-type specific expression patterns. Accordingly, immunostaining for these calcium-binding proteins or exploiting their gene promoters in transgenic animals could be used as an efficient anatomical approach for the selective visualization of interneuron types or as a cell-type-specific manipulation tool, respectively. Whereas most hippocampal GABAergic cell types could be characterized by their respective calcium-binding protein profiles, surprisingly, to date no specific EF-hand calcium-binding proteins have been described in the CB_1 cannabinoid receptor-positive hippocampal interneurons despite the fact that these cells comprise of a significant population among hippocampal interneurons. In light of the widespread cell physiological importance of calcium buffering in neuronal function, we hypothesized the presence of yet undetected calcium-binding proteins in this interneuron population. Therefore, in the present study, we sought to identify the EF-hand calcium-binding proteins expressed by the CB_1-positive interneuron types, with the help of *in silico* analysis of a single-cell RNA-sequencing database and by using immunofluorescence stainings and confocal microscopy. Based on the bioinformatical analysis we first found that the mRNA of the N-terminal EF-hand calcium binding proteins 1 and 2 (NECAB1 and NECAB2) are expressed in high copy numbers in the CB_1-positive hippocampal interneurons. We next performed double immunofluorescence staining on transcardially perfused C57BL/6 mouse hippocampal slices with antibodies against NECAB1 and NECAB2 proteins, and the CB_1 receptor. Confocal microscopy analysis of the colocalization of these proteins at the population level within interneuron cell bodies located in the hippocampal CA1 radiatum subfield revealed that both NECAB1 and NECAB2 are present in all CB_1-positive interneurons (n=50-50 cell bodies/marker/animals, n= 3-3 animals). In accordance, the combination of cell-specific biocytin-labeling of individual morphologically characterized CB_1-positive interneurons with NECAB1 or NECAB2 immunostaining confirmed that these calcium-binding proteins are present in both the perisomatic (basket) and the dendritic (Schaffer-collateral-associated) subtypes of this interneuron family. Taken together, these findings demonstrate that NECAB1 and NECAB2, two previously uncharacterized calcium-binding proteins are present in the CB_1-positive interneurons in the hippocampal CA1 radiatum subfield. Moreover, the results also raise the possibility that the cell-type-specific expression of these calcium-binding proteins may contribute to the various distinct physiological properties of these hippocampal interneurons.

MEMBRANE DISTRIBUTION OF NMDA RECEPTORS IN HIPPOCAMPAL NEURONS: NANOSCALE MAPPING AND REGULATION

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NMDA-type glutamate receptors (NMDAR) are ion permeable channels mediating different forms of synaptic plasticity in the brain, a mechanism thought to be the molecular basis of neuronal development, learning and memory formation. The GluN2A and GluN2B subunits are highly expressed in the hippocampus and exhibit distinct electrophysiological, surface diffusion and expression properties. Here, we used direct stochastic optical reconstruction microscopy (dSTORM) to characterize the surface distribution of GluN2A or GluN2B-containing NMDAR. As a Single Molecule Localization Microscopy technique, dSTORM enables to overcome the resolution barrier due to the diffraction limit of light. We report the first map of surface endogenous NMDAR in rat cultured hippocampal neurons. The super-resolved distributions were compared between cellular compartments, i.e. dendritic shaft and spines, and glutamate postsynaptic densities. We unveil that GluN2A or GluN2B-containing NMDAR have distinct nanoscale organization in synapses. Moreover, the molecular pathways underlying these distributions are also different, involving PDZ scaffold proteins and CAMKII proteins. Together, our data provide the high resolution maps, as well as part of the regulatory pathway, of GluN2-NMDARs expressed in hippocampal neurons, shedding new light on the organization of a key glutamate receptor signaling.

THE EFFECT OF DEEP BRAIN STIMULATION ON REPETITIVE BEHAVIOUR IN RATS OVEREXPRESSING THE DOPAMINE TRANSPORTER

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Tourette syndrome (TS) is a neurological disorder associated with repetitive, involuntary movements and vocalizations referred to as tics. Recent evidence suggests that dysregulation of the dopaminergic system plays an important role in the etiology of repetitive disorders. An increase in presynaptic dopamine activity in the striatum and increased dopamine receptor availability have been found in TS patients suggesting an overactive dopamine transporter (DAT). This study sought to determine the effect of DBS on repetitive behavior in rats overexpressing the DAT receptor following amphetamine administration. Rats (WT = 7, DAT = 6) were injected with amphetamine (2mg/kg) after which they were placed in recording boxes for a 60-min habituation period. Five minutes prior to the start of DBS and stereotypy, a five minute video recording (baseline) was obtained. Subsequently, DBS (130Hz, 100μs pulse width, 150μA current for one minute with a nine minute resting period) was started during the stereotypy phase, and lasted for 30 minutes with a total of five repetitions. Thirteen videos were scored for the six different types of behaviors: no locomotion, locomotion, continuous rearing, continuous sniffing, oral stereotypy, and head movements. DBS caused a decrease in the probability of locomotion and an increase in the probability of no locomotion in both WT and DAT, indicating that rats were less likely to move following the initiation of DBS. The DAT genotype increased the probability of continuous rearing compared to WT with a slight decrease in the probability of locomotion. Over time, the probability of locomotion decreased while the probability of no locomotion increased.

EVOLUTIONARILY CONSERVED MECHANISMS OF ASSOCIATIVE LEARNING AND MEMORY IN THE POND SNAIL

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Associative learning is often the simplest and most efficient way to adapt to environmental changes in a wide range of species. Just like more complex forms of learning, it can also lead to long-term memory (LTM) formation after multi-trial conditioning. However, associative LTM can also be triggered by a single training trial allowing sharply timed sampling of the developing memory trace on multiple levels from behaviour to molecules. Since the mollusc *Lymnaea stagnalis* is capable of this type of learning, it provides a highly valuable experimental model for top-down analyses of adaptive changes leading to LTM. In *Lymnaea*, molecular mechanisms of LTM involve highly conserved signaling pathways (e.g., CaMKII, NMDA and GluA1 receptors) and RNA and protein synthesis. Cellular mechanisms of LTM involve synaptic and non-synaptic plasticity in key modulatory interneurons of the feeding network. More recently, an integrated behavioural, molecular and electrophysiological approach revealed that amyloid beta peptides known to underlie Alzheimer's disease in humans cause memory loss and impairment of single neuronal function in *Lymnaea*. *Lymnaea* can also be used as a novel invertebrate model to develop pharmacological tools to reverse age-related learning deficiency.
Our recent work has concentrated on the period of early consolidation following single-trial training. We identified a number of time points after conditioning when the memory trace becomes vulnerable. Our results suggest that these "lapses" during consolidation are not simply points of weaknesses but they can provide opportunities for adaptive changes that alter the fate of the developing memory trace. We show that intervention by a novel appetitive or aversive stimulus disrupts memory formation but only when applied at the sensitive time points. We also discovered that induction of a new associative memory at lapses, but not at other times, can result in the dominance of the second memory over the original memory trace. The price for this updating is the abolition of the original memory.

PERSISTENT CANNABINOID CONTROL OF GABA RELEASE DOES NOT REQUIRE DIACYLGLYCEROL LIPASE-ALPHA, THE SYNAPTIC ENDOCANNABINOID-SYNTHESIZING ENZYME

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Synaptic strength is modulated by several signaling mechanisms that adjust transmission properties, including neurotransmitter release probability. Retrograde endocannabinoid signaling via the presynaptic CB_1 cannabinoid receptors is a widespread and efficient mechanism that plays an essential role in controlling neurotransmitter release throughout the brain. Recent investigations have unfolded multiple molecular mechanisms of how cannabinoid signaling can control synaptic transmission in a phasic or tonic manner. Importantly, these distinct forms of cannabinoid signaling can be independently affected in brain disorders. For example, tonic cannabinoid signaling is impaired in a mouse model of autism, whereas the phasic forms of endocannabinoid signaling remained intact (Földy et al., 2013 Neuron, 78:498-509). This seminal finding raised the intriguing possibility that phasic and tonic forms of cannabinoid signaling may have different underlying molecular mechanisms. To mechanistically explore the contribution of diacylglycerol lipase-alpha (DGL-alpha), the predominant 2-AG synthesizing enzyme to phasic and tonic endocannabinoid signaling, we performed paired whole-cell patch-clamp recordings between presynaptic perisomatic interneurons and postsynaptic CA1 pyramidal cells in the mouse hippocampus. These physiological investigations have been complemented by the detailed nanoscale analysis of cell-type-specific CB_1receptor numbers using stochastic optical reconstruction microscopy (STORM) super-resolution imaging and by measuring hippocampal endocannabinoid levels employing liquid chromatography/tandem mass spectrometry (LC-MS/MS). The latter approach revealed that 2-AG levels in acute hippocampal slice preparations obtained from DGLα knockout mice are strongly reduced compared to the wild-type littermate controls. Despite the reduced basal tissue 2-AG levels, presynaptic CB_1 receptor numbers on interneuron terminals as well as the baseline amplitude of unitary inhibitory postsynaptic currents (uIPSCs) and success rates of unitary synaptic events remained identical between littermate wild-type and DGLα knockout mice. These findings are consistent with the possibility that tonic 2-AG signaling is still functional in the absence of DGLα. Indeed, we found that the CB_1 receptor inverse agonist AM251 could significantly increase GABAergic synaptic transmission between perisomatic interneurons and CA1 pyramidal cells in both wild-type and DGLα KO mice. In contrast, depolarization-induced suppression of inhibition (DSI), a canonical form of phasic endocannabinoid signaling was absent in DGLα knockout mice, whereas DSI was robust and could be blocked with the CB1 receptor antagonist/inverse agonist AM251 in wild-type animals. Taken together, these findings demonstrate that DGLα produces synaptic 2-AG for phasic endocannabinoid signaling, whereas tonic cannabinoid signaling operates via a DGLα-independent molecular mechanism.

GENETIC ASSOCIATION ANALYSIS OF SYNAPTOSOMAL PROTEIN CODING SNAP25 GENE POLYMORPHISMS WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER

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The synaptosomal-associated protein, 25 kDa (SNAP25) plays a crucial role in neurotransmission, being one of the essential components of the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE complex) which mediates the fusion of neurotransmitter vesicles to the presynaptic cell membrane. Animal studies of the coloboma mouse pointed to the involvement of the SNAP25 gene in hyperkinetic behavior, a core feature of Attention Deficit Hyperactivity Disorder (ADHD). Polymorphisms in the SNAP25 gene have been also indicated as putative genetic components of impulsivity in healthy adults.
There are two widely investigated single nucleotide polymorphisms (SNPs) only 3 base pairs apart in the regulatory 3' untranslated region (3' UTR) of the SNAP25 gene named after the restriction enzymes used in their genotyping methods, MnlI (rs3746544 G/T) and DdeI (rs1051312 C/T). These two SNPs have been jointly studied in haplotype constellations, both variants disrupting the binding site of miR-641 resulted in higher expression in an in vitro reporter gene system compared to the T-T haplotype. Recent meta-analyses concluded that both SNPs may increase the odds of developing ADHD, although the effect of the MnlI was more pronounced in Asian populations. Therefore, we aimed to characterize the combined effect of the haplotype variants of these SNPs in childhood onset ADHD.
Our child psychiatry patient group consisted of 417 children, of which 195 had DSM-IV ADHD as the main diagnosis but additional 60 had ADHD in addition to Tourette syndrome (total of 255 children had ADHD symptoms). An independent cohort of adult ADHD patients (n=95) was recruited to test the association in a persisting ADHD subgroup. The two 3' UTR SNPs in the SNAP25 gene (rs3746544 and rs1051312) were analyzed with a direct haplotyping method based on real-time PCR amplification. The genotype and haplotype frequencies were compared to a control group from the general Hungarian population (n=782).
The SNAP25 3'UTR haplotype G-T was more frequent in patients with ADHD as compared to those patients without ADHD (p=0.016), or to healthy controls (p=0.037). Analyzing the two SNPs separately, there was significant association only with MnlI (rs3746544) polymorphism. There were more G-allele carrier (G/G or G/T genotype) children in the ADHD group (64.3% vs. 52.5% in children without ADHD, 56% in controls). Our findings support the recent meta-analysis that showed more prominent effect of the MnlI polymorphism in ADHD.

This study was supported by the Hungarian Scientific Research Fund (OTKA) F67784 and the National Brain Research Program (NAP) KTIA_NAP_13-2014-0011.

A MICROSURGICAL METHOD TO MODULATE THE SPONTANEOUS POPULATION ACTIVITY AND INTERICTAL-LIKE ACTIVITY IN RAT BRAIN HIPPOCAMPUS SLICES

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Epilepsy is one of the most common neurological disorder, approximately 1 % of the human population is affected. In epilepsy the normal brain functions are disrupted by periodic occurrence of seizures and interictal activity. Despite the importance of interictal spikes in epilepsy diagnosis, little is known about the size of the neuronal population able to generate epileptic discharges.

We aimed to determine the minimal neuronal network required to initiate population bursts, therefore we investigated the network properties of spontaneous sharp waves (SPW), and interictal spikes induced by low Mg^{2+} while diminishing the size of the neuronal population by microsurgery.

Local field potential was recorded with a 24 channel laminar microelectrode array in the hippocampal CA3 region and the entorhinal cortex, and high intensity laser pulses were applied to modulate SPW and interictal-like activity.

In most cases the microsurgical procedure led to a marked decrease in the LFP amplitude and recurrence frequency of the SPW and interictal-like activity. Although the procedure did not reduce the LFP amplitude to baseline in all slices, it eliminated the multiunit activity in the case of interictal-like events. NeuN immunostaining indicated a widespread cell loss presumably caused by the laser cuts, though it failed to visualize them.

We were able to modify the spontaneous SPW activity and interictal-like activity using a laser microsurgery procedure. With this model system, we can estimate the effect of laser microsurgery on physiological and pathological neuronal activity and might aid the development of new neurosurgical techniques in the future.

THE EFFECTS OF SUBSTANCE P ON LEARNING IN MORRIS WATER MAZE TEST IN THE RAT AMYGDALA AND GLOBUS PALLIDUS

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Substance P (SP) belonging to the tachykinin peptide family has long been implicated in learning, memory and reinforcement processes. SP has a preferential affinity for neurokinin-1 (NK1) receptors but it can also act on the other two tachykinin receptive sites, namely NK2 and NK3 receptors. SP immunoreactive elements and NK receptors have been found in limbic structures, i.e. the amygdala (AMY), or in different parts of the basal ganglia, including the globus pallidus (GP). The amygdala plays an important role in learning and memory processes. Serious deficits were observed following lesions of distinct amygdaloid nuclei and its role in conditioned fear has been suggested. GP is also involved in the regulation of memory processes, learning deficits develop in different learning paradigms following electrolytic or excitotoxic lesions of the GP. In our previous experiments SP improved learning in passive avoidance paradigm both in the CeA or GP. The effects of SP on learning and memory in a non-punishing situation, however, haven't been investigated yet.
The aim of our study was to examine the effects of SP injected into the CeA or GP on learning in Morris water maze paradigm. We examined the possible involvement of NK1 receptors in the effects of SP, as well. Male Wistar rats were microinjected with 0.4 µl of 10 ng SP, 100 ng SP or vehicle solution into the CeA or GP. Rats receiving immediate post-trial injection of 10 ng, but not 100 ng SP into the CeA or GP exhibited improved performance over control rats during acquisition trial. To examine the possible role of NK1 receptors in mediation of SP effects, the high affinity non-peptide NK1 receptor antagonist WIN51,708 was applied in 0.4 µl, in an equimolar dose to SP treatments. Prior treatment with the NK1 receptor antagonist blocked the effects of SP on learning in Morris water maze test when injected into CeA, while in the GP could not inhibit promnestic effect of SP.
Our results show that 1) SP facilitates learning in Morris water maze task in the CeA and GP, 2) this effect is dose-dependent and 3) is mediated via NK1 receptors in the CeA, while in the GP NK1 receptors are not involved in this process. The possible role of NK2 or NK3 receptors has to be elucidated.

The project has been supported by the European Union, co-financed by the European Social Fund (EFOP-3.6.1.-16-2016-00004), EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

CORTICOSPINAL TRACT REGENERATION IN CB57/BL6 AND YFP-H MICE FOLLOWING STEM CELL TRANSPLANTATION

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Spinal cord injury is a devastating condition that seriously diminishes patients' quality of life. Functional improvement primarily depends on the regeneration of injured axons. The corticospinal tract (CST) is a major descending pathway, so it has been a prominent target for testing the regrowth of axons after injury. In this study we investigated whether transplantation of neuroectodermal stem cells (NE-4C cell line, ATCC^{TM}2925) was able to induce axonal regeneration after spinal cord injury, and compared the difference in the regenerative capacity between CB57/BL6 (wild type) and YFP-H mice.
A C7 hemisection injury of the spinal cord was performed in wild type and YFP-H mice, then NE-4C stem cells expressing "Tomato Red" were grafted. Control animals underwent the same injury without stem cell transplantation. Biotinylated dextran amine (10 kD) was injected into the motor cortex in both strains to label all the CST axons and compare the transport capacity of axons. After five weeks of survival we determined the extent of axonal regeneration in control and transplanted mice. Detailed immunohistochemical analyses were performed to investigate the fate of the transplanted cells and the environment of the site of injury.
In YFP-H mice we found high numbers of axonal retraction bulbs rostrally to the injury without any regenerating axons. In wild type animals the stem cells supported axonal growth and modified the environment at the site of the injury. Both in YFP-H and CB57/BL6 mice the density of inhibitory ECM molecules was significantly lower as compared with controls. Control animals did not show any signs of axonal regeneration and a high density of inhibitory molecules were found close to the site of the injury. Our labeling studies showed limited axonal transport capacity in YFP-expressing CST fibres. This data suggest that NE-4C stem cells can induce axonal regeneration in wild type but not in YFP-H mice. This failure to regenerate may lie in the limited regenerative capacity of YFP-expressing axons.

SEX-DEPENDENT SOCIAL BEHAVIOUR OF WISKET RATS

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Asociability is a pronounced behavioral feature in schizophrenia. It has been shown that during early childhood pre-schizophrenic children show significantly reduced play behavior. Selectively bred rats after peri-adolescence isolation rearing and subchronic ketamine treatment (WISKET rats) exhibit phenotypes related to schizophrenia, including reduced prepulse-inhibition of startle reflex, decreased acute heat pain sensitivity, cognitive disturbances, disturbed locomotor activity and thermoregulation, and electrophysiological alterations. To further validate our WISKET rat line regarding its translational utility in schizophrenia research, we examined their social behavior. Investigating sex- and age-dependent alterations in schizophrenia may yield important insights into its etiology, thus male and female rats were also investigated in different ages. GABA-ergic system is affected in schizophrenia, that may account for altered social behavior. Considering the contribution of the hippocampal and cortical GABAergic inhibitory circuitry in the altered social behavior, molecular-biological studies were also performed.
Four groups of animals (n=8-22 rats/group) were involved in the experiments: both sexes of naive socially rearing Wistar rats; and the 21st generation of selectively bred WISKET rats with complex treatment (isolation rearing with ketamine treatment). The social interaction test was repeated at the neurodevelopmental stages of postweaning (at the age of 3 weeks) and young adult (at the age of 11 weeks). Weight-matched, unfamiliar pairs of rats with identical treatment were simultaneously placed in opposite corners of the unfamiliar testing chamber. The animals' behavior was recorded for 10 min by using an overhead infrared video camera for off-line analysis. Parameters evaluated for social investigation include: the time spent with sniffing each other defined as social interest; the number of initiating attack, fights, pushing past and crawling over each other with physical contact defined as aggressive behavior; running away defined as escape behavior (avoidance). For non-social exploratory behavior the rearing and self-grooming activities were assessed.
At the younger age we could not observe any differences between the groups. At 11 weeks of age impaired social activity with increased aggressive behavior and avoidance were observed, especially in male WISKET rats compared to naive Wistar ones. Regarding the non-social activities, decreased rearing and increased grooming activities were detected in both sexes of WISKET rats. These behavioral alterations were accompanied with decreased GAD mRNA and protein expression in the prefrontal cortex, however these alterations could not be observed in hippocampal samples.
These results further increase the validity of our WISKET model in schizophrenia research regarding the negative symtoms and molecular-biological findings as well.

ROLE OF DIMINISHED PLASMA TRYPTOPHAN LEVELS IN MEMORY DEFICITS IN TYPE I DIABETES

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Objective: Diabetes mellitus is the most common metabolic health problem, characterized by a hyperglycemia that results from an absolute or relative deficiency of insulin. Animal and human studies have shown decreased plasma TRP and brain serotonin (5-HT) levels in diabetes. Serotonin is known to play an important role in pathophysiology of various neuropsychiatric disorders and other related problems such as cognitive impairment. The present study was performed in subjects with type I diabetes mellitus and healthy controls. The aim of the study was to investigate the relationship between plasma tryptophan and occurrence of memory dysfunctions in male and female diabetics.
Methods: In present study 100 diabetic subjects were selected in which 50 were males and 50 were females. Likewise the controls were also in the same number. A questionnaire was used to evaluate the memory impairment in subjects. Plasma tryptophan was determined by HPLC-EC method.
Results: The present study shows that in diabetes there is considerable memory impairment in the human subjects. Results showed a significant ($p<0.01$) decrease in plasma tryptophan levels in both male and female diabetic patients. Memory impairment was also evident in both diabetic male and female subjects.
Conclusions: Diabetic subjects exhibited occurrence of memory impairment with concomitant decline in plasma tryptophan levels. Present findings indicate that lowered brain 5-HT levels may be responsible for the memory deficits seen in diabetics.

Keywords: Tryptophan, 5-HT, Diabetes, Memory Impairment.

INFLUENCE OF ZBTB33 GENE KNOCKOUT AND BACTERIAL LIPOPOLYSACCHARIDE ON DAILY ACTIVITY DYNAMICS IN MICE

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Kaiso protein (coded by Zbtb33 gene) recognizes and binds methylated cytosine residue in a DNA molecule and causes epigenetic repression of genes that have methylated cytosines in promoters. It has been shown earlier that Zbtb33 gene knockout mice are characterized by increased motion activity in the open field test as well as decrease in expression of depressive-like behavior in the forced swim test. However the role of Kaiso protein in everyday mice life remained unclear. In the present paper we have studied the influence of Zbtb33 gene knockout on daily activity and sensitivity to bacterial lipopolysaccharide (LPS) in mice. The experiments have been conducted on sexually mature male mice of Zbtb33 (KO) gene type knockout and wild type C57BL/6 (WT). All animals were aligned in their weight and had SPF (specific pathogen free) status during the entire experiment. Daily dynamics of motion activity, sleep, food, and water consumption have been monitored with the help of the hardware and software complex (PhenoMaster, TSE, Germany). LPS has been dissolved in sterile physiological solution and administered intraperitoneally at a dose of 0.1 and 1.0 mg/kg. Sterile physiological solution has been administered to control animals. Intact KO and WT mice did not differ in average daily motion activity and sleep duration. However KO mice were less active and spent more time sleeping during night time when mice are more active. Moreover, intact KO mice consumed less food and water compared to WT ones. LPS dose-dependently suppressed motion activity, increased sleep duration and caused anorexia in mice of both genotypes. Nevertheless KO mice were more sensitive to a low dose (0.1 mg/kg) of LPS compared to wild type animals. The obtained results bring to light the biological significance of Kaiso gene and provide verification for the necessity of normal functioning of this gene in natural populations.

The study is supported by Russian Science Foundation (17-15-1032).

CIRCADIAN RHYTHM OF MOTOR ACTIVITY IN DISC1 MUTANT MICE

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It is well known that circadian rhythm is disrupted in patients with psychiatric disorders and that the impaired circadian rhythm is implicated into pathophysiological mechanisms of psychiatric disorders. Hence, we assume that genetic animal models of psychiatric disorders will demonstrate altered circadian activity in comparison with wild-type mice. So, the aim of our current study was to describe circadian rhythm of motor activity in DISC1 mutant female mice, which model schizophrenia-related phenotypes (DISC1-L100P) and depression-like behavior (DISC1-Q31L) in comparison with wild-type (WT) [C57BL/6NCrl] mice. The experiment was performed using IntelliCage system, which recorded the total number of visits for each animal. Mice were kept on a 12:12 light:dark cycle with lights on at 18:00. The obtained results were processed by using cosinor analysis, the classical technique for rhythm detection and parameter estimation in chronobiology. Analysis of circadian rhythm of motor activity revealed that WT mice have acrophase (time of maximal activity) at 9:55±0:15 h, MESOR (Midline Estimated Statistic Of Rhythm) equals 16.7±2.00 visits, and amplitude (peak amplitude) is 10.0±1.46 visits. Circadian rhythm of motor activity parameters in DISC1-L100P mice were as follows: acrophase - 8:40±0:09 h (significantly earlier than in WT); MESOR – 15.6±0.96 visits (not differs from WT); and amplitude - 12.5±1.10 visits (not differs from WT). DISC1-Q31L mice shows acrophase at 8:58±0:16 h (significantly earlier than in WT); MESOR 12.1±0.92 visits (not differs from WT and DISC1L100P); and amplitude 6.6±1.06 visits (significantly lower than both WT and DISC1L100P). Notably, that DISC1-Q31L mice demonstrated the decreased motor activity (low amplitude) than WT animals perhaps, in parallel with depression-related behavior in DISC1-Q31L genetic line. DISC1-L100P mice showed the increased amplitude and later acrophase in comparison with DISC1-Q31L strain, suggesting higher motor activity and different activity pattern. Overall, analysis of circadian rhythms of motor activity revealed that DISC1 mutant mice demonstrate circadian pattern of motor activity which differs from WT animals, also DISC1-Q31L and DISC1-L100P mutants differ between each other by motor activity.

The study is partially supported by Presidium of the Russian Academy of Sciences (program IV.12.1, 2015–17).

ASSOCIATIONS BETWEEN SERUM ZINC LEVELS AND MENTAL HEALTH: FINDINGS FROM THE 2010 KOREAN NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY

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Background: Mental health problems are a major public health issue worldwide, and zinc may be associated with psychiatric symptoms, but such associations have not been investigated extensively. This study was conducted to evaluate the relationship between serum zinc levels and mental health problems in Korean adults.
Methods: We used data from the Korean National Health and Nutrition Examination Survey V-1, a cross-sectional survey of Korean civilians. Data from 1,748 subjects were analyzed.
Results: Serum zinc levels did not differ significantly according to psychiatric symptoms, including sleep duration, stress, depressed mood, suicidal ideation, and whether respondents sought psychiatric consultation. The frequencies and odds ratios of psychiatric symptoms according to serum zinc tertiles were not significantly associated after adjusting for age, smoking, alcohol consumption, physical activity, body mass index, total body fat, and renal function and for daily fat, carbohydrate, and protein intake.
Conclusion: Serum zinc levels may not be associated with psychiatric symptoms in Korean adults without psychiatric disorders.

DIVERSE PHYSIOLOGICAL FUNCTIONS OF NEUROPEPTIDES IN SNAIL BRAIN

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The information processing between cells is mediated primarily by means of chemical signalization including small molecular weight peptides. Peptides exert their effects as hormones released into the circulation or as modulators and neurotransmitters released synaptically or non-synaptically into the synaptic cleft or nearby the target receptors. Neuropeptides often co-exist with other peptides or low molecular weight neurotransmitters and may co-release with them. Working as neurotransmitters, neuromodulators, or neurohormones, neuropeptides are involved in the control of a variety of biological and physiological processes providing an astonishing degree of complexity of the central nervous system. The ongoing evolution of neuropeptides and their receptors has led to an enormous abundancy in mollusks. As usual in one species more than hundreds of neuropeptides are identified and sequenced. The neuropeptide diversity stems from a variety of general molecular mechanisms. The purpose of this presentation is to discuss the physiological significance of neuropeptide diversity present in mollusks, focusing mainly on the gastropods. The multitargeted action of a single peptide is ensured by the presence of different peptide receptors, a variety of second messengers and different target cell and organs. Evidences are presented that structurally related neuropeptides derived from the same precursor molecule are not simply functionally redundant isoforms but they may alter the functioning of the same physiological target with diverse efficiency. Neuropeptides usually are present in the organisms as a mixture of peptides rarely as a single molecule. Several neuropeptides are interphyletically distributed some are more restricted phyletically or even represent vertebrate innovations. Unfortunately, the conclusion that several vertebrate type neuropeptides are also present in invertebrates, including molluscan species, is often based on the observations that mammalian antibodies appear to react with invertebrate cells or tissue extract. The investigation of these events at the molecular level has become a major challenge of peptide research.

This work was supported by the OTKA grant No. 19099 and the National Brain Project No. KTIA_NAP_13-2-2014-0006, EFOP-3.6.1.-16-2016-00004. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

EFFECT OF BODY TEMPERATURE ON THE LEVEL OF BRAIN-DERIVED NEUROTROPHIC FACTOR AFTER NEONATAL ANOXIA

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Anoxia during delivery is a complication that can disturb the infant brain development, leading to various types of neurological disorders throughout life. Although it appears that knowledge of the pathogenesis of brain damage as a result of perinatal hypoxia has been well documented in studies on animal models as well as in clinical trials, so far have not been possible to develop effective therapeutics to prevent various types of complications. Nevertheless, numerous reports confirm that the body temperature can effectively reduce the damage of this organ. Moreover, neural tissue damage is accompanied by increased levels of neurotrophic factors. The most crucial role in neuronal plasticity and regulation of cell survival processes in immature brain is attributed to brain-derived neurotrophic factor (BDNF). According to our knowledge, so far there have been no reports on the relationship between body temperature during perinatal hypoxia and the level of BDNF. Therefore, our experiments aimed at checking the effects of body temperature during simulated perinatal anoxia on the level of the neurotrophic brain-derived factor (BDNF).
Two-day-old newborns were exposed to anoxia in 100% nitrogen atmosphere for 10 min in different thermal conditions, which allow them regulate the rectal temperature at the level of i. 33°C (physiological to rat neonates), ii. 37°C (level typical of healthy adult rats), or iii. 39°C (febrile adult rats). The temperature was controlled for 2 hours. Hippocampal and cortex level of BDNF were determined post-mortem, (1) immediately after anoxia, (2) 3 days, (3) 7 days, and (4) 2 weeks after anoxia.
There were no postanoxic changes in the level of BDNF in newborn rats kept at body temperature of 33°C. In contrast, at hyperthermic thermal conditions the level of the neurotrophin was decreased. The results showed, that the body temperature during neonatal anoxia affects the level of BDNF. Furthermore, these data support the idea that elevated body temperature (hyperthermia or fever) has a destructive impact on the development of the brain in hypoxic newborns. Our findings also suggest that neonatal rats maintaining normal body temperature were protected from above disturbances.

Research on this paper was supported by grant from National Science Centre, Poland, no 2016/21/N/NZ7/00399.

VISUAL CORTICAL ALPHA OSCILLATIONS DURING OBJECT WORKING MEMORY – DISTRACTOR GATING OR ACTIVE MAINTENANCE

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EEG alpha oscillations are frequently associated with inhibitory gating mechanisms in support of prioritizing processing and representation during visual attention and working memory (WM): alpha power in a cortical area shows an inverse relation to the relevance of the part of the visual field represented, being smaller for task-relevant and increased for task-irrelevant stimuli. Alpha power is also known to depend on WM load, and reflects the quality and content of sensory WM representations, also in keeping with recent findings on the role of low frequency oscillations in predictive processing in the visual cortical hierarchy. That is, alpha oscillations are implicated in both the active maintenance of WM representations and the filtering of upcoming distractors. In this work, we look at whether alpha oscillations during WM for complex visual objects (faces) reflect active maintenance, inhibitory filtering, or potentially both. In two experiments, participants maintained complex visual objects (faces) or simple visual patterns (gratings) in working memory, and they also had to withstand a distractor during the delay period in 2/3 of the trials. In each trial, two compound, grating-face overlay stimuli were presented in temporal succession, which were followed by a symbolic retrocue indicating whether gratings or faces were task relevant; the cued category was probed at the end of the trial by asking whether the probe matched the retained items. In the first experiment, no-distractor and face-distractor trials were randomly intermixed, while the second experiment consisted of 30 16-trial blocks, within which either no distractor, a face distractor or a grating distractor was presented in every trial. In both experiments, occipito-parietal alpha power displayed a robust, spatially widespread increase during the pre-distractor delay period when faces were retained in WM as compared to when gratings were memorized. The appearance of the distractor led to a robust event-related alpha desynchronization, during which this WM-related modulation was weakened then gradually rebuilt by the end of the delay period. Strikingly, early distractor-related alpha/beta desynchronization clearly depended on whether the distractor category matched that of the retained item, while no robust effects were found preceding the distractor that would be expected from the modulation of target maintenance activity depending on anticipated distractor gating demands. These results provide support for the notion that alpha oscillations during working memory for complex visual objects primarily reflect the active maintenance of visual cortical stimulus representations.

VOLUMETRIC ANALYSIS OF DEVELOPING HIPPOCAMPUS IN THE HUMAN FETAL BRAINS WITH AGENESIS OF THE CORPUS CALLOSUM

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One of the most common human brain malformation, agenesis of the corpus callosum (ACC), occurs either in isolated form or associated with other brain anomalies. Since ACC can modify brain morphology, we aimed to determine which consequences this congenital defect has on prenatal development of the human hippocampus.
We analyzed 81 in utero fetal brain MRI scans ranging between 20 gestational weeks (GW) to 38 GW, which were divided into three groups; control group (49 cases), 15 cases with isolated ACC and 17 cases with ACC associated with other brain anomalies. Segmentation of both hippocampi was performed manually and values of intracranial volume (ICV) were obtained from automated segmentations on a 3D reconstructed MRI from a set of 2D axial, coronal and sagittal T2-weighted images. Obtained data were statistically analyzed and presented as a nonlinear regression model for each of three groups and for both hemispheres.
Volumes of both hippocampi in all three groups display similar values as well as the same slope of growth curve in first phase of the analyzed period. Moreover, after 25th GW there was a significant decrease in hippocampal volume in ACC associated group compared to controls (left: $p = 0.029$; right: $p = 0.020$), and this difference was pronounced even more in later developmental period. Absolute hippocampal volumes in isolated ACC group start to differ significantly after 28th GW, first for the left hippocampus ($p = 0.007$), and after 29th GW for the right hippocampus ($p = 0.024$) and this difference was more pronounced after 30th GW. In analyzed period, ICVs of control and isolated ACC groups do not significantly differ, while ICVs of ACC associated group showed significantly smaller growth.
Our findings indicate that callosal absence notably interferes with the normal process of the hippocampal development during prenatal period in both analyzed groups with ACC, suggesting that this restricted hippocampal growth could affect learning and memory functions in later life of these infants.

HUMAN INDUCED PLURIPOTENT STEM CELL BASED IN VITRO NEUROTOXICOLOGY MODEL

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The development of *in vitro* platforms for neurotoxicology screenings is driven by the needs of the chemical, food and cosmetic industries and pharma drug development for neurodevelopmental and neurodegenerative disorders. Most developmental neurotoxicity studies are carried out in rodents or zebrafish, resulting in relatively high cost and lower translational value of the results due to the species differences. Major international initiatives have started to convert the traditional animal-based developmental toxicity tests to *in vitro* assays using human cells to detect and predict chemical hazards. However, the highly complex structure of the human brain makes *in vitro* modelling difficult. There is only a limited number of human neuronal cell lines, and human tissue suitable for developmental neurotoxicity studies comes from aborted fetuses and biopsies. Human pluripotent stem cell-based assays are highly capable to fill this niche. Human embryonic stem cells (hESCs) from early embryos or human induced pluripotent stem cells (hiPSCs) reprogrammed with specific transcription factors from somatic cells both offer the advantage that a large number of diverse cells and tissue types (for example kidney, liver, cardiac, neuronal, intestinal etc.) can be created using specific differentiation protocols, in a replicable manner.
We present hiPSC-based *in vitro* toxicology assays that can be used to test toxicity at different stages of the neuronal differentiation. Human iPSCs were differentiated into neuronal progenitor cells (NPCs) and then terminally differentiated towards neurons for 21 days (TD21). Both NPCs and TD21 neurons were exposed to different toxicants (e.g. Paraquat, VPA, acrylamide) and then examined with an ATP-based cell viability assay. Concentration-responses were investigated. Furthermore, the cell cultures were characterized by immunofluorescence staining. The results demonstrated that chemicals affecting different biological processes might have very different toxic concentrations on NPCs vs. TD21 neurons. They represent different stages of developmental neurotoxicology, therefore they can be used in modelling distinct process. Sets of different classes of toxicants shown distinct susceptibility differences on TD21 neurons, indicating that hiPSC-based *in vitro* neurodevelopmental models might be used effectively in various academic and pharmaceutical applications to evaluate neurotoxicity.

This work was supported by an EU H2020 project (EU-ToxRisk H2020-PHC-2015-681002).

MECHANISMS OF SEPTOHIPPOCAMPAL SYNCHRONIZATION WITHIN THE MEDIAL SEPTAL CIRCUIT

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The synchronization of distinct brain regions is crucial for efficient information flow and integration across areas. The septo-hippocampal system is an often studied model system for inter-areal synchrony. Population level manifestation of this synchrony is the hippocampal theta oscillation (4-10 Hz) that typically occurs during exploratory behaviors and REM sleep and has been linked to learning and memory functions of the hippocampus. The medial septal region of the basal forebrain has been identified as responsible for the generation of hippocampal theta oscillation. Cells located here that contain the parvalbumin Ca-binding protein (PV+) are able to establish rhythmical activity in a cell-autonomous manner, and can be tightly coupled to the hippocampal theta phase. According to the leading theory, such individual „pacemaker" cells, firing at their own frequencies, are synchronized to a common frequency and thus give rise to the hippocampal theta. However, experiments in which multiple septal neurons were recorded concurrently are rare and therefore the mechanisms of septal theta synchronization are still debated.
Here we analyzed the activity of simultaneously recorded medial septal neurons from urethane anesthetized rats to clarify the functionality of electrophysiologically distinct classes in synchronization. A group of medial septal neurons showed theta rhythmic firing irrespective of the dominant hippocampal oscillation (putative "pacemaker" neurons). These neurons exhibited increased synchrony during theta; however, their frequencies remained the same across neurons. Cross correlation analysis revealed two, mutually inhibitory subpopulations among them, coupled to the peak and the trough of theta waves, in line with the present knowledge about the PV+ cells. A second group of neurons fired synchronously at delta frequency, also independent of hippocampal states. A third group of cells followed the dominant hippocampal oscillation: delta during delta rhythm and theta during theta rhythm. Our findings contradict the aforementioned theory of frequency-synchronization of septal units as a key mechanism underlying hippocampal theta oscillation, since the putative "pacemaker" neurons fired at the same frequency during non-theta state. As an alternative, increased timing precision of action potentials within a theta cycle may be responsible for the observed stronger synchrony of septal neurons during theta rhythm.

NEUROINFLAMMATION IN THE CNS: FROM MOLECULES TO CELLS

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Neuroinflammation is widely accepted as being a contributing factor to the pathophysiology of neurodegenerative diseases as evidenced by the presence of activated glial cells in the brain of these patients pre onset of the disease symptoms. The role of inflammation in the disease process is for the most part unknown but is highly likely to contribute to the cell death seen in these devastating diseases. Thus, targeting the inflammatory aspect of these diseases could have great benefit to the patient and lead to better outcomes from other therapeutic interventions. In the first instance this study aims to characterise the response of the different brain cells to a range of different inflammatory stimuli Looking specifically at the mechanisms of action of these stimuli on the different cell types. In addition, a focus is given on the interactions between these cells, in order to provide us with a better understanding of the contribution these cells make to the pathophysiology of disease. Here we report on the characterisation and effect of exposure of these cells in a range of different conditions, and especially on pro-inflammatory cytokines and pathways these may activate. Moreover, a focus is given on generating a plethora of different brain cell types of different origins that are present during different developmental stages, and explore what neuroinflammation might mean in each case, as well as the differential response of these cells. This data will allow specific anti-inflammatory interventions to be considered and of particular interest is the vast range of dietary compounds with known properties that could be effective in this role.

COMPLEXITY OF MECHANISMS OF ACTION OF TRPV1 RECEPTOR LIGANDS

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The transient receptor potential channel TRPV1 is a non-selective cation channel, which is activated by many endogenous and exogenous compounds, heat, low pH. TRPV1 receptor is important integrator of multiple noxious and inflammatory signals, so it is considered to be a promising target for pain-relieving drugs. Despite more than 20 years of intensive research, none of TRPV1 antagonists are in routine clinical use yet. This may be due to poor understanding of their mechanisms of action. In the present work we studied substances from two different chemical classes: polypeptide toxins APHC1-3 from sea anemone *Heteractis crispa* and SB-366791 - small-molecule cinnamide TRPV1 receptor antagonist. They both produce significant analgesic effects in different animal models. We made patch-clamp experiments on cultured CHO cells stably expressing rat TRPV1 receptors. Capsaicin and pH drops were used to activate the channels.
We showed that the effects of toxins were dependent on the activation strength of TRPV1 receptors. Responses to low capsaicin concentrations (0.1-0.3 μM) were potentiated while responses to a high capsaicin concentration (5 μM) were inhibited. Our data suggest that in the presence of APHCs the capsaicin affinity is increased but the efficacy is decreased. The currents evoked by drops of extracellular pH from 7.3 to 6.2 were significantly potentiated by application of 100 nM APHCs. This effect disappeared when the channel was activated by stronger acidification (pH 4.5).
Unlike peptide toxins that bind to the extracellular part of the channel SB-366791 binds to capsaicin binding pocket and inhibits capsaicin-evoked currents in a competitive manner. Our study demonstrated that SB-366791 inhibited low pH-evoked currents in pH-dependent but non-competitive manner. Under stronger acidification affinity of the antagonist did not change, but the efficacy decreased.
Thus, there are complex allosteric interrelations between TRPV1 activators and modulating ligands, which bind to different sites. Such complexity may be related to the unique structure of TRPV1 which has two distinct but allosterically coupled activation gate regions. Further structural studies are needed to gain insight the detailed molecular mechanisms of action of TRPV1 receptor ligands.

SELECTIVE AROUSAL PATTERNS EVOKED BY MIDLINE AND SOMATOSENSORY THALAMIC STIMULATIONS

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Fast awakening from sleep is vital to animals in an unpredictable environment and sensory systems have been well adapted to fulfill this requirement. However, the existence of an internal arousal systems has long been proposed and the non-sensory midline thalamic nuclei (MT) has been considered to be a key node in transmitting arousal signals from subcortical centers to the cortex. Taking advantage, that MT neurons selectively express calretinin (CR) within the thalamus, we injected AAV-DIO-ChR2-eYFP into the dorsal part of MT (dMT) of CR-Cre mice and by means of optical stimulation we quantitatively assessed the role of dMT in arousal in naturally sleeping mice. Sleep-wake states were monitored by EEG/EMG signals and video tracking of the animal's movement. Intense, (10 sec, 10 Hz) optogenetic stimulation of dMT during slow wave sleep induced fast (~1 sec latency) and persistent arousal accompanied by locomotion, lasting for several minutes. Short stimulations (0.5-2 sec, 10 Hz), evoked stereotyped microarousal: an immediate desynchronization of EEG, with a characteristic drop in the power of delta (1-3 Hz) and sigma (10-15 Hz) bands, followed by a brief (2-5 sec) head-movement with relatively long (3-4 sec) latency. Microarousals could be evoked probabilistically, which was depended on stimulus duration and intensity. In cases when MT stimulations failed to evoke microarousals, the sigma but not the delta frequency band of the EEG power was selectively disrupted.
In order to compare dMT induced arousal with sensory arousal, we injected syn-AAV-ChR2 into the VB of CR-Cre mice. We found that brief (1 sec) stimulation of VB during slow wave sleep, efficiently induced microarousal with similar duration, however, much faster onset (<0.5 sec), than that of dMT. Interestingly, while brief (1 sec) stimulation of dMT was also capable to induce microarousal during REM sleep, VB stimulation was not effective at all. Our findings indicate significant differences between non-sensory and sensory arousal systems and also that MT is a good candidate to effectively induce state transitions in forebrain systems.

OPRM1 GENE POLYMORPHISM AND ADDICTION TO SMOKING IN PATIENTS WITH SCHIZOPHRENIA

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Various evidence suggests that in people with certain neuropsychiatric disorders, such as schizophrenia, the incidence of smoking is significantly higher than in healthy individuals, which is usually explained by the attempt of patients to alleviate the presence of psychiatric symptoms and emotional stress. On the other hand, studies suggest the involvement of the opioid system in the development of schizophrenia and drug addiction, including addiction to nicotine. In the gene OPRM1 encoding the μ opioid receptor type 1, more than 250 different polymorphisms have been found. The rs1799971 polymorphism (A118G) in the OPRM1 gene has been associated with the development of addiction to heroin, amphetamine, nicotine, and alcohol. This polymorphism results in the replacement of amino acids (Asn40Asp), and consequently changes the expression of the receptor and its affinity for the endogenous opioids. The polymorphism rs510769 is also associated with the risk of developing addictions to heroin and amphetamine. Since this polymorphism is located in the intronic region, it is assumed that it could change the function of the OPRM1gene by influencing the alternative intron splicing. To further investigate the role of the gene coding for the μ opioid receptor type 1 in nicotine dependence and schizophrenia, the aim of this research was to investigate the possible associations of polymorphisms rs1799971 and rs510769, in the OPRM1 gene, with smoking in schizophrenic patients. The proposed study involved 204 subjects of Croatian origin, both sexes (64 males and 140 females) with schizophrenia. Of the total number of respondents, 142 were smokers and 62 non-smokers. DNA samples extracted from the blood of all patients by Miller's isolation method were subjected to the genotyping of rs510769 and rs1799971 polymorphisms in the OPRM1 gene. The significance of deviations from the genotype distribution, as well as the differences in the distribution of genotypes, alleles and carrier polymorphisms rs1799971 and rs510769 located in the OPRM1 gene, were determined by using the $\chi 2$ test or Fisher test. The program «Haploview version 4.2» was used to construct LD matrix and determine the haplotype block of rs1799971 and rs510769 polymorphisms. Statistically significant differences were considered when the probability p was less than 0.05. The results of our research showed association between rs1799971 polymorphism in the OPRM1 gene and smoking in schizophrenic patients. Although these results have not been confirmed for the rs510769 polymorphism, the haplotypic association with smoking in subjects with schizophrenia has been found for combination of rs1799971 and rs510769 polymorphisms. Future aims are to determine the possible association of rs1799971 and rs510769 polymorphisms with smoking in healthy controls to see if these polymorphisms are associated with smoking just in schizophrenic patients or with smoking in general.

INFLUENCE OF CORTICAL INACTIVATION ON TRAINING ENHANCED VISUAL RESPONSES IN THE RAT'S SUPERIOR COLLICULUS

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The possibility of inducing neuronal plasticity at the cortical level is generally acknowledged. In our previous study we observed that few hours of visual training enhanced responses both at the cortical and subcortical level. The reinforcement of responses at the subcortical level, in the superior colliculus (SC), can occur through descending projection from the visual cortex (VCx). Therefore, in the current study we have attempted to examine how inactivation of the VCx affects tectal responses after visual training.
Experiments were performed on anesthetized rats exposed to flashing white-light-emitting diodes placed 10 cm in front of them. Monocular visual stimulation consisted of series of 300 repetitions of light flashes with 2 s intervals, presented every 15 minutes through 3 hours. Visual evoked potentials (VEPs) were recorded using multichannel linear electrode arrays from the primary VCx and the SC, contralateral to the stimulated eye. In order to block the activity of the cortex, after 3-hour visual stimulations a well above the contralateral VCx was fulfilled with xylocaine solution (2.5%). During cortical inactivation a single series of visual stimulation (300 stimulus repetitions) was presented. The SC VEP amplitudes were offline analyzed and peak-to-peak VEP amplitude was taken as a measure of response magnitude.
We observed that xylocaine used for neuronal inactivation resulted in a strong attenuation of cortical VEP amplitudes. However, cortical inactivation did not cause any significant difference in SC VEP amplitudes. Collicular VEPs were at the high level and significantly differed from control recording at the beginning of training. This indicates a minor impact of the VCx on response enhancement in the SC. As the VEP amplitudes in SC didn't show any decline after cortical inactivation, we conclude that the increase of responses in SC after visual training is most likely a result of the enhancement of the retinal input to the SC.

Supported by the Polish National Science Center grant Symfonia 1 (2013/08/W/NZ4/00691).

HIGH-DIMENSIONAL MASS CYTOMETRY CHARACTERIZATION OF THE BRAIN'S IMMUNE COMPARTMENT

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The brain together with its borders, create a highly dynamic microenvironment, populated with immune cells. Yet, characterization of immune cells within the normal brain compartment is still limited. In this study, we used mass cytometry (CyTOF) to characterize the immune populations of the naïve brain using 43 cell surface markers. In addition to confirming previously suggested resident and infiltrating immune populations, we were able to identify novel sub-populations in the naïve brain compartment (e.g., CD27+/IL-2R+ NK cells). Using flow cytometry, we further show the distribution of immune populations between the meninges, choroid plexus and parenchyma. We demonstrate the phenotypic range of resident myeloid cells, and identify CD44 as a marker for infiltrating immune populations. Taken together, this study provides a new approach for a system-wide view of immune populations in the brain, and is expected to serve as a valuable resource for understanding brain immunity.

INDUCTION OF LIFELONG D2R SUPERSENSITIVITY: RELEVANCE TO PSYCHIATRIC DISORDERS

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Repeated ontogenetic treatments (10-28d) of rats with the dopamine (DA) D_2 receptor (R) agonist quinpirole (50 mcg/d – 3 g/d) produces life-long DA D_2R supersensitivity (SS). Thus, in adult rats that had been quinpirole-primed as neonates, acute adulthood treatment with a single dose of quinpirole is followed by exaggerated or abnormal responses, including enhanced quinpirole-induced yawning, sniffing, grooming, gnawing, eating, 'taffy pulling', digging, rearing, paw treading, vertical jumping, and locomotor activity. Neonatal quinpirole treatment also results in an enhanced behavioral response to both psychotsimulants amphetamine and nicotine, and moreover, acute adulthood amphetamine or nicotine treatment of these ontogentically quinpirole-primed rats results in a robust enhancement of evoked (extraneuronal) neostriatal and/or accumbal DA release, as assessed by in vivo microdialysis. In contrast to this observation, repeated ontogenetic treatments of rats with the DA D_1R agonist SKF 38393 fails to produce permanent D_1RSS. Also, ontogenetic treatments with the DA D_3 agonist 7-OH-DPAT fails to produce D_2RSS. However, in ontogenetically quinpirole-primed rats, an ontogenetic DSP-4 lesion of noradrenergic nerves attenuates D_2RSS, indicating that noradrenergic nerves have a permissive effect on expression of D_2RSS. Yet, neonatal 6-OHDA lesioning of dopaminergic nerves does not eliminate the ability of ontogenetic quinpirole treatments to produce D_2RSS.
Because a disordered dopaminergic nervous system is implicated in psychiatric disorders, rats with life-long (≥ 2 years) DARSS were posited as a model for schizophrenia. Ontogenetically quinpirole-primed rats were thus found to have cognitive deficits, a deficit in prepulse inhibition, and low levels of the transcript regulator of G-proteins signaling (RGS) RGS9 in dopaminergic terminal areas of the brain. In addition, behavioral and neural plasticity deficits have been shown to be reversed by the atypical antipsychotic agent olanzapine. In summary, ontogenetically quinpirole-primed rats have no identifiable brain lesion (analogous to humans with schizophrenia), yet have face validity, construct validity and predictive ability for schizophrenia. Ontogenetically quinpirole primed rats appear to be a near-ideal non-primate animal model of schizophrenia.

THE ROLE OF EXPERIENCE IN THE DEVELOPMENT OF BINOCULAR VISION IN INFANTS

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In binocular vision, independent signals from the two eyes are brought together into a single representation of objects. Binocular rivalry arises when the two eye's images are not similar enough due to the abnormalities of binocular vision and/or the tricky stimulation strategies of experimental researchers. Interestingly, as we have demonstrated earlier[1], there is a great deal more to human binocular rivalry than a mere competition between the eyes: the adult brain can unscramble complementary patchworks of intermingled rivalrous images as long as the patchworks are based on some originally coherent images. This result suggests that human binocular vision makes use of our experience with objects and the statistical properties of the environment.
The question arises how much the early development of binocular vision might be dependent on visual experience (or how much is it pre-programmed). Employing a classic paradigm where the cortical responses to rivalrous and correlated phases of stimulation are tracked by EEG, we have compared the onset times for cortical binocularity in preterm and full-term human infants[2]. Surprisingly, we found that the extra two months of postnatal visual experience in preterm human neonates leads to a change in the developmental timing of binocular vision. The onset age of binocular function appeared to be at around the same time after birth in preterm and full-term infants. These data further support that the type of neuronal plasticity necessary for the early development of binocular vision is highly dependent on visual inputs.
The development of the visual system, however, continues into the adolescent years in humans[3]. Recent correlates of perceptual alternations under binocular rivalry based on optokinetic nystagmus have helped us to investigate the late maturation of the underlying neural architecture[4].

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CORTICOTROPIN-RELEASING FACTOR, UROCORTIN 1 AND SEROTONIN SYSTEMS IN A THREE HIT THEORY AND MATCH-MISMATCH HYPOTHESIS BASED DEPRESSION MODEL IN THE RAT

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According to the three hit theory of depression genetic- (GEF), epigenetic (EGF), and environmental factors (ENF) may lead to major depressive disorder. The match-mismatch theory emphasizes the significance of the duration and temporal pattern of stress exposure. The role of hypothalamic corticotropin-releasing factor (CRF) in the hypothalamus-pituitary-adrenal axis is well described, but the significance of extra-hypothalamic CRF systems (i.e. central amygdala; bed nucleus of the stria terminalis) is largely elusive. Role of urocortin1 (Ucn1) peptide produced primarily in the Edinger-Westphal nucleus is still controversial. The importance of serotonin (SER) producing neurons of the dorsal raphe nucleus (DR) in stress (mal)adaptation is well known. Our aims were to combine the two theories of depression in a model, and to assess the behavioural alterations and functional-morphological changes in the CRF, Ucn1 and SER systems.
Wistar rats were subjected to a 60 mins acute restraint stress. Based on their acute corticosterone response (CORTr) they were assigned into low CORTr (LC); middle range CORTr (MC), and high CORTr (HC) groups. Pairs of LC, MC and HC rats were mated. The offspring in the LC, MC and HC litters was considered to carry genetically different stress sensitivity (GF). Half of the litters of each group were exposed to maternal deprivation (MD) during the first 21 postnatal days (EPF). Later, half of all subgroups (MD, non-MD) were exposed to chronic variable mild stress (CVMS) in the 5-15th week (ENF). Consequently, there were groups suffering stress throughout their life, groups without any stress exposure (match); and animals suffering a single period of stress (mismatch). Behavioural tests were performed in the 15th week (forced swim test [FST], sucrose preference test). Finally, animals were perfused, their body- and adrenal weights were measured. CRF-FosB, Ucn1-FosB and SER-FosB double immunofluorescence labelling was performed on brain sections.
CVMS-exposed rats showed increased relative adrenal weight. Their immobility time in FST was increased and in line with this, their sucrose consumption was decreased. No significant morphological changes were found in the peptide expression and activity of CRF and Ucn1 systems. The SER expression in DR was increased upon CVMS in LC group in contrast with in the HC animals this change was not detected. CVMS-exposed MD animals of HC group showed the lowest SER expression.
Based on our results the CVMS was effective. Both GEF and EPF effects modify the efficacy of later life ENF. This model may underline that the temporal pattern of stress affects behaviour, at least in part through the DR-SER. The CRF and Ucn1 systems were not affected in this chronic stress model suggesting that they may contribute to a shorter-term control of stress adaptation.

Acknowledgement: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

BENEFICIAL EFFECT OF KETONE SUPPLEMENTS ON ABSENCE-LIKE EPILEPTIC ACTIVITY AND ANXIETY-LIKE BEHAVIOR IN WAG/RIJ RATS

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Ketone supplementation-evoked nutritional ketosis may be beneficial in several central nervous system diseases including epilepsy. The focus of this study was to determine the effects of ketone supplementation on anxiety-related behavior and absence-like epileptic activity in genetically absence epileptic Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats. In the first part of the study, we tested exogenous ketone supplements administered sub-chronically (7 days, normal food with daily intragastric gavage bolus) followed by assessment of anxiety measures on elevated plus maze (EPM). The groups included standard diet (SD) or SD + ketone supplementation (2.5 g/kg/day). Ketone ester (KE; 1,3-butanediol-acetoacetate diester), beta-hydroxybutyrate-mineral salt (KS), and KS + medium chain triglyceride (KSMCT) were used as ketone supplements. The results revealed that KSMCT reduced anxiety on EPM as measured by less entries to closed arms and more time spent in open arms. In the second part of the study, we tested the effects of sub-chronically applied exogenous ketone supplements (KE, 2.5 g/kg/day; KSMCT, 2.5 g/kg/day; intragastric gavage) on absence epileptic seizures in WAG/Rij rats. We demonstrated that the number of spike-wave discharges (SWDs)significantly decreased after ketone supplement treatments between 3rd and 7th days of gavage. Moreover, blood beta-hydroxybutyrate levels were significantly increased in both parts of the study after ketone supplement gavage. Our data indicate that sub-chronic ketone supplementation not only elevated blood beta-hydroxybutyrate levels, but also reduced anxiety-related behavior and absence-like epileptic activity in WAG/Rij rats.

EFFECT OF OXYTOCIN ON THE SOCIAL BEHAVIOR IN NORWAY GRAY RATS, SELECTED FOR ELIMINATION AND FOR ENHANCEMENT OF AGGRESSIVE BEHAVIOR TOWARD HUMANS

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Hypothalamic neuropeptide oxytocin attracts a lot of attention of neurobiologists, as well as of general public. Increased interest in oxytocin is caused by its positive effect on the social behavior in different species of vertebrates, including humans. Since 1972, Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia) carries out selection of wild Norway gray rats (Rattus norvegicus) for elimination of defensive reactions to humans (tame rats) and for enhancement of aggressive behavior toward humans (aggressive rats). Tame rats are not afraid of humans and are more accustomed to human hands than laboratory rats. Tame rats have decreased intermale aggression as compared to rats of aggressive strain, having same behavioral patterns of agonistic interactions. Aggressive rats have an increased aggressiveness towards humans, as well as towards conspecific animals. In this study we investigated effect of exogenous oxytocin on intermale confrontations in Norway gray rats with tame and aggressive behavior. After daily intranasal application of oxytocin (1 mcg/ml) and control saline solution during 5 days, the standard resident-intruder test was conducted to assess behavior of male gray rats towards male Wistar rats. Aggressive rats, exposed to oxytocin applications, showed decrease in duration of aggressive interactions and boxing, as well as lowering tendency in levels of such parameters as number of kicks and lateral threats, all comrade to aggressive rats treated with saline solution. These data indicate decreased aggressiveness in aggressive rats during intermale confrontations. Nevertheless, oxytocin applications delivered to tame rats were observed to have an opposite effect on their behavior in the resident-intruder test, including prolongation of aggressive interactions and lateral threats and increased number of attacks and kicks. Presumably, the process of selection for elimination or enhancement of aggressive behavior toward humans causes divergent changes in effects of oxytocin on the social behavior. Oxytocin applications did not have significant effect on the dynamics of corticosterone levels in rats after the stress of restriction.

The research was supported by the Russian Foundation for Basic Research (grant no. 16-04-00637

ESTABLISHING A NEW COOPERATION MODEL IN RATS

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Impairment in social cognition is a characteristic symptom in many psychiatric diseases such as schizophrenia, bipolar disorder or autism spectrum disorder. An essential part of social cognition is the ability of cooperation. Our aim was to develop a new rodent paradigm of cooperation, which can be used for the modelling of cooperation deficit, and testing putative cognitive enhancer substances with sufficiently high throughput.
Ten pairs of one year old male Lister Hooded rats were trained to simultaneously perform (within 1 second) a nose-poke response into two separate nose-poke modules in a Skinner box in order to obtain food reward. At the start of a trial both nose-poke modules were illuminated and the first nose-poke to any of the modules started a timer. After this the rats had to complete the second nose-poke to the other module in a predefined time window. If they succeeded, the animals were given a food pellet reward, and a new trial was started. If they didn't, the new trial started after a 5 second time-out. The time window for the simultaneous nose-poke was 5 seconds at the start of training. When more than 40 rewards were obtained under these circumstances during the 20 minutes sessions, the time window was reduced first to 2,5 seconds, and finally to 1 second. The performance of the rats was characterized by three parameters: latency to the first nose-poke, the interval between the „simultaneous" nose-pokes and the percentage of succesful trials. In some of the training sessions the behaviour of the animals was videorecorded for further analysis. In the second phase of the experiment, to check whether the task was solved by the animals via real cooperative strategy we conducted a yoked control task where the members of the pairs had to perform the same task under the same rewarding conditions but in two separate chambers, thus the animals can not detect each other.
All pairs were able to complete the task during eight training days on average. The percentage of successful trials rapidly increased in the 5 s time window stage of the conditioning period then it remained stable (52-59%) during the rest of the training, while the first nose-poke latency and the interval between the „simultaneous" nose-pokes decreased gradually throughout the progress of the training. When the animals were transferred to the yoked control task the number of successful trials significantly dropped compared to the previous days' performance in the normal training session (from 59,4% to 44,6%).
Our results suggest that a real cooperative behaviour can be formed in this paradigm. The model is applicable to test the effects of various impairing and improving interventions on social cognition.

The study was funded by the Hungarian National Brain Research Program (NAP), contract# KTIA_NAP_13-2014-0015.

STEADY-STATE VISUAL EVOKED POTENTIALS TO CONE-ISOLATING CONTRASTS IN AWAKE BEHAVING CATS

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We have previously described colour-opponent blue-ON cells in the lateral geniculate nucleus of cats. The properties of these cells are ideally suited to form the basis of functional, dichromatic colour vision. Cortical neurones responding to the stimulation of „blue" cones have never been described in the cat literature. This negative evidence is, however, based entirely on studies in anaesthetised animals. Here we were interested in detecting visual evoked potentials in response to steady-state modulation of the „green" (ML) and „blue" (S) cone types in awake behaving cats. A cat was trained to perform a simple visual reaction time task with a central spot on a dark background as the target stimulus. The animals initiated trials by pressing a glass plate. Target stimuli appeared following a random delay. The animal was rewarded by homogenised cat food when it pressed the response key within 1500 ms. During experimental sessions, the cat could move freely within a 1×1.5 m box. After the training period, the animal was implanted with fourteen epidural electrodes placed over the superficial regions of primary visual cortex. Following recovery, the dark stimulus background was replaced by a checkerboard pattern continuously reversing in phase at 2 Hz. The two colours of the checkerboard corresponded to either of the cardinal directions in the cat's dichromatic colour space: ML-cone isolating, achromatic, S-cone isolating and S-ML. One of these colour conditions was shown in each experimental session. The electroencephalogram was recorded using a wireless signal transmitter while the cat was working in the experimental box. Steady-state visual evoked potentials (ssVEPs) were calculated by averaging EEG traces triggered to the checkerboard reversal. The peak-to-peak amplitude of the ssVEP was used as response measure. We observed two different patterns of responses. On the more anterior electrodes corresponding to area 18, responses to the S-cone isolating stimulus were significantly lower than the ML-cone isolating response ($p<0.01$, S:ML response ratio 0.61±0.14). Achromatic and S-ML responses were statistically equal to the ML-response. This pattern suggests little influence of the S-cone input. In the region of area 17, S-cone responses were again, lower than ML-responses (S:ML response ratio 0.78±0.08). Achromatic and S-ML responses were, however, significantly higher than the ML-cone response alone (ratio to ML-response 1.22±0.08 and 1.36±0.10, respectively). This pattern suggests a significant contribution of the S-cone input to the population response of area 17. Our results suggest that neurones processing S-cone signals are operational in the primary visual cortex of awake cats.

Supported by grants OTKA K108747 and the Hungarian Brain Research Program KTIA_13_NAP-A-I/11, EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

A VIRTUAL REALITY ARENA TO STUDY VISUAL INSECT NAVIGATION

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Visual navigation experiments under natural conditions are difficult to perform because it is hard to control the available visual information. Laboratory experiments, in turn, in most cases rely on very artificial environments where animal responses are unlikely to be representative of normal behaviour. We study visual navigation in ants and have developed a virtual reality arena in which we can present and arbitrarily modify the animal's natural navigation environment and perform behavioural experiments.
The apparatus consists of a 1m diameter spherical projection device with 20 thousand blue-green LED pixels that can project a fully panoramic image to an animal placed in its centre. In addition, 500 UV LEDs with variable polarisation can deliver the sky polarisation pattern. The animal is tethered on a trackball on which she can walk and rotate freely. The ball is designed to match the inertia of the ant, to allow her to walk normally. 3D reconstruction software was used to create a detailed 3D model of the natural habitat of the animal. A custom-designed 3D projection software can present the scene to the ant. The trackball drives the 3D engine in a real-time closed loop to allow the animal to move around in her virtual environment. A high-speed camera films the head movements and yaw-rotations of the ant and the virtual path of the animal is reconstructed.
The system allows us to change the reconstructed environment, add or remove landmarks, change the sky polarisation pattern and apply transformations to the scenery while monitoring the navigation performance of the ant.

NESFATIN-1/NUCB2 DYSFUNCTION IN INTRAUTERINE PROTEIN MALNOURISHED RATS

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Intrauterine undernutrition predisposes to develop obesity, metabolic syndrome and cardiovascular disease. Obesity related diseases such as glucose intolerance, insulin and leptin resistance have been observed in animal studies using adult offsprings of dams kept on protein reduced diet during pregnancy and/or lactation. Manifestation of the phenotype appears with aging if food restriction is not continued and strongly depends on the model used. The underlying mechanisms are not clear, but the role of central "programming" has been emphasised, as alterations in brain development and in hypothalamic food intake and energy balance regulatory circuits were revealed.
Nesfatin-1 is an N-terminal secreted fragment of the prohormone nucleobindin'2 (NUCB2). Nesfatin-1/NUCB2 is expressed widely in the hypothalamus, and coexpressed with several orexigen and anorexigen neuropeptides in the dorsolateral hypothalamic area (DLHA), the arcuate (ARC), the paraventricular (PVN) and the supraoptic (SON) nuclei. Intracerebroventricularly (icv) applied nesfatin-1 reduces food intake and increases energy expenditure, and fasting reduces the level of the peptide in the hypothalamic paraventricular nucleus. We investigated whether developmental "programming" of the central nervous system affects nesfatin-1/NUCB2 expression and function. Offsprings of dams fed with 50% protein restricted diet (PR) from gestational day zero (day of mating) to delivery were investigated and compared with control, normal nourished mates. PR pups had lower birth weight, but exhibited anaccelerated growth. They developed a higher preference for fat-rich over control diet than controls by the age of 10 weeks. However, when kept on normal rat chow, PR rats remained lean even until the 26th week of age, which was the last investigated timepoint. Hypothalamic nesfatin-1/NUCB2 mRNA expression did not differ at birth, but it was elevated in young PR adults (12 week-old) in the DLHA, ARC and PVN, but not in the SON. Nesfatin-1, injected icv at this age reduced food intake of PR rats less effectively than that of controls. Glucose and insulin tolerance was maintained in young adults, but by the age of 26 week PR rats showed reduced insulin sensitivity compared to controls.
These results suggest that dysfunction in central nesfatin-1 signaling may be one of the early steps in developing metabolic syndrome after intrauterine protein restriction of rats.

Supported by: NKFI K115422 (ZET)

DIFFERENTIATION-DEPENDENT MOTILITY-RESPONSES OF DEVELOPING NEURAL PROGENITORS TO OPTOGENETIC STIMULATION

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During neural tissue genesis, neural stem/progenitor cells are exposed to bioelectric stimuli well before synaptogenesis and neural circuit formation. Fluctuations in the electro-chemical potential in the vicinity of developing cells influence the genesis, migration and maturation of neuronal precursors. The complexity of the *in vivo* environment and the coexistence of various progenitor populations hinder the understanding of the significance of ionic/bioelectric stimuli in the early phases of neuronal differentiation. Using non-invasive optogenetic stimulation, we investigated the *in vitro* motility responses of radial glia-like stem/progenitor populations to ionic stimulation. Radial glia-like neural stem cells were isolated from *CAG^{loxp}Stop^{loxp}ChR2(H134)-eYFP* transgenic mouse embryos. After transfection with Cre-recombinase, ChR2-expressing and non-expressing cells were separated by eYFP fluorescence. Expression of the light-gated channels were checked by patch clamp and fluorescence-intensity assays. Neurogenesis by ChR2-expressing and non-expressing cells was induced by withdrawal of EGF from the medium. Cells in different (stem cell-, migrating progenitor- and maturing precursor) stages of development were illuminated with laser light (λ=488 nm; 1.3mW/mm^2; 300 ms) in every 5 minutes for 12 hours. The displacement of the cells was analysed on images taken at the end of each light-pulse. Results demonstrated that the migratory activity decreased with the advancement of neuronal differentiation regardless of stimulation. Light-sensitive cells, however, responded on a differentiation-dependent way. In non-differeniated ChR2-expressing stem cell populations, the motility did not change significantly in response to light-stimulation. The displacement activity of migrating progenitors was enhanced, while the motility of differentiating neuronal precursors was markedly reduced by illumination.

ASSOCIATION OF OXYTOCIN RECEPTOR POLYMORPHISM WITH PERSONALITY TRAITS

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Early social interactions are essential for brain development and have lifelong effect on physical and mental health. The so-called social neuropeptide systems of oxytocin and vasopressin have important regulatory role on social behavior, thereby their genetic variants can potentially modulate the developing personality, throughout self-monitoring, especially confirmation and disconfirmation in social interactions. During the last decades numerous studies used personality questionnaires, such as the Temperament and Character Inventory (TCI) by Cloninger et al. (1993) to evaluate the heritable components of human personality. According to their psychobiological model the temperament dimensions (novelty seeking, harm avoidance, reward dependence, and persistence) are genetically determined factors. Whereas the character dimensions (self-directedness, cooperativeness, and self-transcendence) shape personality through insightful learning leading to intentional implements.
The present study aimed to evaluate the effect of the most widely investigated oxytocin receptor gene (OXTR) polymorphism on personality traits in a genetic association study among healthy young adults. Interestingly, there is only one type of receptor exist for oxytocin, which is expressed both in the limbic system (amygdale, olfactory bulb), memory associated cortical regions, and hippocampus. The intronic variant rs53576 A-allele has been associated with various social-behavioral phenotypes, including lower reward dependence, emotional task-related amygdala activation, and decreased hypothalamic gray matter, especially in males (Tost et al., 2009). We aimed to replicate their genetic association finding in an independent, larger group of healthy adults. Altogether 703 young adults (411 women and 292 men, mean age = 21.42 ± 2.27) of Caucasian origin gave buccal DNA samples and filled out the short, Hungarian version of TCI. The genotyping of rs53576 was performed with PCR-RFLP and real-time PCR methods. Genetic association analysis of the four temperament and three character dimensions was carried out by multivariate analysis of variance.
Significant association was found between rs53576 and reward dependence (RD) temperament dimension but only in men, where A-allele carriers showed lower RD scores compared to those with GG genotype ($p = 0.009$). Interestingly, opposite direction was observed among women but the difference was only tendentious ($p = 0.08$). Our findings support the previously observed association of the risk A-allele of rs53576 and lower RD, but only among men. These results draw attention to gender differences is the genetic effects of the oxytocin system.

SYNCHRONISATION OF BREATHING RHYTHM AND FIRING OF ORBITOFRONTAL NEURONS

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Oscillations in the local field potential of the olfactory bulb, the piriform cortex, the barrel cortex and the hippocampus have been described to be coherent with the breathing rhythm. We aimed to test whether such a synchronisation between neuronal activity and breathing could also be observed in prefrontal areas of the cortex. To examine this possibility, we performed neuronal recordings with silicone probes in the orbitofrontal cortex of awake, head-fixed mice; simultaneously the local field potentials in the hippocampus and olfactory bulb together with the breathing cycles of the animals were recorded. Our results show that a dominant component of slow oscillations in the orbitofrontal local field potential is synchronous with breathing during immobility as well as locomotion periods at 1.5 - 2.7 Hz and 3.9 - 6.8Hz, respectively. The majority of orbitofrontal neurons show strong, significant coupling to this oscillation, which is synchronous with breathing, during awake, resting state (immobility) and locomotion. We investigate how the activity of neurons in different cortical layers or different areas of the prefrontal cortex are coupled to the breathing rhythm of the animal. Our findings might have implications in the suggested multi-variable, subjective, food-reward-value processing function of the orbitofrontal cortex, by providing a temporal frame work for integration of olfactory and gustatory information.

THE ROLE OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE IN ENDOTOXIN-INDUCED RETINAL DEGENERATION

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Neuroprotective and anti-inflammatory specificity of pituitary adenylate cyclase-activating polypeptide (PACAP) have been shown in a number of studies, which confirm the peptide exerts protective effects against neurodegenerative diseases. PACAP-deficient (PACAP KO) mice respond with enhanced sensitivity to negative external factors and provide evidence that endogenous PACAP shows key role in different insults, such as hypoxia or oxidative stress. However, the proteomic background of the inflammation in PACAP KO mice is not clear yet, therefore our aim was to investigate the functional and morphological changes in endotoxin-induced retinal degeneration. We compared the retinas of PACAP KO and wild type mice (Wt) in healthy condition, and in intraperitoneally treated with 6mg/kg lipopolysaccharide endotoxin (LPS) - induced inflammation. We examined dark-adapted electroretinography (ERG) to detect the dysfunction of the retina. Histological structural analysis and glial fibrillary acidic protein (GFAP) immunoreactivity were also analyzed to support the functional results. During LPS infection the ERG responses of PACAP KO mice were disturbed (a-wave, b-wave amplitudes) compared to the Wt mice. Endogenous PACAP caused a significant protection in different retinal layers such as OLM-ILM, OPL, ONL, INL and IPL by preserving their thickness in LPS injected Wt mice. In LPS-treated PACAP KO retinas, the GFAP expression increased in Müller glial cells compared to the LPS-treated Wt.
These results clearly showed that the endogenous PACAP has a protective, anti-inflammatory role in endotoxin-induced retinal inflammation in mice.

Acknowledgement: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, GINOP-2.3.2-15- 2016-00050 "PEPSYS",PTE AOK Research Grant (KA-2017-15), Supported by the ÚNKP-16-4 new national excellence program of the ministry of human capacities, Bolyai Scholarship. MTA-TKI PACAP Research Group. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

SPATIAL LONG-TERM MEMORY AND MODULATION OF NMDA RECEPTOR SUBUNIT EXPRESSION IN MEDIAL SEPTAL CHOLINERGIC AND NONCHOLINERGIC NEURONS LESIONED RATS

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The present study was designed to investigate the effect of selective immunolesions of cholinergic and GABA-ergic SH projection neurons (using 192 IgG-saporin and GAT-1 saporin, respectively) on spatial memory assessed in water maze and the N-methyl-D-aspartate (NMDA) receptor GluN2B subunit expression in the rat hippocampus. We used water maze training protocol with eight training trials. One day after training, probe test with the platform removed was performed to examine long-term spatial memory retrieval. We found that immunolesion of medial septal cholinergic neurons did not affect spatial learning as exhibited by a decreased latency to find the hidden platform across the eight training trials. In contrast, rats with immunolesions of medial septal GABAergic neurons did not show a decreased latency across training trials in water maze. Trained control rats spent significantly longer than chance (15 s) performances such as swimming time in test sector (where the hidden platform was located). Moreover, they spent significantly longer in test sector than in the opposite sector, confirming the establishment of long-term memory. In contrast, the preference for test sector was abolished in medial septal immunolesioned rats. Because Saporin treated rats learned the location of the hidden platform during training, the results suggest that saporin treated rats could not remember the training a day later. We found that the expression level of NR2B subunit of NMDA receptor in the hippocampus was decreased significantly in the GAT-1 treated group compared with the control and saporin treated groups. In conclusion, our findings suggest that immunolesion of medial septal GABAergic neurons can interrupt hippocampus-dependent spatial learning, possibly through modulation of NMDA receptor subunit expression in the hippocampus. Moreover, our finding that selective lesions of medial septal cholinergic neurons affects probe-test performance but not spatial learning, suggests that septohippocampal cholinergic projections are involved specifically in the consolidation or retrieval, but not in the acquisition of long-term spatial memory.

VESTIBULO-OCULAR CHARACTERISTICS AND MOTION SICKNESS SUSCEPTIBILITY OF AEROBATIC PILOTS IN COMPARISON WITH CONTROL PARTICIPANTS

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The aim of the current study is to evaluate the functional plasticity of vestibular system in aerobatic pilots. We investigate whether aerobatic pilots, as individuals who experience an intense vestibular stimulation, present a modification of the vestibular functioning as compared to normal volunteers. To evaluate the adaptive changes we assess vestibulo-ocular reflexes, motion sickness sensitivity and a level of visual field dependence on the perception of the vertical in 2 groups of subjects: the experimental group consisting of 12 aerobatic pilots and a control group of 17 healthy adult subjects. Vestibular stimulation consists of EVAR - Earth-vertical axis rotation and OVAR - off-vertical axis rotation. The Vestibulo-Ocular Reflex (VOR) parameters can be assessed in 2 ways - with the canal-ocular reflex (COR) evoked by a step-stimulation (rotatory acceleration and deceleration), and the otolith-ocular reflex (OOR) evoked by an off-vertical axis rotation with an inclination angle of the axis of 17° relative to the vertical. The OVAR test is known as nauseogenic, so we also estimate the level of motion sickness symptoms evoked by the test with the help of a Graybiel questionnaire. The motion sickness susceptibility questionnaire MSSQ is also used in the study to obtain the motion sickness susceptibility history of subjects. The perception of the vertical and the field dependence are estimated with the help of "Rod and Frame" test which allows to separate the visual and vestibular cues in the perception of vertical and reveal the impact of the visual reference.
We hypothesize that the aerobatic pilots present a higher VOR gain due to the adaptation mechanisms, smaller sensitivity to motion sickness due to habituation and the more accurate perception of the vertical compared to the control group participants.

EXPRESSION AND INFLAMMATION-INDUCED UP-REGULATION OF HEMOKININ-1 IN THE TRIGEMINAL SYSTEM, AND ITS EFFECT ON CULTURED TRIGEMINAL GANGLION NEURONS

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Objective: The newest tachykinin peptide, hemokinin-1 (HK-1), encoded by the Tac4 gene has substance P (SP)-like structure, but distinct expression patterns and mechanisms of action. It activates the NK1 tachykinin receptor, but through different binding site and signalling pathways than substance P, and a presently unknow target has also been proposed for several of its functions. Our team has proven its mediator role in inflammation, peripheral and central pain mechanisms. Trigeminal nociceptor sensitization is important in migraine, but no data were available about HK-1 in the trigeminal system. Therefore, we aimed to investigate the expression of Tac4 mRNA in the trigeminal ganglia (TRG) and nucleus caudalis (TNC) and its inflammation-induced alterations in vivo, as well as the effect of HK-1 on cultured TRG primary sensory neurons.
Methods: Tac4 mRNA expression was detected by quantitative PCR (qPCR) in the TRG, TNC and peripheral blood mononuclear cells (PBMC) for comparison. Orofacial inflammation was induced by unilateral s.c. injection of 50 µl Complete Freund's Adjuvant (CFA) into the whisker pad of male Wistar rats (n=12), and TRG, TNC, PBMC were collected for analysis on days 1, 3 and 7. Saline-treated animals and contralateral sides of CFA-injected rats served as controls. Mechanical pain thresholds of the orofacial region were determined with a series of von Frey filaments. The effect of HK-1 on TRG cells in comparison with SP was measured by fluorescence Ca2+-imaging in cultured TRG neurons obtained from wildtype C57Bl/6 (WT) and NK1 receptor gene-deleted (NK1-/-) mice to investigate the receptorial mechanism.
Results: HK-1 was detected at the mRNA level in the TRG, TNC and PBMC. CFA induced significant orofacial mechanical allodynia on day 1 with a maximum on day 3. This correlated with the Tac4 gene expression increase in the TRG and interestingly also in PBMCs. In TRG samples on the CFA-injected sides, Tac4 mRNA increased by 3.0 fold on day 1, 7.8 fold on day 3 and 7.0 fold on day 7 compared to saline-treated animals. In the TNC, Tac4 mRNA was not significantly altered with small increases on days 1 and 3 relative to contralateral sides. In PBMC, Tac4 mRNA was significantly upregulated 2.0 fold on day 1, 2.7 fold on day 3 and 2.8 fold on day 7 compared to saline-treated rats. HK-1 (1µM), but not SP, caused a slow and reproducible Ca2+-influx into the TRG neurons obtained from both WT NK1-/- mice.
Conclusion: HK-1 is expresssed in the trigeminal system and remarkably upregulated in the TRG and leukocytes in response to orofacial inflammation. Therefore, it is a potential mediator of the cascade of events resulting in the sensitization underlying migraine headache and the accompanying facial allodynia. HK-1 directly activates the primary sensory neurons via an NK1-independent mechanism. Identification of its target and signalling pathways might open new perspectives in understanding migraine mechanisms.

Acknowledgement: KTIA_NAP_13-2014-0022, GINOP-2016-00050, EFOP-3.6.1.-16-2016-0004. J. K. was supported by TAMOP-4.2.4.A/2-11/1-2012-0001 'National Excellence Program Apáczai Scholarship'. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

LOCAL CONNECTIONS OF EXCITATORY NEURONS TO PARVALBUMIN-CONTAINING INTERNEURONS IN MOTOR-ASSOCIATED CORTICAL AREAS OF MICE

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Parvalbumin (PV)-containing fast-spiking neurons are the largest subpopulation of cortical GABAergic interneurons. PV neurons may serve not only as mediators of lateral, feedforward and feedback inhibition in the cortical circuit, but also as generators of gamma rhythms. For understanding the precise role of PV neurons in the local cortical circuitry, it is important to reveal the inputs that drive the interneurons. In the present study, we morphologically investigated local excitatory inputs to PV neurons in the motor areas, using the transgenic mice in which the input sites, i.e. the dendrites and cell bodies, of PV neurons were specifically labeled with dendritic membrane-targeted GFP. In cortical slices from the transgenic mice, single pyramidal neurons of layers (L) 2/3, L5a, L5b and L6 were intracellularly stained with biocytin. We reconstructed the axon fibers of intracellularly stained pyramidal neurons and counted the number of axon varicosities, which apposed to the input sites of PV neurons (apposed varicosities). The number of apposed varicosities to the total varicosities of L2/3–5 pyramidal neurons was 13–15%, whereas that of L6 pyramidal neurons was significantly more frequent, i.e., 25%. Furthermore, the L6 pyramidal neurons group was subdivided into two types according to the spread of their axon collaterals: slender type (L6s) and wide type (L6w). Interestingly, axon varicosities of L6s pyramidal neurons more frequently apposed to PV neurons (28%) than L6w pyramidal neurons (20%). These results suggest a difference in the local excitatory control of PV neurons between L2/3–L5 and L6 pyramidal neurons.

SINGLE-MOLECULE VIEW OF THE PLASMA MEMBRANE ORGANIZATION FOR SIGNAL TRANSDUCTION

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Single-molecule imaging and tracking of molecules in the plasma membrane of living cells are now providing researchers with the unprecedented ability to directly observe and analyze molecular dynamics and interactions (for a review, see Kusumi et al. Nat. Chem. Biol. 17, 524-532, 2014). My group is primarily responsible for advancing single-molecule observations in living cells and high-speed single-molecule imaging and tracking at time resolutions up to 20 μs.
“Directly seeing” how individual molecules behave right in front of our eyes is as if we were watching a group of ballet dancers in the theater. Therefore, the knowledge gained by single-molecule methods is now revolutionizing our understanding of the dynamics, distributions, and functions of the molecules in/on the plasma membrane. One of the most exciting results we have recently obtained is that signaling in/on the plasma membrane is often enabled by very transient molecular interactions, rather than stable molecular complexes, at variance with the prevalent views described in many textbooks and reviews. I plan to talk about these recent results.

OXYGEN-INDUCED RETINOPATHY IN PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE DEFICIENT MICE

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Introduction: The oxygen-induced retinopathy (OIR) is a well-established model of retinopathy of prematurity (ROP) characterized by vessel obliteration and new, abnormal vessel formation. ROP is one of the leading causes of childhood blindness. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a well-known neurotrophic and neuroprotective protein. Several studies have revealed the presence of PACAP and its receptors in the retina, as well as its retinoprotective effects in ischemic and diabetic lesions. Recently we have shown that local PACAP treatment improve the retinal vascularization and decreases the avascular area in the rat model of ROP.
In this study we aimed to examine the effect of endogenous PACAP in the mouse model of ROP.
Methods: OIR was generated by placing litters of PACAP deficient and wild-type mice in 75 % oxygen concentration from postnatal day 7 (P7) to P12, then returned them to room air. Control mice of both types were kept in room air. On P17 retinas were isolated and the vessels were visualized by isolectin staining. The percentages of avascular to whole retinal areas as well as the number of vascular tufts were measured. Statistical analysis was done by Student's t-test.
Results: Retinas of PACAP deficient OIR mice showed greater central avascular area than those of the wild type (12.33 % vs 4.51 %; p=0.003). Retinal images of controls kept in room air did not show vascular alterations. No changes in the number of vascular tufts were observed between deficient and wild mice.
Discussion: This is the first study to examine the endogenous effect of PACAP in OIR model Previously we have shown the beneficial effect of exogenous local PACAP treatment on the vessel formation and cytokine profile in the rat OIR model. Together with the present findings we suggest that PACAP could be a novel retinoprotective agent in ROP.

This research was supported by EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, GINOP-2.3.2-15-2016-00050 "PEPSYS", PTE AOK Research Grant (KA-2017-15), Supported by the ÚNKP-16-4 new national excellence program of the ministry of human capacities, Bolyai Scholarship, MTA-TKI PACAP Research Group. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

GUANOSINE MAY INCREASE ABSENCE EPILEPTIC ACTIVITY IN WISTAR ALBINO GLAXO RIJSWIJK RATS

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One-third of epileptic patients are drug refractory due to the limited efficacy of antiepileptic therapy. Thus, there is an immense need to find more effective, but safe and well-tolerated antiepileptic drugs. A great deal of results suggests that not only adenosine (Ado) but also non-adenosine nucleosides such as guanosine (Guo) are endogenous antiepileptogenic modulators. To investigate the involvement of the adenosinergic system in Guo-evoked changes in absence epileptic activity, we used a non-selective Ado receptor antagonist theophylline (intraperitoneally, i.p.; 5 mg/kg) in combination with both i.p. 50 mg/kg and 100 mg/kg Guo in Wistar Albino Glaxo Rijswijk (WAG/Rij) rats. We also applied i.p. a selective A_{2A} Ado receptor antagonist SCH 58261 (7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine) (1 mg/kg) and a cyclooxygenase (COX) 1 and 2 inhibitor indomethacin (10 mg/kg) in combination with i.p. 100 mg/kg Guo to decide whether Ado/A_{2A}R/COX/PGE2 system has a role in the Guo-evoked modulation of absence epileptic activity. We strengthened our previous result that lower dose of Guo (i.p. 50 mg/kg) decreased the SWD number, whereas higher dose of Guo (i.p. 100 mg/kg) was found to enhance the number of SWDs in WAG/Rij rats. Combined i.p. injection of theophylline or SCH 58261 or indomethacin with 100 mg/kg Guo decreased the SWD number compared to i.p. 100 mg/kg Guo alone. The results suggest that i.p. 100 mg/kg Guo can increase SWD number by means of the adenosinergic system in WAG/Rij rats, which system may have a role in neuroinflammation- and age-evoked increase in SWD number. Consequently, we argue against the previously suggested usability of Guo as an effective drug for the treatment of absence epilepsy.

MECHANOSENSITIVE ION CHANNEL PATHWAYS ARE CRITICAL IN RETINAL GANGLION CELL DEGENERATION IN GLAUCOMA

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Purpose: Mechanical deformations of the eye can lead to neuronal damage, inflammation and may contribute to optic neuropathies. The molecular mechanisms that transduce strain into dysfunction of retinal neurons remain to be identified but are likely to involve activation of specialized mechanosensors. Our goal is to identify and characterize the molecular mechanisms that drive strain-dependent plasticity underlying the pathophysiology of retinal ganglion cells (RGC) in glaucoma.
Methods: Adult C57BL/6 mouse retinas from normal and elevated intraocular pressure (IOP) cohorts were used for in vivo experiments. Acutely dissociated and immunopanned (IP) retinal cells were used for in vitro studies. IP-Cells were stimulated with cyclic biaxial strain (3%; Flexcell stimulation) in the presence/absence of putative mechanosensitive ion channel inhibitors. mRNA and protein levels were measured with qRT-PCR and Western blots. Immunolabeling and Ca-imaging were applied to visualize and determine the changings in response to mechanical strain.
Results: Mechanical strain had profound, dose-dependent and time-dependent effects on transcript levels of multiple TRP channel isoforms, as well as Piezo1 & Piezo2 and 2-pore potassium (K2P) mRNAs. Stretch-evoked responses were reduced or absent in cells exposed to selective blockers or isolated from KO mice. Immunostaining and Western blots revealed upregulation of proapoptotic proteins, including calpains, caspases, and TRPV4 in glaucomatous retinas and in cultured RGCs exposed to biaxial stretch regimens. The number of caspase 3 positive cells in stretched IP-RGC was significantly increased, in a TRPV4-dependent manner, compared to the control. Consistent with changes in calcium homeostasis, RGCs responded to increased % radial stretch with dose-dependent increases in [Ca2+]i that were suppressed by pharmacological antagonists of TRP channels and mimicked by TRP agonists.
Conclusions: These data demonstrate that IOP elevation and mechanical strain regulate the expression of TRPV4 and downstream Ca2+-dependent mechanisms in RGCs. These mechanisms include prominent proapoptotic pathways (calpains, caspases) that have been linked to neurodegeneration in glaucoma. By identifying the mechanistic links between IOP, mechanical strain, calcium homeostasis, gene expression and cell death, our findings provide insight into the pathophysiological mechanisms associated with compromised RGC function and loss of viability in glaucoma.

BURSTING CHOLINERGIC NEURONS OF THE BASAL FOREBRAIN SHOW SYNCHRONOUS ACTIVITY IN AN AUDITORY DETECTION TASK

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The cholinergic basal forebrain (CBF) has been implicated in diverse cognitive functions through its influence on cortical information processing. Earlier studies differentiated tonic and phasic effects of the CBF based on the varying timescales of cortical acetylcholine release. In accordance, in vitro experiments described an early- and a late firing cholinergic group, likely corresponding to bursting and non-bursting neurons, proposing that bursting neurons convey phasic information while non-bursting neurons set tonic levels of cortical acetylcholine. However, this theory has not been tested in vivo, therefore it remains unclear how bursting and non-bursting groups of CBF contribute to cholinergic effects at different time scales. To address this question we analyzed optogenetically identified and putative cholinergic neurons in the nucleus basalis (NB) (n = 44) and in the horizontal limb of the diagonal band of broca (HDB) (n=12) in mice performing an auditory detection task requiring sustained attention. Our analysis of autocorrelations uncovered three types of cholinergic neurons: ‘tonic’ neurons showing long refractory periods, ‘phasic bursting’ neurons showing classical bursting phenotype and ‘phasic non-bursting’ neurons exhibiting short refractory but no prominent burst shoulders. By analyzing cross-correlations of concurrently recorded pairs of NB neurons (n = 16) we discovered that the bursting ones were synchronously active at a short timescale, unlike the tonically active cholinergic neurons. However, all three cell types were capable of fast and precise responses to behaviorally salient events, which clearly distinguished them from tonically active cholinergic interneurons of the striatum. Thus, bursts of cholinergic neurons likely reflect strong bottom-up excitatory drive that, with the added synchrony, leads to stronger and more widespread cortical activation.

GAPDH BINDER INHIBIT THE TOXICITY OF EXOGENOUS POLYQ-CONTAINING AGGREGATES

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Conformational neurodegenerative diseases, such as Alzheimer's disease, Huntington's disease (HD), amyotrophic lateral sclerosis etc., are increasingly associated with prion diseases due to the toxicity of proteins with incorrect conformation. In the process of neurodegeneration, such molecules can be found in the intercellular space and form toxic complexes involving other proteins, causing the death of neighboring neurons. The horizontal transfer of pathogenic protein complexes in neurodegenerative pathologies implies their appearance in the intercellular space. The mechanisms of the release of such complexes from cells can be very diverse. We assume that a massive release of aggregates is possible from cells dying due to the appearance in their cytoplasm and nucleus of mutant, aggregate prone proteins. In addition to the mutant proteins in the diffusion and development of neurodegenerative diseases often take part normal proteins that are necessary for cell physiology. One of these proteins is the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH). In this work, we demonstrated the possibility of inhibiting the cytotoxic effect of such aggregates through the use of specific drug that bind the GAPDH protein, a well-known participant of aggregation, in the intercellular space. Selected drug RX624 (hydrocortisone derivative) prevented the formation of GAPDH and polyQ containing aggregates in the growth medium of cells synthesizing a fragment of the huntingtin protein pathogenic form, reduced the death of the acceptor cells caused by incubation with medium containing toxic complexes. This approach allows specifically blocking the formation of exogenous pathogenic complexes without affecting cellular physiology.

THE ROLE OF OXYTOCIN AND DOPAMINE INTERACTION IN AMYGDALOID REINFORCING MECHANISMS

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Neuropeptide oxytocin (OT) is involved in social and non-social behavior. The central nucleus of the amygdala (CeA), part of the limbic system, plays an important role in learning, memory, anxiety and reinforcing mechanisms. CeA was shown to be rich in OT-receptors. Our previous findings indicated that in the rat CeA OT has a dose dependent positive reinforcing 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.
Male wistar rats were microinjected bilaterally with 10 ng OT (Sigma: O6379, injected in volume of 0.4 µl). In different group of animals 4 µg DA D2 receptor antagonist (sulpiride: Sigma: S7771 dissolved in sterile saline, injected in volume of 0.4 µl) was applied. Other animals received DA D2 receptor antagonist 15 min before 10 ng OT treatment or vehicle solution into the CeA.
Rats receiving 10 ng OT spent significantly longer time in the treatment quadrant during the test session. Prior treatment with DA D2 receptor antagonist blocked the effects of OT. Antagonist in itself did not influence the time rats spent on the treatment quadrant.
Our results show that in the rat CeA OT has positive reinforcing effects. DA system plays a role in positive reinforcing effects of OT because DA D2 receptor antagonist can block these actions.

The project has been supported by the European Union, co-financed by the European Social Fund (EFOP-3.6.1.-16-2016-00004) and Pécs University, Medical School (PTE ÁOK KA-2015-15). The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

ABHYDROLASE DOMAIN-CONTAINING PROTEIN 4 (ABHD4) IS A VENTRICULAR ZONE-RESTRICTED, PROAPOPTOTIC SERINE-HYDROLASE IN THE MOUSE EMBRYONIC CORTEX

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Cortical development is a very complex process involving a wide variety of signaling molecules and pathways, which fulfill distinct functions at different stages of embryonic maturation. Endocannabinoids, the endogenous ligands of the cannabinoid receptors have been recently implicated in various aspects of corticogenesis. N-arachidonoyl-ethanolamine (anandamide, AEA) was the first endocannabinoid to be discovered. Its presence in the embryonic forebrain and its importance in the regulation of several fundamental aspects of cortical development such as proliferation and neuroblast migration has already been well described. However, NAPE-PLD, the enzyme, which is generally considered as its primary synthesizing enzyme in the brain is not expressed until postnatal ages raising the intriguing possibility that AEA may be synthesized via an alternative synthesis route in the developing forebrain. Abhd4 is a recently discovered serine hydrolase, and it is involved in the metabolism of N-acyl phospholipids, including a potential lipid precursor of anandamide, but its biological function has remained elusive so far. Therefore, in the present study, we sought to explore the presence and function of Abhd4 in the developing neocortex. In situ hybridization demonstrated that Abhd4 shows a strong and highly restricted expression in the ventricular zone of the embryonic telencephalon, where the primary proliferating progenitors of the embryonic cortex called radial glia progenitor cells reside. This peculiar RGPC-restricted expression pattern was consistent in all neurogenic niches in the prenatal and early postnatal brain. Surprisingly, by using loss-of-function approach, we found that embryonic cortices obtained from Abhd4 knockout mice did not show any defect in the well known developmental processes generally associated with the function radial glia progenitor cells, such proliferation, neuroblast migration and cortical lamination. On the other hand, the immediate decay of Abhd4 mRNA in cells leaving the ventricular zone indicated that silencing of Abhd4 expression might be essential for proper cortical development. In accordance with this hypothesis, ectopic expression of wild-type Abhd4, but not of its hydrolase-dead mutant version triggered caspase-dependent cell death both in vitro and in vivo while also arresting neuroblast migration in the subventricular zone/intermediate zone area. These findings together with the highly restricted expression of Abhd4 in RGPCs, but silencing in migrating neuroblasts indicate that Abhd4 has an on-demand pro-apoptotic function which is not necessary during normal development but might be activated after insults to the cortical progenitors. (See also the accompanying poster by Lele et al., presenting that Abhd4 is an essential player for the apoptotic pathway initiated after adherens junction breakdown and maternal ethanol exposure during embryonic cortical development).

IMPACT OF MATRIX METALLOPROTEINASES ON LONG-TERM GABAERGIC SYNAPTIC PLASTICITY IN THE SCH-CA1 HIPPOCAMPAL PROJECTION

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Matrix metalloproteinases (MMPs) play an important role in excitatory synaptic transmission, learning and memory. Recently, we have shown that MMP-9 is involved in LTP induced by the spike timing-dependent plasticity paradigm in the barrel cortex of mouse (Lebida and Mozrzymas, 2016) and that MMP-3 activity supports plasticity via regulation of NMDARs function in stratum radiatum of CA1 hippocampal region (Brzdak et al., 2017). Besides glutamatergic synapses also inhibitory synapses exhibit several forms of long-term plasticity. However, contribution of MMPs on long-term GABAergic synaptic plasticity have not been investigated so far. To address this issue, we have considered both inhibitory long term potentiation (iLTP) and depression (iLTD) in acute hippocampal slices (P18-P21). We made an attempt to induce iLTP either by transient exposure to NMDA (3 min, 20μM) or by reverse spike timing protocol whereas iLTD was induced by forward pairing (in the presence of DNQX, Vh = -40 mV). We found that following NMDA treatment a stable increase in GABAergic mIPSCs took place indicating successful iLTP induction (mIPSC amplitudes potentiation 22±8%, n = 7). In the case of the reverse pairing, a stable iLTP was observed only in a fraction of recordings while in remaining experiments an opposite or no effect were observed. This variability was probably due to recruitment of different sets of interneurons innervating pyramidal cells from which recordings were made. We found that in the presence of bath applied metalloptroteinases inhibitor, FN-439 (180μm), transient NMDA application initially induced an increase in mIPSC amplitudes but this effect was not stable and mIPSC amplitudes returned within up to ten minutes to the baseline level (in FN-439: 98±6%, n = 7 at 22-24 min after NMDA application $p<0.05$ in comparison to control). On the contrary, iLTD induced by reverse pairing was resistant to MMPs inhibitor (CTR 70±6%, n = 15, FN-439 67±5%, n = 13 $p>0.05$). Thus in Sch-CA1 hippocampal projection i-LTD does not depend on the activity of MMPs. In conclusion, we provide the first evidence for involvement of extracellular proteolysis in the GABAergic plasticity.

Supported by Polish National Science Centre grant OPUS/2014/15/B/NZ4/01689.

C-FOS-DRIVEN CENTRAL AMYGDALA MEDIATED MODULATION OF APPETITIVE BEHAVIOR

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The central nucleus of the amygdala (CeA) has primarily been studied as a structure involved in processing of aversive behaviors, whereas its role in appetitively-motivated learning is much less understood. The published data show involvement of the basolateral amygdala (BL), which sends its projections to the CeA, in encoding sensory-specific features during appetitive learning. In contrast, the CeA was implicated in modulation of incentive motivation to pursue an associated external reward. Previously we reported that after appetitive, but not aversive learning, expression of c-Fos, a component of AP-1 transcription factor, is significantly increased in the CeA. Since c-Fos activation is closely linked to synaptic plasticity, learning and memory, we hypothesized that appetitive learning depends on c-Fos expressing neural circuits in the CeA. To test this hypothesis we first compared c-Fos expression pattern in the amygdala following place preference and place avoidance training and examined inputs from the BL on the activated CeA neurons. The c-Fos expression in the CeA was significantly higher following place preference than place avoidance training, with over 90% of the c-Fos positive cells receiving projections from the BL. Then, to test the role of c-Fos expressing CeA circuits in appetitive motivation we optogenetically activated neurons involved in reward learning. We used *c-fos*-driven targeting of channelrhodopsin and trained the animals in an operant conditioning task, in which they learned to associate auditory stimulus with food reinforcement. Optogenetic stimulation of the neurons increased bar-pressing responses but only when the conditioned stimulus was present, suggesting that *c-fos*-expressing neurons are involved in modulation of incentive motivation. To further test the role of *c-fos*-expressing neurons in appetitive learning we locally blocked behaviorally-induced *c-fos* expression using RNAi-based approach, that is, the delivery of a short-hairpin (sh) RNA in a lentiviral vector. This resulted in impairment of appetitively (sweetened water vs tap water) but not aversively (quinine-adultered water vs tap water) motivated discrimination learning and significantly decreased motivation to seek reward. In contrast, blocking *c-fos* expression did not affect reward consumption. Taken together, the results reveal that *c-fos* expression in the CeA neurons is necessary for appetitively but not aversively motivated learning, modulating approach motivation but not reward consumption.

MATERNAL ETHANOL EXPOSURE AND ADHERENS JUNCTION DAMAGE LEADS TO ABHD4-DEPENDENT CELL DEATH IN THE MOUSE EMBRYONIC CORTEX

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Radial glial progenitor cells (RGPCs), the main neural stem cell type of the developing pallial cortex generally divide asymmetrically producing an RGPC and a daughter neuroblast. Under healthy conditions, the stem cell remains embedded into its native tissue environment via numerous types of cell-cell and cell-extracellular matrix connections. In contrast, the neuroblast cell must delaminate and move towards the cortical plate, which requires the breaking of its cadherin-based adherens junctions and laminin-integrin contacts. It is well known that pathological conditions such as congenital malformations, intracerebral hemorrhages or even maternal alcohol abuse severely disrupt adherens junctions and leads to migration defects in the embryonic neocortex. It is conceivable to hypothesize that abnormally detached and still proliferating RGPCs should be eliminated to avoid cortical dysplasias or even tumor formation. Conversely, healthy neuroblasts should escape from this postulated cell death programme and continue their radial migration. In the present study, we tested this hypothesis by exploiting in utero ventricular electroporation of dominant-negative form of N-cadherin (DnCdh2) and by using two mouse models of the fetal alcohol syndrome. We found that DnCdh2-electroporation disintegrated the normally compact ventricular zone and resulted in a massive ectopical dislocation of Pax6-positive RGPCs in the subventricular zone. Moreover, the disruption of adherens junctions and maternal ethanol exposure both elicited caspase-dependent cell death in the embryonic neocortex. To delineate the molecular mechanisms responsible for the "dead-or-alive" cell fate decision during abnormal or normal detachment of RGPCs or their daughter cells, respectively, we investigated the role of Abhd4, a ventricular zone-restricted pro-apoptotic serine-hydrolase, as a novel instigator of cell-death in the developing neocortex (see the accompanying poster by László et al). Notably, we found that ectopic expression of Abhd4 in migrating neuroblasts triggered a migration halt and eventually lead to caspase-dependent cell death. In contrast, neither in utero electroporation of DnCdh2 nor maternal ethanol exposure could evoke cell death in Abhd4-/- embryonic cortices, whereas cell death was rescued by expression of Abhd4 in the Abhd4-knockout animals. Taken together, our results demonstrate that Abhd4 is both sufficient and necessary as an important „guardian" protein executing protective cell death events upon pathological disruption of radial glia progenitor cells in the developing neocortex.

INSULIN-LIKE GROWTH FACTOR I AND ITS BINDING PROTEIN-3 ARE REGULATORS OF LACTATION AND MATERNAL RESPONSIVENESS

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Adaptation to motherhood includes maternal behaviour and lactation during the postpartum period. The major organizing centres of maternal behaviour and lactation are located in the hypothalamic medial preoptic area (MPOA) and the arcuate nucleus, respectively. Insulin-like growth factor I (IGF-I) is an effector of the growth hormone axis; however, its function in the brain is largely unexplored. We identified increased maternal IGF binding protein-3 (IGFBP-3) expression in preoptic rat microarray data and confirmed it by RT-PCR. *In situ* hybridization histochemistry showed markedly elevated IGFBP-3 expression in the MPOA and the arcuate nucleus in rat dams. Prolonged intracerebroventricular injection of IGF-I or antagonism of brain IGFBP-3 with an inhibitor (NBI-31772) using osmotic minipumps increased pup retrieval time, suggesting reduced maternal motivation. Suckling-induced prolactin release and pup weight gain were also suppressed by IGF-I, suggesting reduced lactation. In addition, IGF-I-induced tyrosine hydroxylase expression and its specific phosphorylation in tuberoinfundibular dopaminergic neurons suppress prolactin secretion. Thus, IGF-I may inhibit both behavioural and lactational alterations in mothers. Neurons in the MPOA and arcuate nuclei express IGFBP-3 during the postpartum period to neutralize IGF-I effects. IGFBP-3 can prevent the blockade of maternal behaviour and lactation exerted by IGF-I, suggesting a novel modulatory mechanism underlying the behavioural and hormonal effects during central maternal adaptations.

LIFE-WORK OF ENDRE GRASTYÁN AND ITS IMPACT ON PHYSIOLOGY, BEHAVIORAL NEUROSCIENCE AND PSYCHOLOGY.

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Endre Grastyán was born in 1924 in a small town, Őriszentpéter, close to the Western border of Hungary. He died in Pécs, 1988. He conducted his university studies in the Medical University of Pécs. Impressed by the captivating lectures of his physiology professor, Kálmán Lissák, he decided to direct toward a theoretical career, and as a student joined to the teaching and research team of the Department of Physiology. After graduation he received an appointment to the Department of Physiology and after the retirement of Kálmán Lissák in 1976, Grastyán succeeded him in the chair. In 1983 he became a member of the Hungarian Academy of Sciences.
Two years after the discovery of the brain stem reticular formation by Magoun and Morizzi, Grastyán showed that in addition to the external stimuli, the active state of this system is substantially influenced by the vegetative afferent inflow. This observation turned his attention to the role played by central activation and inhibition in the control of motivated behavior. In the cat he discovered that initial to building an instrumental conditioned response first a theta activity could be recorded from the hippocampus and it was followed by desynchronization. He recognized a closed correlation between the orientation response and theta activity in the early phase of learning. This finding brought a world-wide appreciation to the 35 year old researcher and the publications on this subject are still cited in the literature. Based on his experiments on the cat he built up a new learning theory which was capable to explain the significance of reinforcement in conditioning. He postulated two functional states of a self-sustained complex system, namely the nervous substrate capable of controlling either rewarding, or punishing effects. In his motivation and learning theory inhibition played a positive role (i.e. active inhibition), whereas activation was identical with the moment of release from under inhibition. In connection with the function of the hippocampus and memory formation, he was the first to recognize the significance of the feed-forward inhibitory control mechanism. By spatial separation of the conditioning signal from the reward, he was able to describe an orientation behavior toward the conditioned signal and this indicated that the orientation behavior was one of the key events of learning. His creative experimental analysis and related theories of motivation, learning and emotion are classics; they are parts of textbooks, and important materials in physiology and psychology courses. His last project was to study of play. With this project he had returned to the heart's desire of his youth: to explain the "Homo ludens". The inauguration presentation to his academy membership was „The Neurobiology of Play". He said that the basis of research was fundamentally a playing activity driven by the unknown.

EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

ULK4 DEFICIENCY LEADS TO HYPOMYELINATION

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Brain nerve fibres are myelinated by oligodendrocytes. Key transcription factors for oligodendrogenesis have been identified, however little is known about factors regulating their expression. Hypomyelination are increasingly recognized as a pathology underlying neuropsychiatric/neurodevelopmental disorders, but not much is known about factors causing both phenotypes. Here we report that *Unc-51-like kinase 4 (Ulk4)*, a rare susceptibility gene for neuropsychiatric and neurodevelopmental disorders, acts as a regulator of myelination. A hypomorph Ulk4tm1a/tm1a mutation significantly down-regulates oligodendrogenic transcription factors of *Olig2, Olig1, Myrf, Sox10, Sox17* and *Sirt2*, and reduces myelination by half. Stage-specific factors including Sox17, Mbp and Mog are remarkably down-regulated, suggesting a direct reduction of oligodendrogenesis. Secondary effects of axons are also likely to contribute to the hypomyelination, as axonogenesis and white matter integrity are impaired and reactivated astrocytes and microglia elevated in *Ulk4* mutants. ULK4 may therefore become a novel therapeutic target for white matter diseases associated with hypomyelination.

STUDIES ON THE ROLE OF THE CASR IN HUMAN IPSC-DERIVED NEURONAL CELLS

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In addition to its primary role in maintaining the systemic calcium homeostasis by regulating the parathyroid hormone release, much evidence has shown that the calcium sensing receptor, CaSR, is an important factor in the development and function of the central nervous system (CNS). In fact, several animal studies report the CaSR being involved in the proliferation and maturation of neural progenitor cells (NPCs) into neurons and glial cells, as well as in the regulation of ion channel activities important for neurotransmission (Bandyopadhyay et al., 2010). Interestingly, recent work also suggest that the receptor might be involved in Alzheimer's disease (AD), the most common cause of dementia which is characterized by the extracellular accumulation of Aβ peptide, pathologically cleaved from the amyloid precursor protein (APP), intracellular neurofibrillary tangles of hyperphosphorilated TAU protein and Ca2+ dyshomeostasis. According to this hypothesis, Aβ peptide binds to neurons' and astrocytes' CaSR and activating a set of intracellular signaling pathways which upkeep Aβ intracellular accumulation and oversecretion (Armato et al., 2013). In this context, the difficulty of modelling the complex mechanisms of the human nervous system's development as well as pathogenic aspects of neurological disorders in animals, makes the induced pluripotent stem cell (iPSC) technology a relevant tool for this purpose. In fact iPSCs generated from healthy and neurological disease-affected donors can be differentiated in any kind of cells of human body, like neurons, providing a practical way of studying the human neurodevelopmental events and an opportunity to better model the given disease in a Petri dish. In our study, we differentiated neurons and astrocytes from iPSCs-derived neural progenitor cells of AD patients and healthy controls. Moreover, the expression of APP, BACE1 and TAU protein has been investigated. We showed that the key steps of APP processing are recapitulated in these neuronal cultures. The CaSR expression has been assessed by comparing the receptor's protein level of neurons to that of SH-SY5Y cells transiently transfected with hCaSR cDNA and to that of human kidney lysate. We also tested some positive and negative CaSR modulators, (e.g. R 568 hydrochloride; NPS 2143 hydrochloride), in order to evaluate the effects of the receptor's modulation in the derived neuronal cultures. To the best of our knowledge this is the first study investigating the CaSR in neurons and astrocytes generated from human iPSCs derived CNS cells.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 675228 (CaSR Biomedicine).

HYPOTHERMIA AFFECTS THE TISSUE INTEGRITY AND FUNCTIONAL RECOVERY IN COMPUTER-CONTROLLED SPINAL CORD INJURY MODEL IN MINIPIG

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The efficient hypothermic therapies need to be carried out in well-designed preclinical SCI models closely resembling the most situations after SCI in men. We focused on 1) characterization of a preclinical minipig model of SCI at lumbar level using computer-controlled compression apparatus, and 2) the optimalization of local cooling of the spinal cord at the site of injury. Hypothermia was applied locally up to 30 min after SCI (for 5 hours) via perfusion chamber with 4°C saline solution. The animals were behaviorally assessed during 9 weeks of survival (scoring points 0-20). In the area of SCI, local cooling of spinal cord down to 19 ºC was shown to decrease blood flow by ~35% comparing with control. The computer-controlled compression device produced graded contusion lesions at L3 level with three different degrees of tissue preservation and functional recovery, depending upon pre-set impact parameters. Nine weeks after SCI there was a large contusion lesion at the epicenter, with 47, 67 and 79 % reduction of the gray matter after 8N-, 15N- or 18N- force impact. We have found that saline hypothermia leads to a tissue sparing, and to substantial sparing of neurofilaments in spinal cord segments that are likely to be affected by secondary injury (cranially +3, +2, +1 and caudally -1, -2, -3) from the lesion site. Early and intense local hypothermia increased the gray and white matter preservation at the lesion site in 8N SCI group (by 11% in gray matter and 15%, 7% and 4% in the dorsal, lateral and ventral columns respectively). Major increase in NF-IR profiles was detected after saline hypothermia in lateral columns in the +1 and -1 segments, a finding which strongly correlates with significant white matter preservation. Our findings confirm the strong correlation between neurological outcome, the extent of tissue preservation within the immediate rostro-caudal (+1 and -1) segments, and preservation of neurofilaments, almost excusively found in myelinated axons. It seems that this improvement was essential for modulation of the key spinal microcircuits leading to better functional outcome. No functional improvements were observed after trauma in the group subjected to 15N or 18N SCI. Saline solution administered over the lesion site in the 15N SCI significantly preserved the gray and white matter and protected neurofilaments in the dorsal and lateral columns, however the enhancement of tissue integrity was seen only in caudal segments. In 18N SCI group the hypothermic treatment increased the number of neurofilaments in all columns in comparison to SCI group, however, the improvement was significant only in lateral columns (+1 segment) and in ventral columns (+1 and -3 segments). The results indicate that local saline hypothermia (4°C) applied after SCI for 5 hours could be a promising therapeutic method when combined with clinically-proven surgical and medical treatments for SCI.

Supported by OP VV EÚ, ITMS: 26220220127 and VEGA 2/0168.

CONFORMATION-SPECIFIC COVALENT BINDING OF AZIDO-RILUZOLE TO SODIUM CHANNELS

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The effect of sodium channel inhibitors are known to strongly depend on the conformational state of the channel. However, it is not known whether state-dependent action is due to state-dependent affinity or state-dependent accessibility, whether inhibition is due to channel block or channel modulation, and how drug onset and offset rates relate to conformational transition rates.
In this study we used azido-riluzole, a photoreactive analog of the neuroprotective sodium channel inhibitor riluzole. Upon UV-irradiation this compound is activated and binds covalently to its binding site. In whole-cell patch clamp experiments we synchronized voltage pulses with UV light pulses, and evoked covalent binding in different conformational states of the channel: Resting (UV pulses applied at -150 mV holding potential), inactivated (UV pulses at -10 mV holding potential), and half-inactivated (UV pulses at the $V_{1/2}$ of the steady-state availability curve, where approximately half of the channels are in pre-open-closed state, and the other half is inactivated).
Interestingly, the onset rate of inhibition did not differ significantly, suggesting that accessibility of the binding site was similar in all three conformations. In addition, differences in apparent affinity were much smaller than between resting and inactivated state affinities for either riluzole or azido-riluzole without UV-illumination. No significant difference was found in the extent of modulation either, all three treatment caused similar delay in recovery from inactivated state.
These data indicate that the significant state-dependence of azido-riluzole is not due to its presence *vs.* absence at different conformations of the channel but rather to the orientation of the molecule within the binding site.

ALLOSTERIC A2A-D2 RECEPTOR-RECEPTOR INTERACTIONS IN REWARD BEHAVIOR

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Cocaine, like other addictive drugs, produces a high by *overstimulating* the brain's r*eward* system. Cocaine reward results from the activation of the mesolimbic dopamine (DA) system including DA neuron projections from ventral tegmental area to the nucleus accumbens (NAc) and prefrontal cortex (PFc) [1]. Recent findings highlight an antagonistic interaction between *adenosine* $(A)_{2A}$ receptors and DA D_2 receptors which may have a role to alter cocaine reward [2-3].
The *aim* of the *present study* was to determine *the effects* of the selective A_{2A} receptor agonist and antagonists after their systemic (i.p.) or local (intra-NAc or intra-PFc) injections on intravenous cocaine self-administration in rats. Furthermore, we verified if the postsynaptically localized A_{2A}-DA D_2 receptor heterocomplexes in the NAc are engaged in control over cocaine reward with local microinjection of the transmembrane (TM5) peptide that directly disrupts the membrane association of the above receptor complexes.
Our data indicate that A_{2A} receptors do not exert a constitutive role to control cocaine reward while A_{2A} receptor agonism reduces such behavioral action of cocaine. With using local microinjection technique, we found that the A_{2A} receptor agonist-induced reduction towards cocaine reward depends on stimulation of A_{2A} receptors localized in the NAc, but not in the PFc. Finally, intra-NAc injections of TM5 reverses the A_{2A} receptor agonist-induced inhibition on cocaine reward.
Our results indicate that pharmacological stimulation A_{2A} receptors in the NAc plays a inhibitory control over cocaine rewarding properties. For the first time we show the importance for the A_{2A}-DA D_2 receptor heterodimeric complexes in cocaine reward. As such, A_{2A} receptor agonists may be considered as a new strategy to treat cocaine abuse.

Supported by the grants from the National Science Centre (Poland; 2011/03/N/NZ7/06294), the Vetenskapsrådetgrant (Sweden; 2016-01007) and by statutory funds of the Institute of Pharmacology PAS (Kraków, Poland).

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HOW DOES MATERNAL SMOKING INFLUENCE THE NEUROBEHAVIORAL DEVELOPMENT OF RAT PUPS?

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Numerous studies indicate that smoking during pregnancy may have harmful effects on the newborns. Perinatal smoking can affect fetal development, resulting in delayed cognitive and motor development, retarded locomotor behavior, and an increase in the incidence of psychiatric abnormalities. The aim of the present study was to investigate the influence of maternal smoking during pregnancy on the early physical and neurobehavioral development of newborn rats.
Wistar rats were exposed to whole-body smoke exposure for 2x40 minutes daily from the mating until delivery. For the treatment, TE2 manual closed-chamber smoking system and 2 3R4F research cigarettes per occassion were used. The control group was not exposed to smoke. After delivery animals were cross-fostered. Then the offspring were tested for somatic (eyeopening, incisor eruption, ear unfold) and neurobehavioral development (ear twitch, eyelid reflex, forelimb and hindlimb placing and grasping reflexes, auditory startle reflex, disappearance of crossed-extensor reflex, air righting, gait, negative geotaxis) daily for the first 3 weeks of life. Weight was measured daily.
Motor coordination, grid walking, foot-fault tests and open field activity were also examined. Data were compared to those of the control group by using one-wayANOVA as the statistical method.
Results show that certain physical reflexes of prenatally smoking pups (eyelid and ear twitch reflex) appeared later compared to the control group. We also observed a delay in reflexes indicating the neural maturity - hind limb grasping, fore-and hind limb placing reflexes - in the group of prenatally smoking pups. Although there was no difference between birth weights, the smoker pups gained more weight until the end of our observation period. Second-hand smoker pups moved less in the motor coordination test, and made fewer foot faults compared to the non-smokers. We did not show any considerable differences in novelty seeking activity. This study suggests that maternal smoking during pregnancy has early detectable effects on the neurological development of the rat pups which may indicate the future vulnerability of these individuals.
Although it is well-known that smoking during pregnancy has short and long-term effects on the offspring, but it has not been known if there is an early effect on the neurobehavioral development. In our animal experiment we showed an early delay in some developmental signs. This work may suggest to pay more attention to those individuals who were affected by cigarette smoke during intrauterine life.

Supported by GINOP-2.3.2-15- 2016-00050 “PEPSYS”, the National Research, Development and Innovation Fund K119759, MTA-TKI, PTE AOK KA Research Grant, Bolyai Scholarship, National Brain Research Program (KTIA_13_NAP-A-III/5), TAMOP 4.2.4.A/2-11-1-2012-0001 „National Excellence Program”, The UNKP-16-3-IV, UNKP-16-4-III, UNKP-16-4-IV, New National Excellence Program of the Ministry of Human Capacites, and Centre for Neuroscience, University of Pecs, EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

PROTECTIVE EFFECTS OF PACAP AGAINST THE COMBINED TOXICITY INDUCED BY ALCOHOL AND NICOTINE IN SH-SY5Y CELLS

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is an endogenous 38 amino acid containing neuropeptide with various cytoprotective functions including neuroprotection. Thus, in-vitro and in-vivo studies have provided substantial evidence of PACAP protection against neuronal injury related to ischemia, trauma and various endogenous and exogenous toxic agents. We have earlier reported that exposure of neuroblastoma-derived SH-SY5Y cells to higher concentrations of ethanol results in significant cell loss and that pre-exposure to PACAP can protect against this toxicity. It is well established in both in-vitro and in-vivo studies that nicotine in low concentrations offers neuroprotection. However, it is interesting to note that, based on our experiments, a combination of even low levels of alcohol and nicotine resulted in significant cell loss in SH-SY5Y cells. Treatment of SH-SY5Y cells with a combination of ethanol & nicotine even in low concentrations resulted in a concentration-dependent toxicity where significant cell loss was seen even in low concentrations of the mixture (as low as ethanol 1mM + nicotine 0.1uM). In this study we sought to determine whether PACAP might also protect against the combined toxicity of alcohol & nicotine in these cells and elucidate possible underlying mechanism(s). Pretreatment with PACAP resulted in a dose-dependent reduction in ethanol & nicotine induced toxicity where at 100nM, PACAP completely blocked ethanol & nicotine's maximal effect. The effects of PACAP in turn, were inhibited by PACAP antagonist (PACAP 6-38) where full block was achieved with 1uM antagonist. Cell flow cytometry indicated that the major toxicity by the combination of ethanol & nicotine was apoptotically mediated and that PACAP could block this process. Taken together, these data indicate that PACAP or PAC1 receptor agonist, a major site of PACAP action, could be of therapeutic potential in alcohol & nicotine induced neurotoxicity.

Supports: KTIA_13_NAP-A-III/5, GINOP- 2.3.2-15- 2016-00050 "PEPSYS", OTKA K10498, NKFIH K119759, PTE AOK Research Grant No. KA-2017-01, TAMOP 4.2.4.A/2-11-1-2012-0001 „National Excellence Program", UNKP-16-4-IV New National Excellence Program, EFOP-3.6.1.-16-2016-00004, MTA TKI 14016 Program, Bolyai Scholarship. The present scientific contribution is dedicated to the 650th anniversary of the foundation of the University of Pecs, Hungary. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

BRAIN MICROVASCULAR INFLAMMATION INDUCED BY AMPHETAMINE INVOLVES ANGIOTENSIN II AT1 RECEPTORS ACTIVITY

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An increased expression of Angiotensin II AT1 receptors (AT1-R) in brain microvessels have been described under certain pathological conditions. These receptors are the main effector of a locally acting renin-angiotensin system in brain vasculature involved in the regulation of oxidative stress conditions and inflammatory responses. AT1-R blockers are known for their safety use in hypertension treatment and for their anti-inflammatory effects by normalization of adhesion molecules, cytokines levels and pro-inflammatory transcription factors. On the other hand, the heat shock protein (HSP) family displays anti-apoptotic effect after noxious stimulus exposure. Particularly, HSP70 is over-expressed after its transcriptional factor activation by increased oxidative stress. Psychostimulants, such as amphetamines (Amph), promote enduring alteration over neural circuits that can be observed long after a challenge administration (drug/stress). Their consumption has been associated with brain vascular alterations and inflammatory conditions in cortical brain areas. To this respect we have observed ICAM-1 and AT1-R increased expression in medial cerebral artery one week after repeated exposure to Amph. The present work was aimed to identify brain microvascular inflammatory alterations after an Amph sensitization protocol and to elucidate the possible role for AT1-R in the onset of these alterations.
Male Wistar rats (250-300g) were used. To evaluate the long-term Amph effects over brain vasculature, the animals received a daily Amph (2.5mg/kg, i.p.) injection for 5 days. To study the participation of AT1-R in long-term Amph effects, the AT1-R blocker Candesartan (CV 3mg/kg p.o.) was administered for 5 days prior the repeated Amph administration. The inflammatory markers were evaluated after 1 week of withdrawal in basal conditions or evidenced by a challenge exposure (Amph 0.5mg/kg i.p./cold exposure 4ºC 4hs). Brain microvessels were isolated by sucrose gradient and then immunofluorescence was performed against AT1-R and HSP70.
Amph exposure induced a long lasting increase in AT1-R expression at basal conditions. This increase was prevented by previous CV administration. When the animals received a cold exposure challenge elevated levels of AT1-R in Amph-treated animals were still evident. Meanwhile, after an Amph challenge, the AT1-R expression was exacerbated in all of the experimental groups. Regarding HSP70, there were no differences in its expression at basal conditions. In Amph- treated animals a sensitized HSP70 expression was observed when receiving either an Amph or a cold exposure challenge. Previous AT1-R blockade prevented the development of the sensitized response induced by Amph.
The present results show that Amph has long-lasting inflammatory effects over brain microvasculature, evidenced as increased AT1-R expression and sensitized response of HSP70. The development of Amph-induced microvascular inflammation involved AT1-R activation.

FRACTAL PROPERTY OF MAINTAINED SPIKING ACTIVITY IN PARVOCELLULAR, MAGNOCELLULAR AND KONIOCELLULAR CELLS OF MARMOSET LATERAL GENICULATE NUCLEUS.

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Purpose: Maintained activity in macaque retinal ganglion cells shows Poisson-like renewal statistics at sub-second timescales (Troy & Lee, Vis. Nsci., 1994), but at multi-second timescales, activity shows fractal (self-similar) characteristic in cat lateral geniculate nucleus (LGN) (Teich et al., J. Opt. Soc. Am, 1997). Here we analyse the dynamics of spiking activity across long and short timescales in marmoset LGN neurons, specifically comparing parvocellular (P), magnocellular (M) and koniocellular (K) populations.
Method: Extracellular action potentials of visually-responsive cells were recorded in sufentanil-anaesthetised marmosets using a Neuronexus (16x2) silicon array probe (n = 49) or conventional single-cell recording (n = 105). The visual stimulus was a uniform grey 20 deg. field, intensity ~50 cd/m2. The instantaneous firing rate and variability relative to Poisson process (Fano factor) over time windows between 0.1 s and 100 s were calculated.
Results: The Fano factor of most parvocellular (P, n =36/53), magnocellular (M, n= 21/30) but only minority of koniocellular (K, n=28/71) neurons is close to unity for time windows less than 2 s (i.e. Poisson-like property) but rises monotonically with window width for time windows > 10 s (scale-free/Fractal property). Transition from Poisson-like to Fractal behaviour occurs at shorter time windows for K cells (8.62 s, SD 23.08) than for P (21.9 s, SD 36.52) and M cells (10.01 s, SD 21.10, $p < 0.01$, Kruskal-Wallis test).
Conclusion: Maintained firing activity of K LGN neurons exhibits scale-free property over time windows relevant to active vision, whereas activity of P and M cells does not. This result raises the possibility that K pathway selectively contributes to scale-free (self-organised criticality) properties in lerge-scale brain networks.

IMPACT OF ANGIOGENESIS INHIBITION ON POSTNATAL NEUROGENESIS

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New neurons are continuously added to the olfactory bulb (OB) throughout the life of mammals. Postnatal neurogenesis is a multistep process that includes proliferation, migration, differentiation and integration of neurons into the circuits. Neuronal precursors destined for the OB arise in the subventricular zone and migrate for a long distances along the rostral migratory stream (RMS). In the RMS, neuroblasts move along each other thus forming chains that are surrounded by astrocytes. Moreover, neuronal precursors are able to divide while migrating. Recently, the role of blood vessels in navigation of neuroblasts toward the OB has been shown. In addition to their trophic function, specifically arranged blood vessels serve as a physical support for migrating neuroblasts as well as produce migration promoting cues. We have focused on the examination of an importance of specific vascular arrangement for migration of neuroblasts. The aim of this study was to examine an impact of angiogenesis inhibition on reorganization of the RMS blood vessels to the migratory scaffold as well as on the migration and proliferation of neuroblasts within the pathway. Rats were administered an inhibitor of angiogenesis - endostatin during the first postnatal week and then they survived till postnatal day 14 (P14) or till adulthood. We have found that the inhibition of angiogenesis, during the critical period when the vasculature scaffold in the RMS is establishing, has influenced the blood vessels density in the RMS of P14 rats and adult rats and has prevented blood vessels from a rearrangement to the proper scaffold structure. This resulted in disorganization of neuroblasts migration in the RMS - they migrated untypically, rather individually than in chains. Moreover, in the RMS rostral parts, neuroblasts migrated out of the migratory pathway. In addition to migration, proliferation of neuroblasts was also affected. The quantification of proliferating cells has shown that the inhibition of angiogenesis caused an increase of the number of proliferating cells. We suppose that this increase might be caused by accumulation of proliferating cells in the RMS due to the disorganized RMS vasculature and impaired neuroblasts migration. We can conclude that manipulation with the angiogenesis during the early postnatal development caused the disruption of neurogenic processes in the RMS. By this study we proved that in the RMS, not only the presence of blood vessels itself but their specific arrangement into the vascular scaffold is necessary for proper functioning of postnatal neurogenesis.

Supported by VEGA grants 2/0159/17, 2/0069/15 and grant APVV-15-0239

BEHAVIORAL INVESTIGATION OF THE EFFECT OF TETANUS NEUROTOXIN ON SENSORY TRANSMISSION IN THE RAT FACIAL REGION

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Introduction: Tetanus neurotoxin (TeNT) employs axonal transport within peripheral cholinergic neurons and transcytosis to disrupt inhibitory transmission in the CNS, which induces muscular spasticity and autonomic instability. A similar clostridial toxin botulinum neurotoxin type A (BoNT/A), prevents pain transmission and is clinically used for chronic migraine. Although some in vivo studies reperted that chimeric combinations of TeNT and BoNT/A domains may produce analgesic effect at high doses, the in vivo effect of TeNT alone has not been examined so far. Thus, here we investigated the potential effect of TeNT on acute mechanical thresholds and acute inflammatory pain in the rat orofacial region innervated dominantly by the trigeminal sensory nerve.
Experimental procedure: Low local dose of TeNT (1 ng) was injected into the rat whisker pad or into the trigeminal ganglion via infraorbital foramen. Five days following the TeNT injections, mechanical sensitivity of the orofacial region was assessed by Von Frey filaments. Further, the effect of TeNT on nociceptive transmission was assessed in inflammatory pain evoked by 2.5% formalin injection into the whisker pad.
Results: TeNT injection into the whisker pad induced the local spastic paralyisis of whisker movement, while the intraganglionic injection did not alter the facial whisker movement. Low dose TeNT injected into the rat orofacial region or the trigeminal ganglion did not affect acute mechanical thresholds measured by Von Frey filaments. Duration of the nocifensive behavior evoked by formalin (facial rubbing and grooming) was not altered by either facial or intraganglionic TeNT.
Conclusion: Present results indicate that TeNT does not exert any analgesic or hyperalgesic effects in the sensory nociceptive system at doses which exert local spasticity. The data do not support significant effect of TeNT on sensory transmission within primary sensory neurons, or toxin transcytosis into central sensory regions. In conclusion, the data suggest lower affinity of TeNT for sensory neurons in comparison to BoNT/A.

Supported by Croatian Science Foundation (HRZZ), project no. IP-2014-09-4503

HEMISPHERIC SPECIALIZATION IN SEMANTIC PROCESSING AND ITS RELATIONSHIP WITH HIGHER COGNITIVE LOAD

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Previous studies have already demonstrated a right visual hemifield (RVF) advantage in semantic information processing (i.e. left hemispheric specialization). We tested whether the same effect is found when dual-task performance is required. We designed a semantic categorization task combined with a divided visual field paradigm in two task conditions: single-task and dual-task conditions. In the single-task condition, participants (N = 16) were presented visually with words in two types of semantic category: living and non-living objects. They performed the task under monocular viewing condition and were asked to make a semantic categorization in each trial. In the dual-task condition, simultaneously with the semantic categorization of the visual stimuli, participants were asked to memorize auditory information (words). We predicted to find that participants (N = 16) categorize semantic information more easily when semantic stimuli are projected to RVF. Second, considering the higher cognitive load under the dual-task condition, we assumed a higher advantage of RVF presentation in the dual-task condition than in the single-task condition. Third, we investigated the pattern of change in participants' performance as a function of time spent with the task (i.e. Time-on-Task effect). Moreover, we also analyzed the effect of eye dominance. The results revealed more accurate and faster performance in RVF but showed no difference between the single-, and dual-task conditions. However, RVF advantage seems to increase as a function of Time-on-Task in the dual-task condition (i.e.: the left hemisphere's advantage in semantic processing is greater in the dual-task condition). A possible explanation of this obtained result is that the cognitive control over hemispheric inhibition of right hemispheric contributions to semantic processing under higher cognitive load is impaired. In addition, the performance was better, when the participants saw the target with their dominant eye. This suggests that the dominant eye dominates not only the ocular movements but also the perceptual recognition of the visual stimuli, because no eye movements were required during the task.

Source of Funding: Hungarian Scientific Research Fund (OTKA K-120012).

PRENATAL EXPOSURE TO MARIJUANA ALTERS BEHAVIOR IN MICE OFFSPRING

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Marijuana is widely used by women during pregnancy. Frequently, mothers-to-be presume that marijuana has no effects on fetal progress, though it is known that marijuana exposure may cause adverse outcomes on fetus, including behavioral and neurological disorders that eventually may be manifested lifelong. Moreover, the presence of various other chemicals in its smoke may mediate some effects observed on offspring. Therefore, in this study we have evaluated how maternal marijuana exposure, via inhalation at a low dose (0.03% Δ^9-THC), could imply behavioral differences between groups (control and cannabis) during the childhood and adulthood period of offspring.Ten pregnant mice 60 day-old were exposed to marijuana smoke [0.2 g of *Cannabis*] (Cannabis group-CA; n=5) or filtered air (FA group; n=5) in the exposure system from 5.5 to 17.5 GD during 5 min daily. The dams were allowed to give birth naturally and the pups were randomly assessed (4 animals per litter) for muscle strength, motor coordinator and reflex, respectively by the following the tests: Hind- limb suspension (PND 2, 4, 6, 8, and 10), Surface righting (PND 4-6) and Negative geotaxis (PND7-11). At the age of weaning (PND 21) the pups were separated from their mothers and groups of males and females were formed. Two male e two females from each litter were assessed when juveniles (PND 30-33) and reassessed when adults (PND 60-63). The animals were evaluated by the Open-field (locomotors activity); Elevated Maze (anxiety) and Object recognition tests (memory). All animals were weighed weekly from PND 1 to PND 60. Statistical analyses were performed using SPSS software, version 17.0 and applying the significance level of p=0.05. The results revealed that FA group animals gained significantlymore weight than Cannabis animals. Females had significantly less weight increase than males from the fourth week on of their lives. Neonatal behavioral tests showed more muscle weakness on CA group animals (PND10). Overall, animals from CA group have made more attempts to lift their bodies using their hind limb muscles. The negative geotaxis has shown that FA pups were slower, but more successful in righting themselves compared to CA pups. The CA righting reflex was significantly slower (PND4) than FA. The adults behavioral tests showed a significantly ansiolitic effect on CA group as well as a deficit in long term memory at PND60. As a result, these effects suggest that maternal exposure to marijuana smoke, even at a low dose, may affect mice neonate's and, later on, adult's behavior. Ultimately, the results shown throughout our paperindicate the many potential harms of using marijuana during pregnancy.

RELATIONSHIPS BETWEEN THE PATTERN OF THE C-FOS-POSITIVE NEURONS AND LOCOMOTION HINDQUARTERS PROPERTIES IN CATS

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The spinal cord contains distributed neural networks responsible for generation and controlling of the basic locomotor patterns in different directions. The purpose of the present work was to investigate the structure and function of spinal neuronal networks controlling forward (FW) and backward (BW) locomotion. The c-Fos immunostaining technique was used for defining the activating interneuronal population. In addition, we performed the comparative analysis of the patterns of c-Fos-positive (FOS+) cells with: 1) the distribution of the motoneuronal pools innervating the specific hindlimbs muscles (based upon the comprehensive study by V. Vanderhorst and G. Holstege [1997])) with the individual patterns of muscle activity and movements kinematics. We obtain a non-uniform dynamic of the rostrocaudal FOS+ cells distribution, over the lumbar and sacral segments (L1-S2). Both FW and BW stepping cats have 2 peaks of FOS+ cells. The first peak is located in L4-L5 segments corresponded to hip flexor motoneuronal pool. The second peak is found in L6-L7 segments and corresponded to multiple motoneuronal pools of the muscles responsible for hip extension, knee flexion, ankle flexion and ankle extension. We found that the second peak is similar in BW and FW cats, while the first peak is generally lower in BW stepping cats. We analyzed the amplitudes of flexion-extension and angle range in different hindlimb joints during stepping and obtained a high and significant correlation between normalized amplitude of the first FOS+ cells peak and the amplitude of hip motion ($p<0.01$; $r = 0.93$ for FW cats; $r = 0.71$ for BW cats). All animals from BW group have generally lower amplitudes of the first FOS+ peak compared to the FW cats that corresponded to dominated flexion and significantly reduced angle range in a hip joint. The inter-peak reduction region at level of L5-L6 segments is corresponded to motoneuronal pool of the adductor muscles. We suppose that it was due to the fact that cat's trunk was fixed in restrained condition, and it didn't require the active balance control substantially dependent upon the adductor muscles activity. The amplitudes of the FOS+ peaks are differed from cat to cat but well correlated to quality of the stepping movements of individual animals. Thus we have found that c-Fos positive neurons distribution over the lumbosacral enlargement depends on direction of stepping, and upon the hindquarters kinematic properties and the pattern of muscles activity.

The study was supported by the Russian Foundation for Basic Research (RFBR grant №16-04-01791 and RFBR grant №17-04-01822), grant of President of Russian Federation (MD-1018.2017.7), and by Russian Science Foundation (RSF grant №14-15-00788).

TOWARDS A BETTER UNDERSTANDING OF INTRA- AND EXTRACELLULAR NEURAL SIGNALS AND THEIR RELATIONSHIPS

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Aims. A novel experimental method is presented for simultaneous recording of extra- and intracellular activity. In spite of the widespread use of multi-channel extracellular electrodes, very limited knowledge is available about the intracellular validation of these signals.
Methods. Whole cell patch clamp recordings were used to detect intracellular single cell activity, in rat hippocampal slices. Simultaneous extracellular signal was detected from the vicinity of the same neuron with a newly developed, multi-channel laminar edge-probe. Electrophysiological measurements were completed with subsequent histological analysis. Dendritic and axonal morphology of the intracellularly recorded and filled cell was revealed by three dimensional reconstruction performed with the aid of the NeuroLucida system.
Results. The presented method allows the investigation of single cell contribution on the extracellularly recorded signals. Furthermore, our experimental setup let us determine the exact Euclidean cell-electrode distances.
Conclusion. The knowledge about the exact spatial location of the cell compartments and the electrode contacts can help to determine the impact of single cell activity on extracellular recordings. The simultaneous, multimodal signals recorded with our system can yield additional information for various model-based calculations of neuronal dynamics.

IN UTERO LABELLED LATE-BORN SPINAL DORSAL HORN NEURONS ARE INVOLVED IN NOCICEPTION

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Neurons in the superficial spinal dorsal horn arise from late-born progenitor cells and it is widely accepted that they are assembled into circuits underlying nociceptive information processing. We found previously, that these neurons are born in a short time interval around E12.5 in mice embryos, and migrate radially and then in a non-radial (intra-laminar) manner for building the superficial laminae of the spinal cord. How these late born neurons differentiate further and what roles they have in the processing of nociceptive information remains unanswered however. To gather information about their adult morphology and function, we labelled neurons in the lumbar and cervical spinal cords of mice embryos at E12.5 with GFP by in utero electroporation and then allowed mothers to give birth to the electroporated pups. At the age of P18-P30 we performed patch-clamp recordings, morphological analysis and phospho-histone (pS10H3) assay after acute burn injury in the spinal dorsal horn of these animals. Electrical stimulation of the dorsal roots revealed a subset of the GFP labelled neurons that received monosynaptic inputs from C or A-delta fibres. We also found numerous pS10H3 immuno-positive GFP labelled neurons after a short burn injury of the limbs. Our data indicate that late born neurons migrating into the superficial spinal dorsal horn might be involved in nociception upon acute tissue injury.

This research was supported by the Hungarian Scientific Research Fund (OTKA PD 108467), the Hungarian Brain Research Program (KTIA_NAP_13-1-2013-0001 (ZM) and KTIA_NAP_13-2-2014-0005 (PSz)) grants of the Hungarian Government and the Hungarian Academy of Sciences (MTA-TKI 242).

NETWORK MECHANISMS OF PATHOLOGICAL SYNCHRONIZATION IN THE THALAMOCORTICAL CIRCUIT

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Absence seizures consist of a sudden and brief impairment of consciousness accompanied by a lack of voluntary movements and generalized, bilaterally synchronous 'spike and wave discharges' (SWDs) at 2.5-4 Hz in the EEG. Although childhood and juvenile absence epilepsy are considered relatively benign, they have been shown to be associated with learning difficulties, behavioral disorders and other psychiatric and neurological conditions, therefore a thorough mechanistic understanding of the cellular and network mechanisms involved in its generation is of crucial importance. Similar SWDs are exhibited by diverse genetic rodent models and investigations in these animal models have provided direct evidence for the crucial involvement of cortico-thalamo-cortical networks in SWD generation. Specifically, the primary somatosensory cortex (S1) temporally leads other sensory and associational cortical areas and the thalamus in the expression of SWDs. However, the activity of various neuron types during SWDs has remained relatively unexplored. By performing multiple field potential recordings in the ventrobasal thalamus, and various cortical sites (S1, auditory cortex, motor cortex, medial prefrontal cortex) together with single unit recordings in S1 of awake behaving stargazer mice during spontaneous SWDs we show that the activity of identified interneurons in S1 is significantly altered 1-2 seconds before the onset of SWDs in the EEG. The activity of various interneurons was also correlated with SWDs on a faster timebase. Specifically, most neurons exclusively fired action potentials around the trough of the SWD. The activity of a subset of S1 interneurons was correlated with the extent of inter-areal synchronization quantified as the delay between SWDs measured at various cortical sites. These results highlight the role of neuronal heterogeneity in cortical networks in the generation of pathological synchronized activity of non-convulsive epilepsy.

KINASE/PHOSPHATASE ACTIVITIES BALANCE IN ANXIETY- AND MEMORY-RELATED BEHAVIOURS IN ANIMAL MODELS

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Brain mechanisms of memory and anxiety involve neuroadaptive changes underpinned by the same phenomenon called neuroplasticity. It is the ability of the brain to be continuously reorganized on a functional and morphological level. The molecular basis of neuroplasticity includes two contradictory processes: long-term potentiation (LTP) and long-term depression (LTD), both of which are dependent on specific Ca2+ influx, however, LTP is linked with activation of protein kinases, while LTD requires activation of protein phosphatase.
Hence, the aim of the study was to evaluate the importance protein kinase/phosphatase activities balance in anxiety-related behaviours in 5 dpf larval zebrafish and memory-related effects in adult mice.
Zebrafish larval behaviour analysis was evaluated using the ZebraBox system manufactured by ViewPoint. Thigmothaxis was used as an index of anxiety. Zebrafish larvae displaying thigmotactic behaviour avoid the center of an arena (inner zone) and prefer to stay close to the boundaries of a well (outer zone). Moreover, larval locomotion was measured using simple locomotor assay as well as light on/off assay. Kinase/phosphatase activities balance in larval behaviour was investigated using acute FK-506 (a potent calcineurin inhibitor) and SL-327 (a selective MAPK/ERK kinase inhibitor) in a wide range of doses from 1 to 20 μM.
To assess memory function in mice we used passive avoidance test (PA test). The test is based on the association formed between an aversive stimulus (a foot shock) and a specific environmental context. The apparatus consists of two compartments, light and dark, separated by a guillotine door. The entrance of animals to the dark box was punished by an electric foot shock. Short- and long-term memory performance was evaluated using acute FK-506 (1.0, 5.0 and 10.0 mg/kg ip) and acute SL-327 (3.0, 10.0 and 30.0 mg/kg ip).
Our results indicate that kinase/phosphatase activities balance is involved in anxiety-related behaviours in larval zebrafish. In term of memory-related effects in mice, only long-term memory was found to be dependent on both calcineurin inhibition and MAPK/ERK signaling cascade.

INTERSUBUNIT INTERACTIONS MEDIATED BY LOOP C AND F MOVEMENT AND ANION INTERACTION SITE IN SHUT $\alpha_1\beta_2\gamma_2$ $GABA_A$ RECEPTOR HOMOLOGY MODEL.

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Pentameric ligand gated ion channels (pLGICs), including $GABA_A$ receptors ($GABA_A$Rs), play important role in synaptic transmission. Kinetics of these receptors has been extensively studied using electrophysiological methods, but molecular mechanisms underlying receptor functioning are not clear. Here we present a homology model of $\alpha_1\beta_2\gamma_2$ $GABA_A$R based on homomeric glycine receptor cryo-electron microscopy template in shut, strychnine bound state. Resulting model depicts the $GABA_A$R in shut, ligand-free state. To relax the structure molecular dynamics simulation (MDs) was performed. Most prominent movements were expected in binding sites (BSs) areas as the result of strychnine removal. In the extracellular domain (ECD) two subunit interfaces (β_2+/α_1-) are forming orthosteric BSs. During MDs these sites opened by loop C and F outward movement, similar movement (but less pronounced) was observed in allosteric BS at α_1+/γ_2− interface. On the contrary, loops C and F at non-binding interfaces (α_1+/β_2- and γ_2+/β_2-) moved in the inward direction closing those sites. Those phenomena led to disruption of the symmetry of interfaces observed in homopentameric template. At the BS interfaces interactions with ligand molecules were replaced with stronger interactions with solvent molecules, whereas in closed interfaces new interactions between residues were formed. At α_1+/β_2- interface this interplay was mediated by Glu208α_1 (loop C) and Arg117β_2 (β-strand 5). This interaction dragged loop C toward inner part of the interface. Simultaneously Asp172β_2 (loop F) moved toward Lys192β_2 (β-strand 9) influencing the opening of neighbouring subunit interface (β_2+/α_1-). At the second non-binding interface, γ_2+/β_2-, similar shutting interactions were observed, but impact on the neighbouring binding interface was negligible. In the transmembrane domain (TMD) three constriction points of the ion pore were observed. First at the level of Leu9' ring, (channel gate), second one at level of -2' residues (selectivity filter) and not previously known at the level of the 20' charged residues. In order to examine its role another MDs with chloride ion placed in 20' site was performed, expecting electrostatic interactions to be observed. As result spontaneous ion fluctuations at 20' residues area were detected. In addition, those interaction led to stable and permanent widening of the top part of the ion pore. Final ion pore profile was highly similar to pore profiles of other pLGICs. Our results are clearly indicating significant differences between homo- and pentameric pLGICs, mainly at subunit interfaces. These observations may shed light on such properties of $GABA_A$R as e.g. binding sites affinity or cooperativity with modulatory site. In addition, we propose that chloride ions may be found within the ion pore even if the receptor is in the shut state and its presence may play important role in establishing TMD helices conformation.

Supported by NCN grant DEC-2015/18/A/NZ1/00395.

NEW FEATURES IN VIVIDSTORM PROMOTE CORRELATED SUPER-RESOLUTION AND CONFOCAL MICROSCOPY

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Single-molecule localization microscopy not only achieves comparable localization precision of target proteins to immunogold electron microscopy, but the higher labeling intensity, the possibility of multiplex imaging, the faster tissue handling procedures and the automated molecular data analysis tools together represent potential major advantages over electron microscopy in several neuroscience applications. In addition, we have recently shown that combining Stochastic Optical Reconstruction Microscopy (STORM) with confocal microscopy makes rapid and efficient nanoscale molecular imaging possible within a morphologically and neurochemically defined cellular and subcellular context even in complex brain circuits. To further facilitate correlated confocal and super-resolution imaging, we have also developed the open-source software VividSTORM, which is capable of the simultaneous visualization and analysis of both pixel-intensity-based (e.g. confocal, widefield, TIRF, STED, SIM) and single-molecule localization-based (e.g. STORM, PALM) microscopy data. Here we introduce multiple recently implemented new features in VividSTORM, which are not yet available in any other microscopy software packages. The original workflow includes i) alignment and overlay of the pixel-intensity-based image and the molecular localization coordinates; ii) delineation of the labeled target cell or subcellular structure as region-of-interest (ROI); iii) selection of those specific single molecule localization points, which belong to the identified target profile; iv) coordinate-based analysis of the super-resolution data. VividSTORM is also equipped with a graphical user interface, hence these workflow steps can be easily performed even by novice users. The novel features freely available in VividSTORM promote this process in many ways. For example, we implemented both manual and automatic alignment methods, which are fiducial-marker-based or image-based, and can be used to overlay images even with low-copy-number protein localizations. We have extended the unbiased ROI selection feature by including automated 3D segmentation of the pixel-based image using a modified Morphological Active Contours Without Edges (MACWE) algorithm. We also integrated a Bayesian clustering approach for the nanoscale analysis of molecular density distribution, which uses an unbiased statistical selection of clustering parameters compared to the original DBSCAN method, which is based on clustering values predefined by the user. Finally, the batch analysis of pre-selected ROIs is now also supported in the latest VividSTORM version available at: http://katonalab.hu/vividstorm2/. These new features together further facilitate high-throughput correlated confocal and super-resolution data analysis and functional interpretation of quantitative molecular observations within identified cellular and subcellular structures in the brain.

THE POSTNATAL DEVELOPMENT OF THE LATERAL GENICULATE NUCLEUS AND THE PERIGENICULATE NUCLEUS OF THE VISUAL SYSTEM OF THE CAT: SMI-32 STUDY

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The development of mammalian visual system consists of two periods: the first lasted in prenatal and early postnatal life allows to envelope an initial visual map and connections based upon mainly inherent genetic program. The second, during definite part of the postnatal life, is characterized by high neuronal plasticity (the so-called critical period) and is needed for refinement of the initial maps into complete neuronal configuration under visual environment. In the cat used in our study as a model animal, the critical period proceeds from 3 to 12 postnatal weeks, with maximal plasticity at 4-5 weeks. The one of main visual processing region is visual thalamus consisted of several nuclei. In present work, we examined the postnatal maturation of two of them: the dorsal nucleus of the lateral geniculate nucleus (the LGNd) and the perigeniculate nucleus (the PGN). The LGNd is the main relay station providing the transmission of visual information from retina to the primary visual cortex, and the PGN is one of the most important modulators of the LGNd. In the experiment, we used 18 cats aged 0 (in-born and preterm born), 4, 10, 14, 21, 28, 34, 62, and 122 days. SMI-32 antibodies were used allowing selectively label a specific neuronal population of large principal neurons of the LGNd (supposed to be Y neurons) and large inhibitory cells of the PGN (modulating the LGNd). We found that SMI-32 positive (SMI-32(+)) neurons of the LGNd tend to locate in the layer CM, the lower part of A1 layer adjacent to the CM layer, and in the lower and upper parts of A layer adjacent to the PGN. This cell distribution is highly expressed in birth and becomes less pronounced during development. The number of neurons: 1) does not change considerably in the CM layer from birth; 2) grows in the A layer until 10 days; 3) grows to 34 days and decreases after this age in the A1 layer. The number of SMI-32(+) cells in the PGN is growing rapidly from 10 days, has the maximal amounts from 14 to 34 days, and decreases in 62 and 122 days of life. Based on the obtained data we can suggest that the SMI-32(+) cells in the LGN develop heterochronically; the layers A and A1, which are considered to be functional equivalents have different patterns of cell distribution; and that the PGN can play an important role during the critical period of visual system development.

FLUOXETINE-INDUCED PLASTICITY IN THE PREFRONTAL CORTEX ENHANCES EFFECTS OF BEHAVIORAL TREATMENT OF ABNORMAL AGGRESSION IN RATS

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Violence and crime are strongly associated with childhood adversities and social neglect during sensitive periods of early life; however, the processes that mediate this relationship remain largely unknown. We have earlier demonstrated that post-weaning social isolation of rats - a laboratory modell for early social neglect - resulted in escalated and abnormal forms of aggressiveness and over-activation of several brain regions, including the medial prefrontal cortex (mPFC) during aggressive encounters in laboratory rats. Deficits in prosocial behavior were eliminated by resocialization during adulthood, but abnormal aggression was resilient to this treatment. We assumed that escalated aggression was refractory to the corrective effects of re-socialization because of the adulthood-specific reduction in neural plasticity; therefore, here we investigated whether fluoxetine - that has been shown to reactivate juvenile-like state of plasticity - makes animals receptive to the effects of re-socialization in this model. The hypothesis was tested by combining psychosocial and plasticity-related pharmacological treatments (re-socialization and fluoxetine, respectively) in male rats submitted to post-weaning social isolation. According to our results, post-weaning social isolation induced abnormal and escalated forms of aggression in adulthood that were eliminated by the combination of 3-week long fluoxetine therapy and re-socialization, but neither treatment alone. To study treatment-induced changes in neural plasticity, we investigated gene expression profiles in brain regions relevant for aggression control by qPCR. Re-socialization and fluoxetine, albeit did have independent effects on neural plasticity in some brain regions, interactively modulated neural plasticity in the infralimbic cortex of the mPFC: the expression levels of BDNF 1 and 4 were down-regulated by post-weaning social isolation in the infralimbic cortex and restored by the combined but not by individual treatments. Moreover, the behavior improvement after the combined treatment was dependent on TrkB activity. Additionally, input from the ventral hippocampus to mPFC was specifically strengthened by the combined treatment alone, revealing the importance of this pathway in the beneficial effects. In summary, rats submitted to post-weaning social isolation model, fluoxetine dramatically enhanced the effects of re-socialization, indicating a synergistic interaction between positive social experiences and enhanced neural plasticity. The mPFC emerged as important mediator of the beneficial effect. This suggests that aggression problems may be diminished by plasticity-related pharmacological treatments to promote the efficacy of psychotherapy.

INCREASING THE SENSITIVITY OF A NEW, WEB-BASED AMBLYOPIA SCREENING APPLICATION

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Stereoblinds often have amblyopia in the background, therefore this finding in childhood always requires medical attention. The Euvision® screening system is a test especially designed for assessing stereopsis in children. The test can be performed at home, using any computer or mobile device and requires no assistance of healthcare professionals. Since it is based on an anaglyphic technique, red-green filter glasses are needed for channel separation. The aim of our present study was to minimize the false negative rate to increase the sensitivity of our test in detecting lack of stereopsis, that helps to identify ophthalmologic conditions potentially leading to amblyopia. According to our previous findings, the false negative measurements mostly arise from the monocularly detectable visual cues (i.e., artifacts) that may be present in the stimulus set, due to variations in display characteristics or individual biases in the color vision of the patient. In order to detect the presence of these monocular artifacts in the static and dynamically updated random dot stimuli we recruited young, healthy individuals (n=21). Participants performed the Euvision® test in the following conditions: A. wearing no filter-glasses: 1. with both eyes 2. with right eye 3. with left eye B. wearing filter-glasses: 1. with both eyes (red-green) 2. with right eye (green) 3. with left eye (red). Participants had to identify the orientation of 10 stereo-Snellen E optotypes for each condition. The proportion of correctly identified directions (i.e., hit ratio) was registered by the system. The passing level of the tests was based on Bernoulli probability ratio and it was set at $p<0.001$ or 8/10. All participants except an amblyopic patient passed both static and dynamic tests by using filter-glasses. None of the participants passed the test when the images were viewed monocularly either with filter-glasses on or off regardless of the dynamic property of the images. In binocular viewing conditions without filter-glasses, four and five participants passed the tests in the static and dynamic conditions, respectively. In conclusion, the presence of monocular artifacts can be clearly ruled out. Passing the test in the binocular condition without filter-glasses may arise for three possible reasons: 1) individual differences of the red-green isoluminance properties for the two eyes; 2) the different color filtering properties of LCD monitors from different viewing angles or 3) slightly different color filtering properties of the two corrective eye glasses (e.g. photo grey lenses). These binocular artifacts, however, will probably not influence false negativity, therefore they will not affect sensitivity.

Supported by EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

PITX2+ NEURONS ARE THE SOURCE OF C-BOUTONS ON CRANIAL NERVE NUCLEI

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The motor control circuitry in the central nervous system must be flexible. Rhythmic motor behaviors, such as locomotion and respiration, need to adapt in order to suit the varying demands of different states and environments. This flexibility is partly provided by neuromodulatory systems, which adjust the output of motor circuits by modulating the properties of neurons within them. Respiratory rhythm, for example, needs to be modulated according to the mechanical and metabolic demands of different behavioral states or conditions (e.g. sleep-wake state, exercise, hypoxia or hypercapnia). One such type of modulatory system is the C-bouton synapse. C-boutons are large, cholinergic inputs on motor neurons. In the spinal cord, we have shown that they have a crucial role in task dependent increases in motor neuron activity and muscle activation, with acetylcholine acting as the neuromodulator at C bouton synapses via activation of muscarinic receptors on motor neurons. C-boutons originate from interneurons marked by the expression of the transcription factor Pitx2. In this study, we focused on the C bouton system within the brainstem. By using genetically modified mice, in which fluorescent proteins are conditionally expressed in Pitx2+ neurons, we confirmed the presence of Pitx2+ interneurons and mapped their location. More specifically, we found Pitx2+ neurons in three different regions of the brainstem, namely the lower medulla, the pons and the midbrain. Importantly, we have identified that cholinergic Pitx2+ neurons are only located in the lower medulla, intermingled with non-cholinergic Pitx2+ interneurons. We have also demonstrated that the column formed by Pitx2+ neurons in the spinal cord continues into the lower medulla and contains both cholinergic and non-cholinergic neurons. Moreover, we have also shown that C-boutons on motor neurons of the brainstem (accessory, hypoglossal, ambiguous, facial, trigeminal nuclei) only originate from cholinergic Pitx2+ neurons. These results point to the same neuronal origin of C-bouton neuromodulation in both spinal cord and brainstem.

NEUROSTEROID REGULATION AT THE GABA-ALPHA4 RECEPTOR OF NEURONAL EXCITABILITY AND TONIC INHIBITION

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γ-amminobutyric acid type-A receptors (GABAARs) have a pentameric structure and are important for mediating the majority of inhibition in the central nervous system (CNS), producing both phasic (synaptic) and tonic (extrasynaptic) inhibition. Phasic and tonic inhibition are mediated by GABAARs with different subunit compositions, phasic receptors typically containing α1-3βnγ whereas tonic receptors consist of α4-6βnδ. Dysfunctional inhibition causing disruption to the excitation-inhibition balance has been implicated in many psychological disorders such as epilepsy.
Neurosteroids are important regulators of GABAAR function, and act by positivvely or negatively modulating GABAARs. Neurosteroids are locally produced steroids derived from hormones and released as a response to stress, inducing a potentiation of the GABAergic inhibition. Neurosteroids most potently modulate delta subunit containing receptors and thus are able to alter the tonic inhibition mediated by extrasynaptic receptors. Following the discovery of the binding site for neurosteroids on GABAARs (Hosie et al. 2007), a new mouse line with a knock-in mutation at position 246 in the α4 has been generated, in which the conserved glutamine (Q) is changed to a methionine (M). This amino acid substitution is supposed to prevent neurosteroid binding at its potentiation site, and thus allows us to investigate the functional role and properties of neurosteroids at defined GABAAR subtypes containing the α4 subunit.
Initial recombinant work in HEK293 cells validated the functional effects of the Q246M mutation, specifically disrupting the binding of THDOC to the NST potentiation site of the alpha4 receptor without affecting any of the other properties of the receptor. An electrophysiological characterisation of the new mouseline in various brain areas reveals that the Q246M mouse has a reduced sensitivity to NST potentiation for the tonic current, leaving the synaptic potentiation mostly intact. Current Clamp experiments reveal the importance of the alpha4 receptor in mediating the NST induced change in excitability in the hippocampal circuitry.
Future experiments will focus on the behavioural relevance of alpha4 mediated neurosteroid regulation, focussing on epilepsy, anxiety and anaesthesia.

ELUCIDATION OF VARIOUS INFLAMMATORY PATHWAYS IN EXPERIMENTAL PARADIGMS OF STZ-INDUCED DIABETIC NEUROPATHY

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Diabetic neuropathy affects more than 50% of diabetic patients. Rutin has been demonstrated in number of pharmacological activities including anti-diabetic, anti-oxidant and anti-inflammatory activities. Streptozotocin (STZ, 55 mg/kg) was administered intraperitoneally (i.p.) to overnight fasted rats. Naive and diabetic rats were randomly selected and divided into eight groups of six animals in each group. Rutin (100 and 200 mg/kg,i.p.) and Nimesulide (5 and 10 mg/kg, i.p.). All the behavioural parameters (Measurement of body weight, Mechanical allodynia, Cold allodynia, Mechanical hyperalgesia, Thermal hyperalgesia) were performed on day 0, 2^{nd}, 4^{th}, 6^{th} and 8^{th} week. On last day (of 8th week), blood was collected retro-orbitally and mean nerve conduction velocity was assessed. The animals were then sacrificed sciatic nerves were isolated for further biochemical estimations, TNF-alpha and caspase-3 activity estimated by ELISA. Rutin(100 and 200 mg/kg) for 8 weeks significantly protected all the behavioral alterations, oxidative damage and change in mean nerve conduction velocity induced by STZ. Further, combination of Rutin (100 and 200 mg/kg) with Nimesulide (10 mg/kg) significantly reversed all the behavioural, biochemical and changes in nerve conduction velocity as compared to their effect per se in STZ-induced diabetic neuropathy. The present study suggests the protective effect of *Rutin* against STZ-induces diabetic neuropathy. Study further provides an evidence that rutin produces better effect in combination with nimesulide against STZ-induces diabetic neuropathy.

TRANSLATIONAL EEG REACTIVITY MEASURES FOR ASSESSING THE POST-ISCHEMIC BRAIN

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EEG assessment of diffuse ischemic brain injury remains methodologically challenging. We reported that in comatose patients with „burst-suppression" (BS) EEG, the decrease in suppression ratio during visual stimulation, referred to as BS reactivity, correlated with injury severity.
A reversible BS state can be induced by deep general anesthesia. The aim of this study was to assess the anesthetic BS reactivity to photic stimulation following experimental global cerebral ischemia (GCI). Adult male Wistar rats accommodated to a 12 h dark/night cycle were subjected to a 5-minute GCI by „4-vessel occlusion".
Continuous telemetric EEG/EMG monitoring within 48 hours of reperfusion indicated no seizures, or abnormal slowing of resting EEG rhythms. Sleep-wake cycles recovered, showing only an attenuated nocturnal increase in wakefulness, consistent with reduced nocturnal activity levels measured by video-tracking. EEG investigations under anesthesia did not detect abnormalities in visual evoked potentials. Nevertheless, reactivity of anesthetic BS patterns measured over 1 min, was only half than in controls due to increased accommodation to stimulation. By convoluting an alpha function with the binary BS signal and optimizing the function time constant, we found that after GCI there was an increased time-to peak from 3 to 7 sec. A similar delay was observed in the peak of the hemodynamic response function, measured by burst-triggered averaging of the laser-doppler signal recorded over 30 minutes.
Our data suggest that the impaired BS reactivity reflects a global impairment in vascular reactivity. This opens the possibility of using anesthetically induced BS to derive translational markers of ischemic injury.

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

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Temporal lobe epilepsy (TLE) is the most common form of focal epilepsies and often related with hippocampal sclerosis (HS) that is visible with MRI. In lower number of cases, TLE is associated with malformation of cortical development (MCD), and occasionally MCD is coincided with HS. In a few cases, MRI cannot detect any morphological alteration in TLE. In previous studies, decrease of the number of parvalbumin-immunoreactive (PV-IR) cells has been observed in HS. Despite the loss of PV-IR cells, the preservation of axo-somatic synapses has been demonstrated. In our present study, the aim was to examine PV-IR cells and axons in the dentate gyrus (DG) of TLE patients with different etiologies.
Tissue samples contained the hippocampal formation of therapy resistant TLE patients which were obtained from the Dept. of Neurosurgery. According to the MRI results, patients belong to the following groups: HS, HS+MCD, MCD, MR-negative. Patients were considered MR-negative when no abnormality could be observed with the routine imaging technique. Immunohistochemistry detecting PV in the DG was examined with light and transmission electron microscopes (TEM).
PV-IR cells were observed mostly in subgranular location in the hilus of the DG in MCD and in MR-negative cases, and their morphology and number were similar to that found in controls. In the HS and MCD+HS group, decrease of PV-IR cells was observed in the hilus, and large numbers of ectopic PV-IR neurons were detected in the molecular layer of the DG and along the hippocampal fissure. In HS and MCD+HS groups PV-IR axons running perpendicular to the granule cell layer were observed in the molecular layer of the DG and their localization suggested that PV-IR axon terminals sprout and terminate on the dendrite of granule cells. The sprouting PV-IR axons were frequently observed in patients who had infant febrile seizure in their history of the disease. No direct correlation was observed between sprouting of PV-IR axons and the location of PV-IR cells. TEM examinations proved sprouting of PV-IR terminals, which were terminating on distal dendritic shafts and spines of granule cells.
Our results indicate alteration of target profile of PV-IR cells in TLE associated with HS, and we propose the role of infant febrile seizure in pathomechanism of this process.

Our work was supported by the NAP (KTIA_13_NAP-AII / 11), and by the EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

EFFECT OF EXERCISE TYPE AND INTENSITY ON SUSTAINED ATTENTION: A CATECHOLAMINERGIC HYPOTHESIS

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Moderate physical exercise increases catecholamine release and can reduce response times (RTs) to sustained attention tasks. This effect is supposed to result from the sympathetic stimulation that occurs when exercising, as attentional processes are relying on the pattern of noradrenaline (NA) release by the locus coeruleus and the NA pathway it activates. This decreasing effect can be observed until a particular intensity, achieved around the ventilatory threshold (VT) and correlated to a catecholaminergic threshold. Moreover, the type of exercise might also modulate attention during exercise by affecting the pattern of NA release by the locus coeruleus. Monotonous tasks appear to affect more RTs than varying tasks. This study hypothesized that cycling at a constant intensity would induce less effect on RTs than cycling with variations around this intensity.
The effects of intensity and type of exercise on a sustained attention to response task (SART) were investigated in twelve trained male subjects while using near-infrared spectroscopy (NIRS) to monitor the activity of the NA-dependent fronto-parietal attentional network. Sympathetic stimulation was estimated by measuring salivary α-amylase concentrations. Participants performed the same protocol at rest, at a low constant intensity, at a moderate constant intensity below VT, and at a moderate variable intensity.
RTs during the SART decreased when exercising at moderate intensity and even more during varying than during constant exercise. A correlation was observed between the salivary α-amylase concentrations and the results to SART. NIRS results indicated higher cerebral oxygenation in the regions of the fronto-parietal attentional network during both moderate intensity sessions than during the control sessions.
This study confirms the decreasing effect of exercise intensity on RTs in a sustained attention task and suggests an even more speeding effect of exercise when intensity is varying.

SOME PROPERTIES OF RECENTLY CHARACTERIZED GROUP OF FOUR LOOSELY ATTACHED TO NEURONS EXTRACELLULAR METALLOPEPTIDASES (NEMPS); PROTECTION OF SYNTHETIC PEPTIDES FROM NEMPS

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Recently it was found that in mammalian brain (rat, cow), a group of low specific extracellular neuron bound metallopeptidases (NEMPs) is present (Kropotova ES and Mosevitsky MI (2016) Neurochem. Res., 41, 2666-2674). NEMPs can be detached from neurons by a low concentration of Triton X-100 (0.05-0.1%, 5 min, 0^{o}C). This group consists of four enzymes: carboxipeptidase (NEMP1), aminopeptidase (NEMP2), endopeptidase I (NEMP3) and endopeptidase II (NEMP4). Some specific properties of NEMPs were revealed. So, NEMP1 molecules are very small (8-10 kDa), but highly inclined to a self aggregation. NEMP3 needs for its active state in the presence of an "activator". Some amino acids (glycine, alanine, lysine, arginine, histidine, phenylalanine) can be the activators, some other (glutamate, aspartate, tryptophan, methionine, cysteine, d-histidine) can't activate NEMP3. An allosteric interaction of NEMP3 with an activator seems likely. All NEMPs are low specific. Due to this property, NEMPs are able to control a wide range of neuropeptides and to perform their catabolism. This is their probable physiological function. However, the low specificity of NEMPs makes these enzymes dangerous for the introduced in brain therapeutic peptides. It is well known that therapeutic effect of these peptides is very short, because they are quickly destroyed. Basing on our earlier results showing that carboxipeptidase NEMP1 and aminopeptidase NEMP2, being the dipeptidases, can't split dipeptide beta alanine-histidine (carnosine), we flanked synthetic peptides (ala-lys-phe, analogs of enkephalin, bradikinin and other neuropeptides) with beta alanine. These "protected" peptides were incubated (37^{o}C) in a suspension of isolated axonal endings of neurons (synaptosomes). These presynaptic parts of synapses carry all four NEMPs on their surface. It proved that protected peptides were an order more stable than the non-protected analogs. The analgesic effects of enkephalin and protected enkephalin were studied on Wistar rats. Equivalent dose of enkephalin or protected enkephalin (0.04 μM) were introduced intranasally. After a certain interval of time, an intraperitoneal injection of 0.7% acetic acid was done. The number of writhes was analyzed for 20 min. In the presence of enkephalin, analgesic effect was shown for 40 min, while In the presence of protected enkephalin this effect continued for 300 min, that is 7-10 times longer. It should be noted that the addition of flanking beta alanine residues not influenced negatively: in the both cases the initial analgesic effects were similar. This result opens new opportunities to use therapeutic peptides in the treatment of nervous diseases.

This research was supported by Russian Basic Investigations Foundation (grants 09-04-01571-a and 12-04-00505-a (MM) and 14-04-00587-? (MK))

ISOLATION OF PRIMARY CORTICAL AND SPINAL CORD RAT MICROGLIA FOLLOWED BY CHARACTERIZATION OF MICROGLIA DERIVED EXOSOMES

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Exosomes are extracellular vesicles (EVs) of 50-100nm diameter, released by CNS cells as a consequence of the fusion of multivesicular endosomes or multivesicular bodies (MVBs) with the plasma membrane. With the capability to transfer nucleic acids, proteins and lipids, exosomes can influence numerous functional and pathological aspects of both donor and recipient cells. The main goal of present study was to characterize exosomes derived from primary cortical and spinal cord rat microglia under normal and inflammatory conditions.
Primary cultures were isolated from neonatal Wistar rats (P3) brain cortex, and spinal cord followed by magnetic microglia separation with CD11b/c MicroBeads. After reaching confluency both cortical positive CD11b/c cells and spinal primary cultures were stimulated with LPS (500ng/mL) for 24h to activate microglia and enhance release of the exosomes. Using centrifugation and ultracentrifugation steps we isolate exosomes from each primary cell culture conditioned medium. Using the NanoSight technology, mean and modal diameter size of the exosomes were measured, as well as the concentration of particles. To examine morphology and size of exosomes, we involved field emission scanning electron microscopy analyses (JEOL FE SEM JSM-7000F). For protein analyses of exosomes, we have performed SDS- PAGE electrophoresis and mass spectrometry (nanoHPLC-MS/MS).
Our results indicate, that CD11b/c MicroBeads separation yielded a highly purified Iba1 positive microglia population (95-98% of cells). Stimulation of positive CD11b/c microglia cell populations with LPS resulted in secretion of exosomes with specific proteins content in relation to microglia cells origin, cortex or spinal cord: exosomes proteins profile of cortex microglia stimulated show an inflammatory profile (Psma1, Rpl13a), besides exosomes proteins profile of spinal cord microglia which are orientated through regeneration.
Present data confirmed that microglia cells origin influence exosomes content and that microglia derived extracellular vesicles are extremely important considering the key roles that microglia play in development and neurological disease. Supported by APVV 15-0613 (DC), Stefanik (MS), SK-FR-2015-0018 (DC), ERANET- AxonRepair.

NEUROCHEMICAL AND ELECTROPHYSIOLOGICAL FEATURES OF EXTENDED AMYGDALA PROJECTING NEURONS LOCATED IN THE PERIAQUEDUCTAL GREY AND DORSAL RAPHE NUCLEUS

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Periaqueductal grey (PAG) and dorsal raphe nucleus (DRN) are brainstem structures, which are involved in a wide range of physiological processes including defensive responses, nociception or social interaction. Recent studies reported that vPAG/DRN dopaminergic neurons project to the extended amygdala, particularly to the lateral part of the central amygdala (CeL) and to the bed nucleus of stria terminalis (BNST). Vasoactive intestinal polypeptide (VIP) and cholecystokinin (CCK) containing neurons, which can be found in this region, also innervate both CeL and BNST. Given the confounding and partially overlapping information available on the cytoarchitecture and projections of the vPAG/DRN nuclei, we aimed to explore the neurochemical content of vPAG/DRN neurons, focusing on those ones, which project to CeL and/or BNST.

To this end, we intracranially injected retrograde tracers FastBlue and FluoroGold into the BNST and CeL, respectively, of BAC-CCK-DsRed and VIP-ZsGreen1 transgenic mice, to visualize the projecting cells and identify their neurochemical content using immunocytochemistry. Our results indicate a wider distribution of CCK^+ neurons within the whole PAG. In contrast, VIP^+ neurons are confined to the lateral and ventral parts adjacent to the 4th ventricle. We found that 39% of VIP^+ neurons co-expressed CCK. In tracing experiments using CCK-DsRed mice (n=2) we revealed that more than 60% of projecting cells in vPAG/DRN contained only CCK, 2% contain only VIP and 25% express both neuropeptides. We found 13.3% of cell projecting to both nuclei.

Using in vitro whole-cell patch clamp technique in vPAG/DRN containing sections of VIP-ZsGreen1 mice, we recorded the firing pattern of VIP^+ neurons and analyzed their intrinsic properties, which where compared to those data obtained from inhibitory neurons sampled in VGAT-ZsGreen1 mice. Our electrophysiological data show that vPAG/DRN VIP^+ neurons have similar features than those described for dopaminergic neurons, as well as clearly different membrane properties than local inhibitory neurons.

In summary, we provide a map for the distribution of CCK and VIP containing neurons, as well as the overlap in their neurochemical content in vPAG/DRN. We show that VIP^+neurons have different membrane properties compared to local inhibitory neurons and display similar single cell features as dopaminergic cells. Finally, we demonstrate that both $CCK^+$and VIP^+ cell types project to the CeL and BNST, and interestingly that some projecting cells send collaterals to both regions, providing the structural basis for the simultaneous control of the CeL and BNST function.

ADULT DELETION OF SRF IN THE EXCITATORY NEURONS AFFECTS MORPHOLOGY OF THE DENDRITIC SPINES AND SPECIES-TYPICAL BEHAVIORAL TASKS

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Serum Response Factor (SRF) is a major transcription factor in the brain that regulatesexpression of, i.e., immediate early genes and genes encoding cytoskeletal proteins. In this study, we aimed to investigate the role of SRF in the adult brain using inducible knockout animal model (SRF KO). Our previous data of global gene expression analysis showed that deletion of SRF in the adult neurons caused very limited alteration in basal gene expression. In this study, we confirmed that one of previously identified SRF target gene, β-actin, was downregulated in *dentate gyrus* (DG) field of the hippocampus. To address the consequences of this downregulation, we focused on hippocampal morphology. Nissl staining of hippocampal sections from control and KO mice showed no major neuroanatomical differences. To more precisely investigate neuronal morphology we analyzed dendritic spines' shapes and density in the DG field of the hippocampus. The spines' density was not altered in the SRF KO animals. However, we observed a significant increase in spines length and area in SRF KO animals, when compared to their WT littermates. Moreover, using staining for zinc transporter ZnT3, we found a significant increase in the length of the infrapyramidal mossy fibers in the hippocampus of SRF KO animals. To check behavioral consequences of changes observed in the hippocampus of SRF KO animals, we performed three hippocampal-dependent species typical behavioral tasks. We found that mutant mice were impaired in rodent-typical behaviors such as digging, marble burying, and nesting. The animals performed less digging bouts, spent shorter time digging, buried fewer marbles and built none or poorer nests, as compared to their appropriate controls, but there were no differences in latency to start digging. Our results suggest that SRF plays a role in regulation of dendritic spine morphology in the adult brain and the lack of SRF leads to impairments in rodents-typical behaviors.

The research was financed by Polish National Science Centre (NCN); decision no: 2012/07/E/NZ3/01814.

ALPHA7 NACHR AGONIST AND POSITIVE ALLOSTERIC MODULATORS DIFFERENTIALLY MODULATE THE SPONTANEOUS AND NMDA-EVOKED FIRING ACTIVITY OF RAT HIPPOCAMPAL CA1 NEURONS IN VIVO

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Rising incidence of neurocognitive disorders (NCD) and related neurodegenerative diseases (e.g., Alzheimer's disease, AD) infer a major public health issue worldwide. One of the possible novel strategies for treatments of NCD is targeting the cholinergic system by the activation of α7 nicotinic acetylcholine receptors (nAChR) with highly selective agonists combined with positive allosteric modulator (PAM) compounds. In the present experiment, we aimed to study the local, *in vivo* electrophysiological effects of an α7 nAChR agonist (PHA-543613) and two PAM compounds (PNU-120596, NS-1738) on hippocampal neurons.
Extracellular firing activity of neurons was recorded in hippocampal CA1 region of anesthetized rats. PHA, PNU and NS were locally administered in discrete time intervals using microiontophoretic technique. We investigated the effects of α7 nAChR ligands on both the spontaneous firing activity and on NMDA-evoked excitations.
We found that the delivery of PAMs predominantly increased the spontaneous firing rate of neurons (NS - increase: 22/26, decrease: 1/26; PNU - increase: 6/6), while after PHA administration we also detected notable decreases in some cases (increase: 7/13, decrease: 3/13). Furthermore, NMDA-evoked firing responses of the neurons were also differently modulated by PHA. Forty-six percent of the 13 investigated neurons showed increased NMDA-evoked firing rate, but almost half of the neurons (31%) showed decrease in the NMDA-evoked firing rate after the application of PHA. On the other hand, NS predominantly exerted the increase of NMDA-evoked firing activity. Eighty-five percent of the examined 26 neurons showed increase in the NMDA-evoked firing rate, while we only detected a decrease in case of 4% of the neurons after the delivery of NS. Similar pattern was seen after the iontophoretic administration of PNU (NMDA-evoked firing rate increased in 5 cases out of 6). The average NMDA-evoked firing rate increased from 40.1±3.4 Hz to 56.5±7.8 Hz ($p=0.065$, N.S) after PHA administration. On the contrary, local delivery of the α7 nAChR PAM NS resulted in a more definite increase of the NMDA-evoked firing frequency (from 49.7±3.6 Hz to 72.9±5.6Hz, $p<0.001$). In most cases, the NMDA-evoked firing responses to simultaneously iontophoretized PHA and NS showed a higher increase compared to the independent administrations, even if PHA exerted an inhibitory effect on NMDA-excitations when administered separately. Thus, the PAM reversed the blocking effect of the agonist.
These results represent that α7 nAChR agonists and PAMs have different effects on the sensitivity of CA1 neurons to NMDA and on their spontaneous firing activity. Probably, the different effects of agonists and PAMs can be accounted to the desensitization activity of α7 nAChR agonists. These results suggest that simultaneous activation of the α7 nAChRs with agonists and PAMs can be a more effective opportunity to enhance cognitive functions than using the agonist itself.

Support: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL FEATURES OF DISTINCT BASKET CELL TYPES IN THE MOUSE PREFRONTAL CORTEX

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Prefrontal cortex plays a pivotal role in several higher order cognitive functions, including decision making. Even though a significant number of studies has been addressed to clarify the principles of neural operation in the prefrontal cortex, its microcircuit organization, a necessary prerequisite to understand how a network processes incoming information, is largely unknown. In this study, we aimed to reveal the features of two types of basket cells expressing parvalbumin (PV) or cholecystokinin (CCK) in the prelimbic part of the mouse prefrontal cortex.
Using whole-cell patch clamp technique, we recorded and intracellularly labelled interneurons expressing fluorescent proteins in slice preparations that were prepared from the prefrontal cortex of mice expressing EGFP and DsRed under the control of PV and CCK promoter, respectively. This targeted patching allowed us to selectively investigate the feature of the preferred interneuron types that were *post hoc* morphological identified. We observed that the two basket cell types differed significantly in passive and active membrane properties that were evaluated by intracellular injection of current steps. PV-containing basket cells showed a fast spiking phenotype and had low input resistance, while regular spiking and higher input resistance characterized CCK-expressing basket cells. These two interneuron types also showed differences in their morphological appearance. CCK-containing basket cells irrespective of their soma location had wide dendritic and axon arborization. In contrast, PV-expressing basket cells showed large morphological variability that partially depended on the layer were the soma was located. To quantify the ratio of axon and dendritic length in distinct layers for individual interneurons, we defined the layers in CCK-DsRed mice by labeling superficial pyramidal cells using an antibody developed against wolframin (WFS1) and a portion of deep pyramidal cells using a CTip2 antibody. The analysis revealed that PV-containing basket cells could have a restricted axon arbor in a given layer or they may project widespread covering all layers were pyramidal cell somata can be found.
Our results suggest that the two types of basket cells differ both in single cell properties and morphological features. The variability in the electrophysiological characteristics and arborization patterns may support the distinct function they play in circuit operation of the prefrontal cortex.

TRKB AND P75 RECEPTORS IN GENETICALLY DEFINED AGGRESSIVE BEHAVIOR

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Brain-derived neurotropic factor (BDNF) is known to play an important role in the pathogenesis of many disorders of the nervous system. This neurotropic factor is synthesized from mRNA as a precursor protein proBDNF that may be converted into mature BDNF by endoproteases. Both mature BDNF and proBDNF affect the brain functioning sometimes in opposite ways. It is known that mature BDNF initiates neurogenesis via TrkB receptors, whereas BDNF precursor proBDNF acts via p75 receptors and initiates apoptosis. Moreover, there are some data indicating that BDNF also able to induce neuronal death via truncated form of TrkB receptor. Thus, BDNF is undoubtedly crucially involved in the mechanisms underlying neural plasticity and, hence, in the control of various kinds of normal and pathological behavior.
Among others, aggressive behavior attracts particulate attention. This is mainly due to two major reasons: (i) excessive aggression is a base of human asocial and criminal behavior, and (ii) genetic predisposition to the lack of aggressiveness is the ground for one of the prime events in the human life - animal domestication. There are some data indicating the involvement of BDNF in the mechanisms of aggressive behavior. However, these results pretty scarce and contradictory. This is likely caused by the complexity of the BDNF maturation and signaling. Moreover, there is a lack of data on the expression of BDNF receptors in genetically defined aggressive behavior.
The aim of the study was the investigation of the TrkB and p75 receptors expression in the brains of rats selectively bred for 85 generations in the Institute of Cytology and Genetics SD RAS, Novosibirsk, Russia, for high level of defensive aggression and its absence.
We have found that genetic predisposition to high level of aggressiveness is associated with considerable structure-specific changes in both TrkB and p75 receptors expression. Interestingly, that these alterations were observed both in mRNA and protein levels. Moreover, the important for apoptosis truncated form of TrkB receptor was increased in some brain structures of highly aggressive rats compared to tame animals. Taking into account the considerable role of p75 receptor and truncated form of TrkB receptor in the regulation of apoptosis, observed changes in the BDNF receptors expression allowed us to suggest implication of BDNF-induced apoptosis in the development of aggressive phenotype in investigated animals.
Thus, considerable structure-specific changes in the expression of key receptors for BDNF in the brain of the rats with genetically defined aggressive behavior were found for the first time. Moreover, the increase of truncated form of TrkB receptor was revealed in the brain of aggressive animals. Obtained data allow to suggest that both TrkB and p75 receptors could play an important roles in the mechanisms underlying genetic predisposition to aggressive behavior.

The study was supported by the RFC grant # 17-15-01021.

A NEUROERGONOMICS APPROACH OF CAR DRIVING ANALYSIS

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Although recent on time scale of humanity, car driving is a major human activity that concern a very large number of individuals every day. Thus, for most of us car driving is a familiar activity. Despite being familiar for most individuals, car driving is a complex activity because several tasks must be performed in parallel in an uncertain driving environment. The completion of those tasks is implying a variety of cognitive processes ranging from perceptual-motor skills to decision making through attentional and memory processes for instance. Driving behaviours are traditionally analysed under the scope of cognitive ergonomics, the discipline focusing on human activities study in real life situations. Over the years, cognitive ergonomists agreed on the existence of three different levels of control in car driving: strategical, tactical and operational. With the development of brain imaging technologies such as functional Magnetic Resonance Imaging (fMRI) neurosciences have offered a new understanding on human behaviour. The objective of the current contribution is to combine the strengths of ergonomics with those of neurosciences. To do so the brain regions associated to strategical, tactical and operational driving tasks were investigated based on an exhaustive meta-analysis of studies mixing fMRI with simulated car-driving. Results revealed different brain networks associated with the three levels of car driving control. Strategical tasks were found to be associated with inferior and superior frontal gyri as well as inferior and superior temporal gyri known to be implied in planning movements and high level decision making based on previous memories. Tactical tasks were associated with middle frontal and middle temporal gyri known to be involved in attention reorientation and planned actions adjustments depending on environmental cues. Operational tasks were associated to brain regions known to be involved in complex visual scene analysis, procedural memory and movement control. The brain regions engaged for the three different levels of car driving control were found to be independent. This reinforces the three levels of control categorisation and supports the idea that different driving tasks controlled at different levels can be performed in parallel. The collected results are used to discuss both ergonomics and neuroscientific car driving models.

FEEDFORWARD INHIBITION IS RANDOMLY WIRED FROM INDIVIDUAL GRANULE CELLS ONTO CA3 PYRAMIDAL CELLS

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Feedforward inhibition (FFI) is crucial for the neuronal communication between the DG and CA3 region, because it can effectively sparsify and shape the memory- and spatial navigation-related activities of these hippocampal regions. However, our understanding of this prototypical FFI circuit lacks essential details, as the wiring arrangement of the FFI is not yet mapped between individual DG granule cells (GCs) and CA3 pyramidal cells (PCs). Importantly, theoretically opposite network contributions are possible depending on whether the directly excited PCs are differently inhibited than the non-excited PCs. Single GC-triggered disynaptic inhibitory postsynaptic events (diIPSCs) provide functional insight into the specificity of the FFI wiring schemes. Therefore, to better understand FFI wiring, we compared the prevalence of diIPSCs between pairs of individually recorded GC axons or somas and PCs, some of which were connected by monosynaptic excitation, while others were not. We found single GC-elicited diIPSCs with similar probabilities irrespective of the presence of monosynaptic excitation. This observation suggests that the wiring of the FFI between individual DG and CA3 principal cells is not specific. Therefore, the randomly distributed FFI contributes to the hippocampal signal sparsification by setting the general excitability of the CA3 depending on the overall activity of GCs.

BRAIN NEUROPEPTIDE INTERACTIONS FOR THE SAKE OF SOCIO-EMOTIONAL BALANCE

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Brain neuropeptides such as oxytocin (OXT), vasopressin (VP), corticotrophin releasing factor (CRF) and neuropeptide S (NPS) are important modulators of socio-emotional behaviours with differential and partly even contrasting effects on anxiety, stress coping, aggression, and various types of social interactions. Selective targeting of these neuropeptidergic systems has appeared to be a promising option for the treatment of psychopathologies, which are mostly accompanied by both emotional and social dysfunctions.
In this context I will present data demonstrating anxiolytic effects of OXT and NPS in a rat model of high emotionality, i.e. in rats selectively bred for high versus low anxiety-related behaviour, and in a murine model of chronic psychosocial stress. Moreover, both OXT and NPS systems play an important role in the extinction of cued fear as shown in models of cued fear conditioning. Further, both OXT and NPS are major players in the promotion of social interactions. For example, social defeat-induced avoidance of conspecifics is reversed by each of these neuropeptides[1,2]. In a mouse model of social fear conditioning (SFC), both OXT and NPS reversed social fear and reinstated social preference behaviour. In case of OXT, this effect has been linked to the lateral septum[3]. We could also show that activation of the endogenous OXT system as seen in lactation is accompanied by lack of SFC-induced social fear. Thus, enhanced OXT signaling specifically in the lateral septum seems responsible for enhanced resilience of lactating mice against trauma.
As OXT and NPS share many behavioural effects, we tested possible interactions between these neuropeptide systems. Thus, NPS activated hypothalamic OXT neurons, which express the NPS receptors. Both NPS and OXT exert an anxiolytic effect directly within the PVN, but in case of NPS, this essentially requires local OXT activity: When local OXT actions were prevented (either pharmacologically or chemogenetically) the anxiolytic effect of NPS was prevented. [4]
Thus, the multiple neuropeptide effects described within the brain in particular modulating socio-emotional behaviours likely rely on their context-specific interactions.

Supported by DFG, BMBF and EU.

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INDUCTION OF ENDOPLASMATIC RETICULUM STRESS BY ALCOHOL IN SEVERAL DIFFERENT TUMOR CELL LINES

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Alcohol is the most widely and frequently used psychoactive drug in the world. The Alcohol dependence causes many social problems, like car accidents, violence, family conflicts, physical and mental abnormalities, which places a financial burden on the society, therefore it is in the centre of the scientific interest. The chronic alcohol consumption leads to dangerous and irreversible changes at cellular level, it induces the endoplasmic reticulum stress (ER) and the programmed cell death (apoptosis) as well. During ER stress, abnormally folded proteins are accumulated in the lumen of the ER and it can lead to apoptosis through the activation of several signal transduction pathways. The CREB transcription factor is a key mediator of the alcohol effects and the addiction. The goal of our study was to examine the function of this leucine-zipper transcription factor in the alcohol induced stress.
Wild type rat pheochromocytoma (wtPC12) and CREB overexpressing stable PC12 cells (wtCREB) were studied after 48 hours long, high concentration (toxic for humans) ethanol treatment. The apoptotic changes of the nuclei were detected by Hoechst staining and DNA fragmentation assay, while the signal transduction pathways were analysed by Western blotting, the cell viability was tested by ATP assay.
Results: A decreased apoptosis was detected in the wtCREB overexpressing cells after 48 hours long treatment during the nuclear fragmentation analysis, while the wtPC12 cell were more sensitive to the same effect. The Western-blotting results showed an increased activation of stress signal transduction pathways in wtPC12 cell line. The expression level of Caspase-3 and proapoptotic Bcl-2 family member proteins was elevated in wtPC12 cells, while under same conditions the antiapoptotic Bcl-2 family member proteins expression was higher in wtCREB cell line. The cell viability assay showed the wtCREB cells less sensitive to 48 hours long high alcohol concentration treatment.
Conclusion: The high concentration ethanol treatment induces the stress pathways and apoptosis in wtPC12 cells, while in wtCREB overexpressing cell line these processes are less activated. Consequently, the CREB transcription factor has an important role in the inhibition of alcohol induced stress.

This work was supported by EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

THE ROLE OF COMT VAL158MET POLYMORPHISM IN PTSD SYMPTOMATOLOGY

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Prefrontal association cortex (PFC) plays an important role in moderating human behaviour, controlling emotions, cognitive and different executive functions. Exposure to stress impairs PFC functions and leads to reckless behaviour, loss of impulse control and the ability to focus attention, all characteristic symptoms of post-traumatic stress disorder (PTSD). Stress affects PFC function by increasing catecholamine release. Catechol-O-methyltransferase (COMT) is one of the enzymes that degrade catecholamine neurotransmitters. A common *COMT* polymorphism, rs4680 (Val158Met), plays an important role in cortical dopamine degradation by affecting COMT enzyme activity. The valine (Val) variant of the *COMT* Val158Met has been associated with greater dopamine degradation and less synaptic dopamine, compared to the methionine (Met) variant. The *COMT* Met/Met genotype has been associated with more efficient PFC activation and better cognitive performance. The study included 455 male war veterans (303 smokers and 152 non-smokers) with current and chronic combat-related PTSD. The Positive and Negative Syndrome Scale (PANSS) was used to evaluate the structure and severity of symptoms in PTSD patients. We assessed three original PANSS subscales (positive, negative and general psychopathology subscales), and additional PANSS derived subscales (excitement, cognitive, psychotic and depressive subscales). Genotyping of the *COMT* Val158Met polymorphism was performed with TaqMan Drug Metabolism Genotyping Assay.
Results showed a significant difference in PANSS total (P=0.027) scores between smokers and non-smokers which could be explained by the self-medication hypothesis. Therefore, all further analyses were done separately for smokers and non-smokers. Opposed to the lack of any association in smokers, significant differences were found between different COMT genotype carriers and the PANSS total (P=0.046), PANSS cognitive (P=0.014), PANSS excitement (P=0.013) and PANSS psychotic (P=0.023) subscale scores in non-smokers. The observed differences were due to the more severe symptoms found in Val/Val carriers than in other genotype carriers. These results were confirmed after comparing non-smokers who were Val allele carries to the Met/Met homozygotes. Higher PANSS total (P=0.017), PANSS cognitive (P=0.015), PANSS excitement (P=0.003) and PANSS psychotic (P=0.006) subscale scores in Val allele carriers compared to Met/Met carriers were detected.
These results indicate that *COMT* Val allele, associated with lower dopamine availability, could contribute to the more severe PTSD symptomatology (including cognitive, psychotic, depressive and mania-like excitement symptoms). These data support the hypothesis that lower dopamine availability, seen in Val allele carriers, leads to reduced hippocampal volume after traumatic exposure and consequently more severe symptoms in PTSD.

This work was supported by Croatian Science Foundation grant IP-2014-09-4289.

SURVIVAL AND REGENERATION OF INJURED MOTONEURONS INDUCED BY GRAFTED NEUROECTODERMAL STEM CELLS FOLLOWING VENTRAL ROOT AVULSION INJURY: THE EFFECT OF VARIOUS ADMINISTRATION ROUTES

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Spinal motoneurons are severely injured and destined to die after a ventral root avulsion injury. Reimplantation of the avulsed ventral root induces limited motoneuron survival and reinnervation. On the other hand, earlier studies from our laboratory have provided evidence that transplantation of a neuroectodermal stem cell line, NE-4C (ATCC: CRL-2925) induces significant motoneuron survival and regeneration of the injured axons, but the effect of different administration routes remained yet to be investigated.
In our experimental model the left lumbar 4 (L4) ventral root of the spinal cord of SD rats was avulsed and reimplanted immediately after injury. Increasing numbers of NE-4C cells were grafted into the L4 segment of the spinal cord or injected into the blood-stream. In control animals only the L4 ventral root was avulsed and reimplanted without stem cell transplantation. After 3 months survival the L4 spinal nerve was labelled with Fast Blue crystals and the transplanted cells were detected by immunohistochemical markers.
The intraspinally grafted stem cells induced a dose-dependent survival and regeneration of the host motoneurons and they differentiated into neurons and astrocytes settled mainly in the injured side of the L4 spinal segment. The motoneuron-rescuing effect was considerably lower in the case of intravenous application without a dose-response effect.
Our results provided evidence that both the intravenous and intraspinal administrations of stem cells are able to promote the survival and regeneration of the host motoneurons, but transplantation of an increased number of stem cells is not effective in the case of intravenous application.

DIFFERENCES IN THE MOLECULAR STRUCTURE OF THE BLOOD-BRAIN BARRIER IN THE CEREBRAL CORTEX AND WHITE MATTER: AN IN SILICO, IN VITRO AND EX VIVO STUDY

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The blood-brain barrier (BBB) is the main interface controlling molecular and cellular traffic between the central nervous system (CNS) and the periphery. It consists of cerebral endothelial cells (CECs) interconnected by continuous tight junctions, and closely associated pericytes and astrocytes. Different parts of the CNS have diverse functions and structure and may be subjects of different pathologies, in which the BBB is actively involved. It is largely unknown however, what are the cellular and molecular differences of the BBB in different regions of the brain. Using *in silico*, *in vitro* and *ex vivo* techniques we compared the expression of BBB-associated genes and proteins (i.e. markers of CECs, brain pericytes and astrocytes) in the cortical grey matter and white matter. *In silico* human database analysis (obtained from recalculated data of the *Allen Brain Atlas*), qPCR, western-blot and immunofluorescence studies on porcine and mouse brain tissue indicated an increased expression of GFAP in astrocytes in the white matter in comparison to the grey matter. We have also found increased expression of genes of the junctional complex of CECs (occludin, claudin-5, α-catenin) in the white matter in comparison to the cerebral cortex. Accordingly, occludin, claudin-5 and α-catenin proteins showed increased expression in CECs of the white matter in comparison to endothelial cells of the cortical grey matter. In parallel, barrier properties of white matter CECs were superior as well. These differences might be important in the pathogenesis of diseases differently affecting distinct regions of the brain.

EXPRESSION OF PATTERN RECOGNITION RECEPTORS AND ACTIVATION OF INFLAMMASOMES IN BRAIN ENDOTHELIAL CELLS AND PERICYTES

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Neuroinflammation is a common characteristic of the majority of central nervous system (CNS) diseases and is dependent on both infiltrating immune cells and intrinsic cells of the brain parenchyma. Most prominent cells of the neurovascular unit (NVU) involved in inflammatory response are microglia, astrocytes and neurons, but cerebral endothelial cells (CECs) and pericytes can also participate in this reaction. Therefore, we systematically tested pattern recognition receptor expression of CECs and brain pericytes. We detected expression of several Toll-like receptors (TLRs) and NOD (nucleotide-binding oligomerization domain)-like receptors (NLRs), among which TLR2, TLR4, TLR6, NOD1, NOD2, NLRC5, NLRP1, NLRP3, NLRP5, NLRP9, NLRP10 and NLRX mRNAs were expressed in both endothelial cells and pericytes. These receptors confer responsiveness to diverse microbial components and cellular danger molecules released upon tissue injury. Activation of TLR2/6 led to an increased BBB permeability accompanied by downregulation of occludin and claudin-5 expression and disappearance of these tight junction proteins from the cell membrane.
In addition, inflammatory cytokines had stimulatory effect on the transcription of many of these receptors, including inflammasome-forming NLRs. Inflammasomes are multiprotein complexes promoting the maturation of some interleukins (ILs), including IL-1beta, one of the most potent inflammatory cytokines. In CECs, expression of key inflammasome components (NOD2, NLRP3 and caspase 1) along with caspase-cleaved interleukins IL-1beta and IL-33 could be induced by priming with lipopolysaccharide (LPS) and activation with muramyl dipeptide (MDP) or adenosine triphosphate (ATP). In addition, combined priming and activation treatment resulted in active IL-1beta secretion in a caspase- and ERK1/2 kinase-dependent manner. However, we could not detect canonical inflammasome activation in pericytes. On the other hand, pericytes secreted active IL-1beta in response to non-canonical inflammasome activation, i.e. intracellular LPS of phagocytized E. coli bacteria or uptaken bacterial outer membrane vesicles.
Our findings demonstrate that pattern recognition receptors and inflammasomes can be activated in endothelial cells and pericytes of the brain. Cells of the NVU might have an important and well-controlled regulatory role in neuroinflammation.

NEUROVASCULAR UNIT ALTERATION IN SOMATOSENSORY CORTEX AND ENHANCEMENT OF THERMAL NOCICEPTION INDUCED BY AMPHETAMINE INVOLVES CENTRAL AT1 RECEPTOR ACTIVATION

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The use of psychostimulants, such as amphetamine (Amph), is associated with inflammatory processes, involving glia and vasculature alterations. Brain Angiotensin II (Ang II), through AT1-receptors (AT1-R), modulates neurotransmission and plays a crucial role in inflammatory responses in brain vasculature and glia. Our aim for the present work was to evaluate the role of AT1-R in long-term alterations induced by repeated exposure to Amph. Astrocyte reactivity, neuronal survival and brain microvascular network were analyzed at the somatosensory cortex. Thermal nociception was evaluated as a physiological outcome of this brain area. Male Wistar rats (250-320g) were administered with AT1-R antagonist Candesartan/vehicle (3mg/kg p.o., days 1-5) and Amph/saline (2.5mg/kg i.p., days 6-10). The four experimental groups were: Veh-Sal, CV-Sal, Veh-Amph, CV-Amph. On day 17, the animals were sacrificed and their brains were processed for Nissl staining and immunohistochemistry against glial fibrillary acidic protein (GFAP) and von Willebrand factor. In another group of animals, thermal nociception was evaluated using hot plate test, in the four experimental groups, on day 17. Data were analyzed with two-way ANOVA followed by Bonferroni test. Our results indicate that Amph exposure induces an increase in: neuronal apoptosis, astrocyte reactivity and microvascular network, evaluated as an augmented occupied area by vessels, branching points and their tortuosity. Moreover, Amph exposure decreased the thermal nociception threshold. Pretreatment with the AT1-R blocker prevented the described alterations induced by this psychostimulant. The decreased thermal nociception and the structural changes in somatosensory cortex could be considered as extended neuroadaptative responses to Amph, involving AT1-R activation.

NECDIN, A NESFATIN-1/NUCB2 BINDING PROTEIN IN THE RAT HYPOTHALAMUS

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Necdin (neuronally differentiated embryonal carcinoma-derived protein) is a growth suppressor, expressed predominantly in postmitotic neurons. It was primarily considered as a nuclear protein, but according to recent data it is also distributed in the neuronal cytoplasm. Necdin has been associated with promotion of mitochondrial biogenesis, hence the prevention of mitochondria-associated neurodegeneration. The absence of the necdin gene in case of 15q-q12 chromosome deletion has been observed in Prader-Willi syndrome, whose main symptoms include compulsive eating and overweight. Interestingly, in vitro studies showed that necdin interacts with a calcium-binding protein NEFA/nucleobindin-2 (NUCB2), the precursor of nesfatin-1. Nesfatin-1 is involved in many homeostatic functions, like regulation of food-intake and energy expenditure, volume and stress regulation. The exact expression pattern of necdin in the brain and its relation to NUCB2 expression is not known. Additionally, physiological roles of necdin remain to be elucidated. Therefore we compared the expression of NUCB2 and necdin mRNAs during postnatal development by radioactive in situ hybridization in the rat hypothalamus, and investigated whether food or water deprivation affects necdin mRNA expression.
Necdin and NUCB2 mRNA expressions observed from the day of birth (day 0) until day 21 showed many similarities. Both peptides were generally expressed in cells on postnatal day 0. The expressions during development became restricted to specific hypothalamic nuclei, including the supraoptic, paraventricular, ventromedial, arcuate and ventral premammilary nuclei. The ependyma was strongly positive for NUCB2 mRNA from day 0, whereas negative for necdin mRNA. NUCB2 mRNA expression in the ventromedial nucleus and in cells located in the optic tract was transient and diminished by weaning. However, necdin mRNA expression was permanently strong in the ventromedial nucleus and absent from the cells in the optic tract. To assess the function of necdin in the hypothalamus, mRNA changes were measured after 48h fasting or water deprivation using quantitative in situ hybridization method. Food deprivation did not cause any alterations in any of the measured areas. A significant increase in necdin mRNA expression was found in the supraoptic nucleus after water-deprivation. Double immunohistochemistry revealed that the majority of the cells here were double positive for necdin and NUCB2/nesfatin-1.
Our data suggest that during postnatal development necdin is coexpressed with NUCB2 in most of the hypothalamic nuclei. We found no evidence supporting participation of necdin in the regulation of food-intake, but it is probably involved in volume regulation. Further investigations are needed to clarify its exact role in fluid homeostasis and the mechanism of action.

Supported by: NKFI K115422 (ZET).

LATERAL HYPOTHALAMUS GABA NEURONS ASSOCIATED WITH GOAL-ORIENTED BEHAVIORS ARE SPECIFICALLY REACTIVATED DURING REM SLEEP

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In mammals, the sleep-wake cycle and feeding are conserved behaviors engaging a broad range of brain regions. The hypothalamus is a key hub for the integration and regulation of these two behaviors as it receives information from central and peripheral origins and modulates brain activity through widespread projections. Here, we investigate how single GABAergic and glutamatergic cells in the lateral hypothalamus (LH^{vgat}, LH^{vglut2}, respectively) modulate food intake, sleep and arousal. We first measure the neuronal activity of LH^{vgat} or LH^{vglut2} neurons during sleep and food intake paradigms by targeting the expression of the calcium sensor GCaMP6s to the LH of vgat-IRES-Cre or BAC-vglut2::Cre mice, respectively, and subsequently imaging Ca^{2+} transients from single neurons with a miniaturized fluorescence microscope in freely-behaving mice. We found that a large proportion of LH^{vgat} and LH^{vglut2} neurons show maximal activity during REM sleep, while other subsets of cells were active during wakefulness. When the animals were subjected to a free-access feeding paradigm we found that most LH^{vgat}neurons showed maximal activity during food approach or -intake, whereas LH^{vglut2} neurons were predominantly active in behaviors unrelated to feeding. When comparing the functional identity of the neurons for sleep and feeding, we found that LH^{vgat} cells associated with food approach or food intake identity were significantly more likely to be active during REM, but not NREM, sleep episodes. No such specialization was observed for LH^{vglut2}neurons. Interestingly, food-approach and food-intake LH^{vgat} neurons showed a sequential activation profile according to the animal behavior, however, they were reactivated during REM sleep in a random fashion, independently of their functional identities.
These findings indicate that LH^{vgat} neurons are multi-functional encoding aspects of both, sleep- and metabolic function. Furthermore they propose that LH^{vgat} neuron activation in REM sleep may modulate circuits for homeostatic integration via non-experience dependent mechanisms.

RECURRENT SYNAPSES BETWEEN CA2 PYRAMIDAL CELLS

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The CA2 region is unique in the hippocampus. CA2 neurons receive strong external input (*Chevaleyre et al.*, 2010) and modify their firing pattern to fit subtle environmental changes (*Mankin et al*., 2015). Moreover, the CA2 region is essential to social memory (*Hitti et al*., 2014), and triggers sharp-wave ripples associated to memory consolidation (*Oliva et al*., 2016). Although recent studies have focused on the function of the CA2 region, its local circuit structure has not been extensively investigated, mostly because the region is too small to deal with. It is assumed that CA2 pyramidal cells have dense recurrent connections, based on their neurite arborizations (*Dudek et al.*, 2016), but electrophysiological evidence is still lacking. Here we used quadruple whole-cell patch-clamp recordings from CA2 pyramidal cells. We found unitary synaptic transmission between CA2 cell pairs. The connection probability was approximately 2.0%. Thus, CA2 pyramidal cells densely connect to each other.

TASK DEMANDS PREDICT A DYNAMIC SWITCH IN THE CONTENT OF AWAKE HIPPOCAMPAL REPLAY

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Reactivation of hippocampal place cell sequences during behavioural immobility and rest has been linked with both memory consolidation and navigational planning. Yet it remains to be investigated whether these functions are temporally segregated; occurring during different behavioural states. During a self-paced spatial task, awake hippocampal replay occurring immediately before movement towards a reward location, or just after arrival at a reward location, preferentially engaged cells consistent with the current trajectory. In contrast, during periods of extended immobility, no such biases were evident. Notably, the occurrence of this switch between task-relevant and less focused reactivations predicted the accuracy of subsequent spatial decisions. Thus, we conclude hippocampal reactivations can dynamically and abruptly switch operational modes during a constant behavioural state in response to task demands, and that such a ‘switch' contributes to accurate spatial behaviour.

IN-VIVO MODULATION OF TRANSFERRIN RECEPTOR PROTEIN-1 BY A COMPLEX VITAMIN MOLECULE NORMALIZES NEUROCHEMICAL SIGNALING AND REDUCES ALZHEIMER'S-TYPE PATHOLOGY

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Metal ions are crucial for normal neurochemical signalling, while neural ions dyshomeostasis and corresponding oxidative impairment are associated with neurodegenerative processes in Alzheimer's disease (AD). Hypothesizing that metal chelators, antioxidants and anti-inflammatory agents could improve disease outcomes via modulation of transferrin receptor protein-1 (TfP-1)-an important metal ion regulator in the brain, we investigated the efficacy of a formulated complex vitamin supplement (CVS) in reversing behavioural deficit, neurochemical signalling impairment and molecular degeneration in rat model of AD. Eight weeks-old Wistar rats were administered CVS (400 mg/kg/day) orally for two weeks before or after AlCl3 (100 mg/kg)-induced neurotoxicity. Rats were assessed for standard behavioural functions mainly related to cognition, learning, memory and anxiety. The prefrontal cortex (PFC), hippocampus and amygdala were prepared for spectrophotometry, histology, histochemistry and immunohistochemistry. Our data showed that CVS significantly reversed reduction of exploratory/working memory ($p<0.05$), frontal-dependent motor deficits ($p<0.01$), cognitive decline ($p<0.005$), memory dysfunction ($p<0.05$) and anxiety ($p<0.01$). These findings correlated with CVS-dependent modulation of TfP-1 expression within the PFC, hippocampus and amygdala that were accompanied by significant reversal of neural oxidative stress in expressed superoxide dismutase ($p<0.01$), neuronal nitric oxide ($p<0.05$), catalase ($p<0.005$), glutathione peroxidase ($p<0.01$) and malondialdehyde ($p<0.01$). We found that through modulation of TfP-1, CVS inhibited neural bioenergetics dysfunction as increased labelling of glucokinase within PFC and hippocampus (but not amygdala) correlated with increased glucose-6-phosphate dehydrogenase ($p<0.01$) and decreased lactate dehydrogenase ($p<0.05$) expressions. These were related to inhibition of over-expressed acetylcholinesterase and increased total protein synthesis ($p<0.05$) in the three cortical areas. Histomorphology of thin sections from understudied brain areas demonstrated in H & E and Nissl stains corroborated roles of CVS in reversing AlCl3-induced AD-like pathology and were accompanied by related changes in astrocytes and neurofilaments (cytoskeleton) immunohistochemical analyses. Overall, we showed that CVS modulates neural TfP-1 overexpression, thereby normalising neurochemical signalling pathways linking concurrent progression of oxidative stress, bioenergetics deficits, synaptic dysfunction, cytoskeletal dysregulation and cellular hypertrophy in AD.

MECHANISMS OF THE HYPERTHERMIC RESPONSE TO PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE IN RODENTS

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Administration of pituitary adenylate cyclase-activating polypeptide (PACAP) into the central nervous system causes an increase in deep body temperature (T_b), but the involved physiological mechanisms need further clarification. To address this issue, we studied the thermal consequences either of PACAP administration or of the absence of PACAP. In our first approach, we infused PACAP intracerebroventricularly to rats and measured their T_b and autonomic thermoeffector responses. We found that PACAP-induced hyperthermia was brought about by increased thermogenesis and tail skin vasoconstriction through a central site of action. In our second approach, we studied how the absence of PACAP influences T_b in mice lacking the *Pacap* gene (*Pacap*$^{-/-}$). The *Pacap*$^{-/-}$ mice had higher locomotor activity throughout the day and elevated deep T_b during the light phase, but their resting metabolic rate and basal T_b were lower compared to their wild-type littermates. The number of c-Fos positive cells was markedly higher in the medial preoptic area (MPO) of *Pacap*$^{-/-}$ mice than in controls, suggesting that the absence of PACAP results in an increased activation of neurons in the MPO. This brain area is known to tonically suppress non-shivering thermogenesis and its neurons also participate in thermal responses to agonists of the transient receptor potential vanilloid-1 (TRPV1) channel. Therefore, we administered PACAP to TRPV1 knockout mice and found that PACAP-induced hyperthermia and hypermetabolism were exaggerated in these animals. These results suggest that TRPV1 channels play a limiting role in the development of the hyperthermic response to PACAP. We conclude that TRPV1-expressing, presumably GABAergic, neurons in the MPO contribute to the development of PACAP-induced hyperthermia by suppressing the magnitude of the response.

This research has been supported by the Medical School, University of Pecs (KA-2016-15), the New National Excellence Program of the Hungarian Ministry of Human Capacities (UNKP-16-4-III), GINOP-2.3.2-15-2016-00050 "PEPSYS", MTA-TKI, and EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pecs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

NEURONAL AND PROLACTIN HORMONAL ACTIVATION IN SUCKLED MOTHER MICE

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Nursing has important consequences on mothers as it contributes to prolactin release, suppression of fertility, reduced responsiveness of the hypothalamo-pituitary-adrenal axis, increased food and fluid intake, reduced anxiety, maternal aggressiveness. Prolactin mediates some of these actions while others are directly regulated by neuronal inputs from the pups. To separate the actions of these different mechanisms, neurons directly activated by prolactin were visualized by pSTAT5 immunohistochemistry in relation to Fos-expressing neurons in suckled mother mice. In response to suckling following a 22 h pup deprivation, we found a markedly elevated number of pSTAT5-containing neurons in numerous brain regions, including the lateral septum, the periventricular, medial preoptic, paraventricular, arcuate and ventromedial nuclei of the hypothalamus, the medial amygdaloid nucleus, the subparafascicular area, the caudal periaqueductal gray, the dorsal raphe, the lateral parabrachial nucleus, and the nucleus of the solitary tract. Suckling also induced Fos expression in all these brain regions except for the arcuate and ventromedial hypothalamic nuclei. We established the degree of co-localization for pSTAT5 and Fos, which ranged from 8 to 80% in the different brain regions. In addition, both pSTAT5 and Fos were also double labeled with estrogen receptor alpha (ERα) in suckled mother mice, which revealed a very high degree of co-localization between pSTAT5 and ERα in several brain regions with much less potential interaction between Fos- and ERα-containing neurons. The results suggest that most neurons responding to suckling in mother rats, and likely to be involved in maternal responsiveness, 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, and estrogen-sensitive neurons are more likely to be affected by prolactin than by direct neuronal activation.

The work was supported by the OTKA K116538 and the KTIA_NAP_B_13-2-2014-0004 Research Grants for AD.

PHARMACOLOGICAL EFFECTS ON REACTION TIME AND PERFORMANCE MEASURES IN A SIMPLE COGNITIVE PARADIGM IN AGED RHESUS MONKEYS

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Cognitive decline and incidence of neurocognitive disorders (NCD) show a great correlation with age posing a growing need for potent disease modifying therapies. For drug-developmental use, valid pharmacological models are vital. To date, only a few studies are available using naturally aged animal models. In the present study, we set out to develop a procedural cognitive test battery parallel in young human participants and in aged non-human primates (NHPs) as their performance is directly comparable and data obtained with NHPs would greatly increase the translational power of non-verbal cognitive tests originally designed for humans. Here we applied the reaction time based psychomotor vigilance task (PVT) on a touch screen system (Cambridge Neuropsychological Test Automated Battery, CANTAB) to investigate general performance indices and pharmacological effects of known cognitive enhancers. In the PVT, a single target-stimulus appearing at a random latency (1 to 9s) had to be selected. Sustained attention was assessed by recording reaction time (RT) and computing overall performance rate (proportion of correct responses, premature responses and lapses). The tests were validated in three aged rhesus macaques by means of systemic treatments with NMDA antagonist memantine or cholinesterase inhibitor donepezil. Results indicate that acute memantine treatment significantly increased RT, without exerting any beneficial effects on performance rate. Furthermore, longer delay to target stimulus did not have any measurable effects on reaction time and did not show interaction with the memantine treatment. The effects of single and repeated dose donepezil will be further evaluated. Based on the present results we conclude that modified psychomotor vigilance task in our touch screen system is suitable for testing novel cognitive enhancing drug candidates in aged rhesus monkeys.

Support: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

FUNCTIONALLY DISTINCT POPULATIONS WITHIN ANATOMICALLY SIMILAR CCK-EXPRESSING HIPPOCAMPAL INTERNEURONS

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Action potential firing of nerve cells show large diversity, but the general notion is that they are homogeneous within a given type. Therefore, the typical firing properties are often used for the identification and functional characterization of recorded neurons. Here we provide evidences for an exception for the cell-type specific firing patterns by demonstrating markedly different firing properties within an anatomically stereotyped group of hippocampal GABAergic cells.
First, using patch-clamp recordings and anatomical verification, we distinguished two populations within CCK-expressing GABAergic interneurons (CCK-IN) in hippocampal acute slices based on state-dependent firing properties. The first subset of CCK-INs (TOR) showed delayed firing patterns during sustained current injections depending on the preceding membrane voltage. The second subset of morphologically indistinguishable CCK-INs did not show state-dependent spike inhibition (non-TOR cells) and their firing was regular, irrespective of the preceding membrane potential preceding the activity. Voltage-clamp recordings revealed that TOR CCK-INs have substantial A-type currents that activate and inactivate at negatively shifted voltages, which potentially explains state-dependent firing. The pharmacological sensitivity and voltage-dependence of this K^+-current were consistent with the properties of Kv4.3 subunit-containing channels. Next, to explore the two subgroups, we analyzed the transcriptome (22376 genes) in individually recorded TOR and nonTOR cells using single cell RNAseq. This analysis suggested a distinguishing marker, SATB1, whose presence was verified in TOR, but not in non-TOR cells using immunolocalization in recorded and functionally characterized neurons. Furthermore, the results revealed that albeit the Kv4.3 RNA and proteins can be similarly detected in TOR and non-TOR CCK-INs, the different levels of auxiliary subunits of Kv4.3 channels, the KChIPs, potentially explain the distinct properties of the A-currents and firing properties of these two CCK-IN subgroups. Finally, using realistic computer simulations we explored how the availability of this low voltage-activated A-current adjusts the excitability of otherwise similar neurons during various physiologically relevant activity regimes, such as theta-modulated excitatory input drives.
Our results revealed a novel level in the diversity of hippocampal circuits by showing that different availability of an A-type K^+-conductance could render different function among anatomically similar CCK-INs.

D2 DOPAMINE RECEPTOR ANTAGONIST SULPIRIDE PREVENTS THE ANXIOLYTIC AND REWARDING EFFECTS OF NEUROTENSIN IN THE VENTRAL PALLIDUM

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Neurotensin (NT) acts as a neurotransmitter and neuromodulator in the central nervous system. Anxiolytic and rewarding effects of activation of ventral pallidal NT receptors has been shown earlier by our research group. It is known, that NT exerts its effects in interaction with dopamine (DA) receptors in several other brain structures, however, DA-NT interactions in the ventral pallidum (VP) have not been investigated yet.
The aim of the present experiments was to investigate, whether antagonism of D2 DA receptors of the VP can modify the above mentioned effects of NT.
The rewarding effect was examined by means of conditioned place preference test. Hundred ng dose of NT induced conditioned place preference. D2 DA receptor antagonist sulpiride in 4 µg dose did not influence place preference by itself (the result did not differ from the control group). Sulpiride pretreatment (15 min before NT), however, prevented the rewarding effect of 100 ng dose of NT.
The anxiety was investigated by means of elevated plus maze test. Anxiolytic effect of NT has been reproduced, i.e. time spent at the open arms and at the end of the open arms was increased by 100 ng NT dose of NT. Sulpiride by itself did not influence anxiety, however sulpiride pertreatment prevented the anxiolytic effect of NT.
Our present results show that the activity of the D2 dopamine receptors is a necessary requirement for both rewarding and anxiolytic effects of NT, because both effects could be prevented by D2 DA receptor antagonist sulpiride.

The project has been supported by the European Union, co-financed by the European Social Fund (EFOP-3.6.1.-16-2016-00004) and the ÚNKP-16-3-III New National Excellence Program of the Ministry of Human Capacities. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

DEPRESSION IDENTIFICATION USING MACHINE LEARNING BASED ON REWARD-RELATED ACTIVATION PATTERNS DURING MUSIC LISTENING

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Depressive disorders are among the leading causes of functional impairment worldwide. Functional abnormalities in dopamine-rich brain areas associated to reward processing are among the most prominent biomarkers identified in past research, probably linked to the anhedonia component of depression. Different types of stimuli have been used to elicit brain reward responses in the fMRI environment and recently, based on evidence from neurasthenics, musical stimuli have also been used to identify reward abnormalities. Only relatively recently Machine Learning (ML) techniques have been applied, as classification models, to fMRI data. In spite of the increased potential of ML over the standard approaches, the number of studies approaching depression diagnosis by means of ML is limited. Furthermore, the number of ML models applied is even more limited. Most of the studies have exploited the applicability of Support Vector Machines (SVM). Other models that have been applied are Relevance Vector Machines and Gaussian Process integrating or not Decision Trees. In the present study, we aimed to test the performance of novel ML models based on Relational Association Rules (RAR) and Gradual Relational Association Rules (GRAR), their very recent extension, in detecting clinical depression based on activity patterns in canonical brain regions implicated in reward processing during a music listening task. A data set consisting of fMRI scans of 19 unmedicated participants with a diagnosis of Major Depressive Disorder (MDD) and 20 never-depressed healthy controls was used. The data set was obtained from the Open fMRI database (accession number ds000171). Data was pre-processed and then analyzed with statistical parametric methods using FSL. A contrast of positive music minus tone was introduced in an independent two sample t test (mixed effects, flame 1, with an uncorrected p=0.05). Parameter estimates were extracted for 131 voxels that indicated a significant activation for the depression > control cope from each participant. Further, two automatic classifiers based on RAR and GRAR have been applied. Their performance have been evaluated using leave-one-out, which is the unanimous evaluation methodology applied in related studies. We obtained an accuracy of 0.9744 for both of them. With RAR we obtained a sensitivity of 0.9474 and a specificity of 1, while with GRAR we obtained a sensitivity of 1 and a specificity of 0.95. The experimental results indicate that both of them have a considerably better accuracy as compared to previous work. Additionally, we have tested an Artificial Neural Network model which also has an experimental performance which exceeds the average performance of the ML methods used in past studies on depression detection using fMRI. Moreover, a comparative evaluation on the present data with the widely used SVM further supports that the novel ML models we propose are promising to be used, in conjunction with fMRI, for depression detectio.

EFFECTS OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE ON SMALL INTESTINAL INT 407 CELLS

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Pituitary adenylate cyclase activating polypeptide (PACAP) is an endogenous neuropeptide having a widespread distribution both in the nervous system and peripheral organs including the gastrointestinal tract. It has been shown to exert actions on intestinal functions, mainly affecting glandular secretion and motility. PACAP has different effects on cell survival depending on the cell type and the applied stimulus. Its influences on small intestinal epithelial cells are not yet elucidated. The aim of the present study was to investigate the effects of PACAP on intestinal epithelial cells having high turnover (INT 407) against different harmful stimuli, such as oxidative stress, in vitro hypoxia and gamma radiation. We tested the effect of PACAP on proliferation and cell survival using MTT assay. Moreover, various cancer-related factors were evaluated by oncology array. PACAP did not influence the proliferation rate of INT 407 cells. Its cell survival-enhancing effect could be detected against oxidative stress, but not against in vitro hypoxia or gamma irradiation. Clonogenic survival assay was performed to analyze the effect of PACAP on clonogenic potential of cells exposed to gamma radiation. Surprisingly, PACAP enhanced the clone-forming ability decrease induced by irradiation. Obtaining further information on the molecular background, western blot analysis of ERK1/2 phosphorylation was performed. Our data showed phospho-ERK1/2 supression of PACAP in irradiated cells. Furthermore, the role of endogenous PACAP against oxidative stress was also investigated performing ADCYAP1 small interfering RNA transfection. We found significant difference in the cell vulnerability between cells undergoing silencing and cells without transfection suggesting the protective role of the endogenously present PACAP against oxidative stress in INT 407 cells. In summary, PACAP seems to be able to exert contradictory effects in INT 407 cells depending on the applied stressor, suggesting its regulatory role in the cellular household.

Supported by: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pecs, PTE AOK Research Grant KA-2016-03, KTIA_13_NAP-A-III/5, Bolyai Janos Scholarship, GINOP-2.3.2-15-2016-00050 "PEPSYS", MTA-TKI. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

SIGNATURES OF HIERARCHICAL INFERENCE IN STIMULUS-DEPENDENCE OF CORRELATIONS IN V1

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Noise correlations in the visual system have a rich structure and display intricate stimulus-dependence. Yet, it has remained unclear how stimulus-dependence of noise correlations might serve efficient processing of environmental stimuli. We argue that in a hierarchically organized model of natural images, which reflects the characteristics of the hierarchical structure of the visual stream, stimulus-dependence of noise correlations is a natural consequence of performing inference on noisy or ambiguous stimuli. Assuming that neuronal activities at different layers of processing represent the presence/absence or intensity of increasingly complex stimulus features, statistical inference of high-level features influences the inference of low-level features by establishing a context, which implies the modulation not only of the mean responses but that of noise correlations as well. We designed experiments to measure the fine structure of noise correlations and to assess the dependence of patterns in correlations on stimulus identity and more broadly on stimulus statistics. Measuring multiunit activity from a population of primary visual cortical neurons in awake behaving monkeys, we show that the fine structure of noise correlations, but not their mean, is dependent on the identity of natural images. Further, we demonstrate that, in line with predictions of hierarchical inference, stimulus-dependence of noise correlations is characteristic of natural images, but this dependence can be eliminated by using synthetic image stimuli in which only statistics relevant to V1 is retained. In a follow-up experiment we also demonstrate that using synthetic images that are characterized by statistics that V2 is sensitive to, the stimulus-specificity of noise correlations can be reestablished. In summary, we demonstrate that stimulus-dependent noise correlations are signatures of optimal processing and systematic changes in noise correlations provide insights into the computational processes taking place in the visual cortical hierarchy.

THE EXPRESSION OF PERINEURONAL NETS IN THE FETAL AND PERINATAL HUMAN BRAIN

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Perineuronal nets (PNNs) are specialized brain extracellular matrix consisting mostly of different chondroitin sulphate proteoglycans that condenses around certain neurons and their processes. Today it is known that the PNNs have a variety of roles in the normal functions of the brain, as well as in many of the brain diseases. In this research, the PNN of the post-mortem human fetal and children brains were visualized using a histochemical staining with Wisteria floribunda agglutinin (WFA), which is a well-established marker of the PNNs. The aim of the study was to determine the time of appearance, the spatial-temporal distribution, and regional allocation in the human telencephalon, focusing primarily on the telencephalic wall, in particular in the transient subplate zone (SP). In the 12th post conception week (PCW) the PNNs are expressed in the pre-subplate zone as a perisomatic staining in the dorso-lateral telencephalic wall. At 16th PCW the characteristic perisomatic staining can be seen around cells mostly in the deep subplate zone of the lateral telencephalic wall. After that, until the 27th PCW, the PNNs can be found scattered throughout the SP, with frontal sections having them expressed more in the dorsal wall regions (presumptive dorsolateral prefrontal region) whereas the caudal ones in the presumptive temporo-lateral regions of the telencephalic wall. At 28th PCW the WFA staining of the PNNs can be seen much stronger than in the previous stages and around more cells all along the SP. After the 33rd PCW the remains of the SP also has the PNNs expressed quite strongly under the entire cortical plate, a pattern observed also postnatally in specimens from 3rd postnatal month. These results show an early prenatal appearance of the PNN that suggest their importance during normal development and their possible involvement in pathogenesis of different diseases and neurological conditions of neurodevelopmental origin if their development underwent perinatal lesson, altogether pointing out directions for future research.

This work is supported by Croatian Science Foundation projects No.5943 (ZP), and UNIZG BM0054 and HIMRICO Adris (NJM)

Keywords: Wisteria floribunda agglutinin, chondroitin-sulphate proteoglycans, subplate zone

ON THE NEUROCOGNITIVE ORIGINS OF HUMAN TOOL USE: TOWARD A REASONING-BASED APPROACH

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Tool use is a defining feature of human species. So, the issue of the underlying neurocognitive bases should be intensively explored. Yet, this issue has received very little interest, notably because of the profound belief that tool use is based on learned motor programs, as if it did not require any reasoning skills (1). In this talk, we will discuss recent advances in neuropsychology and cognitive neurosciences that have contributed to revise the idea that these so-called motor programs are central to tool use.

First, we will present a series of neuropsychological studies that have shown a strong link in left brain-damaged patients between the ability to use everyday tools and novel tools to solve mechanical problems (2). Studies using voxel-based lesion-symptom mapping have also revealed that the left inferior parietal cortex is involved in both everyday and novel tool use (2). These findings suggest that any tool use activity might be supported by a common neurocognitive mechanism.

Second, we will review neuroimaging data on the topic. In a recent meta-analysis including neuroimaging studies in healthy participants, we demonstrated that the cytoarchitectonic area PF, within the left inferior parietal cortex, is preferentially activated when participants have to focus on the mechanical action involving the tool and the object (3). By contrast, judging whether a handgrip is compatible with the manipulation of a tool involves more dorsal areas of the parietal cortex, within the intraparietal sulcus (e.g., phAIP).

Based on these findings, we present a new theoretical approach, namely, the reasoning-based approach to tool use (4). In this framework, technical reasoning is fundamental to understand mechanical actions between tools and objects. The left inferior parietal cortex, particularly the area PF, might play a key role in this reasoning. Furthermore, the ability to select appropriate movements to carry out the mechanical action generated by technical reasoning might involve a production system, mainly located within the intraparietal sulcus. This approach opens new avenues for future research, stressing that human tool use does not need learned motor programs.

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PERTURBED CA2+-DEPENDENT SIGNALING OF DYT2 MUTANTS OF HIPPOCALCIN AS POSSIBLE MECHANISM OF AUTOSOMAL RECESSIVE DYSTONIA DEVELOPMENT

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Dystonia is a common movement disorder characterized by twisting or repetitive movements with or without tremor. Dystonia occurring with no other neurological signs on clinical examination and normal neuroimaging is currently classified as 'primary isolated dystonia'. Recent research has demonstrated that autosomal recessive form of primary isolated dystonia (DYT2) may be developed due to the point mutations in the neuronal Ca2+ sensor (NCS) protein, hippocalcin (HPCA). HPCA translocation from the cytosol to plasma membrane controls many neuronal mechanisms including slow afterhyperpolarization (sAHP) and long-term depression (LTD) that contribute to modulation of neuronal activity and may potentially underlie DYT2. Two HPCA mutations, associated with DYT2, are located in Ca2+-binding domain, EF-hand 2, suggesting perturbed Ca2+-dependent signaling of these mutants compared to wild-type HPCA. To test this hypothesis, we first studied biophysical properties and Ca2+ buffering capabilities of HPCA and its mutants tagged by different fluorescent proteins simultaneously in the HEKa cells. Decay constants and amplitudes of mutant translocation transients to the plasma membrane in response to Ca2+ uncaging were substantially decreased compared to HPCA, indicating that mutations lead to lowering HPCA affinity to Ca2+ simultaneously preserving a mechanism of Ca2+-myristoyl switch. At the same time [Ca2+]i transients were drastically different near basal levels of Ca2+ in case of the mutants, suggesting that mutations might play a role in disturbed Ca2+ regulation. Later we studied Ca2+-dependent translocation of HPCA and its mutants in the same cultured hippocampal neurons. Analysis of mutant translocation transients to the plasma membrane in response to depolarization-induced [Ca2+]i transients yielded similar results to the HEKa cells. Moreover, short bursts of action potentials did not result in translocation of N75K mutant while HPCA did robustly translocate. Stimulating neurons with theta rhythms produced gradual HPCA accumulation in the plasma membrane while mutants couldn't decode this type of neuronal activity. We conclude that HPCA point mutations found under DYT2 dystonia result in loss-of-function in HPCA Ca2+-dependent signaling that may produce abnormalities in patterns of neuronal activity and lead to the development of this disease.

Support:
Grant NASU (# II - 1- 12) to PB
Grant NASU (#67/15-H) to PB
Osteopathic Heritage Research Foundation to VV

METABOLIC CONTROL OF TRANSCRIPTIONAL MEMORY IN THE "LITTLE BRAIN"

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Besides the endodermal epithelial cells, which secrete digestive juices and absorb nutrients, the alimentary canal contains mesodermally-derived smooth muscle and regulatory interstitial cells, as well as neuroectodermal cells of the enteric nervous system (ENS), which together execute the motor functions required for digestion. For its complexity and size, the ENS is often referred to as the "little brain". Control of tonic and phasic smooth muscle contractility by the ENS involves mesenchymal regulatory cells including interstitial cells of Cajal (ICC), the electrical pacemaker cells of the gut, which also mediate cholinergic excitatory and nitrergic inhibitory neuromuscular neurotransmission, and the so-called "fibroblast-like cells" (FLC), which are only involved in purinergic inhibition. ICC depletion commonly underlies gut neuromuscular dysfunction occurring in a variety of conditions and disorders such as diabetes. However, the mechanisms and the fate of ICC remain unclear. Using genetic lineage tracing and ablation, we have identified impaired ICC differentiation from local stem/precursor cells (ICC-SC) and transdifferentiation into FLC as key mechanisms underlying diabetes- and age-associated ICC loss. Central to these phenotypic processes is a reversible transcriptional switch between KIT and PDGFRA receptor tyrosine kinases driving cell type-specific gene expression programs. By integrated transcriptional and multi-parameter epigenomic profiling, creERT2-loxP- and CRISPR-Cas9-mediated genome and epigenome editing, RNA interference, and epigenetic pharmacology, we have identified the super-enhancers underlying the ICC phenotype and their mitotically heritable but reversible repression by polycomb-mediated mechanisms in ICC-SC and transdifferentiating ICC. Our current efforts are directed toward understanding the role of mitochondrial metabolites in setting up the aberrant epigenomic states in the ICC lineage. Our results reveal a metabolic-epigenetic regulation of phenotypic transitions in the ICC lineage and may identify novel therapeutic options for gastrointestinal complications of diabetes.

CURCUMIN INDUCES APOPTOSIS VIA TRPM2 CATION CHANNEL INDEPENDENT PATHWAYS IN A CELLULAR MODEL OF GLIOBLASTOMA

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Transient Receptor Potential (TRP) channels superfamily has mostly calcium (Ca2+) permeable non-selective cation channels. In human, six different subfamilies (Ankyrin, Canonical, Melastatin, Mucolipin, Polycystin and Vanilloid) and 28 subtypes of the channels are expressed. Transient receptor potential melastatin subtype 2 (TRPM2) are widely expressed in central nervous system such as brain, dorsal root ganglia (DRG) neurons, hippocampus as well as other tissues. Calcium (Ca2+) is one of the most important ions for cell survival. Cell viability and cellular functions are ameliorated by balance between cytosolic and extracellular Ca2+ levels. Transient Ca2+ signals are ways of adjusting of the intracellular Ca2+ levels and pathological changes in calcium signaling play important role in the etiology of the neurological diseases and cancer. Intracellular Ca2+ concentration ([Ca2+]i) may change depend on cationic channels activation from intracellular stores or extracellular liquid to cytosol. Curcumin as natural antioxidant shows phenolic structure, synthesized by Curcuma longa L. (turmeric), has powerful non-enzymatically antioxidant effects. Recent studies have showed that curcumin has also anti-cancer activity on different cancer cell types both in vivo and in vitro experiments. Curcumin administration can decrease calcium signaling via TRP channel inhibition and prevent elevation of [Ca2+]i levels. Hence, in this study it was aimed to investigate that effects of various concentrations of curcumin on apoptosis and cell viability (MTT), reactive oxygen species (ROS) production, mitochondrial membrane potential levels, caspase 3 and 9 enzyme activities in DBTRG-05MG glioblastoma cell line. TRPM2 mediated Ca2+ signaling was analyzed by fura-2 method. For this aim, firstly time and dose dependent manner was evaluated by MTT test and four different doses (5, 10, 25 and 50 μM) and 24 h incubation time were determined. We found that curcumin reduces cell viability on the concentration dependent manner. It was also observed that curcumin induces apoptosis via caspase 3 and 9 related pathways. However, it was not found any direct relationship between increased concentrations of curcumin and inhibition or activation of TRPM2 mediated Ca2+ signaling. Cytosolic free calcium concentration was lower in 5 μM group compare to control group. Curcumin plays significant role on decreasing mitochondrial membrane potential and intracellular ROS production. Moreover, curcumin treatment markedly supports intracellular reduced glutathione (GSH) concentrations. In conclusion, in this study we observed that increased curcumin doses strongly inhibit cellular viability and induce apoptosis through TRPM2 cation channel independent caspase 3 and 9 enzyme activity pathways.

CURCUMIN MODULATES OXIDATIVE STRESS INDUCED TRPM2 CHANNEL ACTIVATION, CALCIUM INFLUX AND APOPTOSIS IN SH-SY5Y NEUROBLASTOMA CELLS

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Transient Receptor Potential (TRP) channels are generally calcium ion (Ca^{2+}) permeant cation channels. Transient Receptor Potential Melastatin subtype 2 (TRPM2) is expressed neuronal tissues such as brain, dorsal root ganglia (DRG) and hippocampus. The SH-SY5Y neuroblastoma cells are frequently used as in vitro model of Alzheimer's and Parkinson's diseases. Curcumin, shows phenolic structure, synthesized by *Curcuma longa* L. (Indian saffron), has powerful non-enzymatically antioxidant capacity compared to Vitamin E. Hence, we aimed to investigate that effects of curcumin on TRPM2 cation channel currents by using the Patch-Clamp technique, Ca^{2+} signaling analysis, apoptosis and cell viability (MTT) assays, reactive oxygen species (ROS) production, mitochondrial membrane potential levels, caspase 3 and 9 enzyme activities in TRPM2 transfected SH-SY5Y cells. For this aim, we designed four experimental groups which named; control, curcumin, transfected and transfected + curcumin groups. Cytosolic free Ca^{2+} concentrations was higher in transfected group compared to curcumin and transfected + curcumin group. Moreover, this data examined with whole-cell recordings of single cells in all groups. ROS levels were significantly higher in transfected group than in transfected + curcumin group. Apoptosis levels in transfected + curcumin group lower than in transfected group. Procaspase 3 and 9 expression levels measured by western blotting and caspase 3 and 9 levels by spectrophotometric methods show that TRPM2 transfected cells are more prone to apoptosis. In conclusion, curcumin strongly induces modulator effects on TRPM2 mediated Ca^{2+} influx caused by ROS and caspase 3 and 9 processes in SH-SY5Y neuroblastoma cells.

MICROSTRUCTURAL WHITE MATTER CHANGES IN ALZHEIMER'S DISEASE

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Introduction: Alzheimer's disease is a neurodegenerative disorder characterized by cognitive decline. Current study used diffusion tensor imaging data from the Alzheimer's Disease Neuroimaging Initiative 2 database to examine microstructural white matter changes in individuals with Alzheimer's disease relative to healthy controls.
Methods: Participants data were collected from 23 individuals with Alzheimer's disease and 35 similarly aged controls. Diffusion weighted images were corrected for distortions related to eddy currents. Fractional anisotropy maps were created using dtifit and input into Tract Based Spatial Statistics. Voxel wise statistical analyses were performed using randomise with threshold free cluster enhancement to correct for multiple comparisons. Individuals with Alzheimer's disease were compared to controls.
Results: Tract Based Spatial Statistics revealed that individuals with Alzheimer's disease had reduced fractional anisotropy and increased mean and radial diffusivity relative to controls in the left and right anterior thalamic radiation, minor and major forceps, left corticospinal tract. In the right corticospinal tract we saw only increased radial diffusivity and decreased fractional anisotropy relative to controls.
Conclusions: Diffusion tensor imaging holds potential as an Alzheimer's disease biomarker given its sensitivity to detect microstructural white matter changes in the brain. Because of Tract Based Spatial Statistics limitations, further diffusion tractography will be necessary for analyzing fibers integrity more accurate.

EFFECT OF RILUZOLE TREATMENT ON THE EXPRESSION OF KCC2 IN INJURED MOTONEURONS

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The K+-Cl-cotransporter-2 (KCC2) is a well known member of the electroneutral cation-chloride cotransporters. 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 cytoplasmatic and membrane-bound KCC2 expression of injured motoneurons with or without riluzole treatment.
In our experimental model the left lumbar 4 (L4) ventral root of the spinal cord was avulsed. Animals were treated with riluzole (4 mg/kg) for 2 weeks. Riluzole is a compound that acts to block voltage-activated Na+ and Ca2+ channels. 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 ventral root was avulsed without riluzole treatment. Expression of KCC2 in the injured motoneurons and in the affected side of the L4 spinal segment were detected 5, 10, 16, and 21 days after the injury with immunohistochemistry followed by confocal microscopy and dSTORM imaging.
In the affected L4 spinal segment, strikingly different immunostaining patterns were observed between the groups. 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 ventral horn in both groups. A dense uniform KCC2 labelling was detected in the the dorsal horn at each time point. To examine further the cytoplasmatic and plasmalemmal expression intensity of KCC2, we used dSTORM to image KCC2 in injured motoneurons. The quantitative analysis of mean fluorescence cytoplasmatic signal revealed that KCC2 staining remained stable in the injured motoneurons of both groups 5 and 10 days after the injury. and decreased from day 16 in the injured group. In contrast, the cytoplasmatic staining intensity increased in injured motoneunons of riluzole-treated animals on day 16 and 21 after the injury. Stronger KCC2 intensity was present in the membranes of injured motoneurons treated with riluzole on day 5 after the injury compared to controls. At later time points KCC2 expression was sporadic or could not be located in the plasma membrane in both groups.
Taking together, the present results indicate that pharmacological blockade of voltage activated Na+ and Ca2+ channels increases the cytoplasmatic density of KCC2 in injured motoneurons but does not influence the membrane expression of KCC2 during the examined period of time.

MPTP TREATED MICE RESPOND POSITIVELY TO PHYSICAL TRAINING AND DISPLAY NO MOTOR IMPAIRMENT

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Introduction: Motor impairment is fundamental feature of Parkinson's disease (PD). There are several reports on the beneficial effect of physical training on the PD symptoms reduction, however the mechanisms underlying this improvement are not known. The detection and experimental quantification of mouse motor impairments that truthfully mimic the anomalies of Parkinsonian patients has proved difficult. One of the most reliable means for the induction of extensive loss of dopaminergic neurons in the substantia nigra pars compacta is the systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in both rodents and primates alike. In contrast to robust pathological phenotypes following MPTP exposure, deficits in movement and motoric actions are hardly discernible in mice engaged in simple motor tasks, even though frank cell loss in the substantia nigra may level at more than 80%.
Aim: The purpose of the study was to estimate the extent of motor disorders in chronic MPTP mice model of parkinsonism and examine the efficacy of physical training in reversing the expected motor impairment.
Method: C57BL/6 mice were treated for five weeks with 12,5 mg/kg MPTP in combination with 250 mg/kg probenecid. Mice were subdivided into: (1) control sedentary; (2) control trained (10 weeks), (3) MPTP sedentary (non-exercised with PD); (4) early trained MPTP (10 weeks: before, during, and after the induction of PD), and (5) late trained MPTP (10 weeks, started after the induction of PD). To assess motor performance rotarod, open field and inverted horizontal grid tests were performed before MPTP treatment, after the completion of intoxication and when the training was finished.
Results: MPTP did not impair motor function. Only MPTP early trained mice at each stage of the rotarod test were able to increase their velocity. In open field test MPTP mice, which exercised on the treadmill (early and late training) increased the total moved distance. In horizontal grid test MPTP exercised mice increased mean step velocity of hind paws (early and late training) and total number of steps (only early training). Some enhancement of motor skills in rotarod test was observed also for MPTP non-exercised mice. In horizontal grid test the only parameter significantly influenced by MPTP treatment was the total number of touches and we did not observe the impact of physical training on the reduction of this parameter.
Conclusions: We did not observe the impact of either MPTP or physical training alone on motor performance in mice model of parkinsonism. However, there has been a certain improvement in some of the motor parameters in both groups of MPTP treated mice, which performed physical training. Surprisingly, enhancement of motor learning and function in mice occurred only when MPTP treatment and physical training were applied jointly but none when applied separately.

Sponsored by NCN UMO-2014/15/B/NZ4/05041 grant and WisTa Laboratories Ltd.

EVALUATION OF EPIDERMAL NEURAL CREST STEM CELLS IN THE INJURED ORGANOTYPIC SPINAL CORD SLICE CULTURE

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Spinal cord injury (SCI) is a devastating condition causing long lasting consequences. Among various therapeutic strategies employed for SCI, stem cell therapy is a potential treatment. By far variety of stem cells have been evaluated which epidermal neural crest stem cells (EPI-NCSCs) is one of the attractive types. Although these multipotent stem cells have been assessed in several SCI models, so many works remain to be done to clarify all aspects of its therapeutic effects. Here, EPI-NCSCs in combination with valproic acid (VPA), a well-known histone deacetylase inhibitor was evaluated in ex vivo model of injury. To do so, the contusion was stimulated in organotypic spinal cord slice cultures. Subsequently, 5 μM VPA was administered to the injured slices one hour after injury. Then, green fluorescent protein- expressing EPI-NCSCs were grafted following treatment with the VPA. The treated slices were assessed with immunohistochemistry and immunoblotting seven days after transplantation. Obtained data revealed that grafted stem cells can survive on the injured slices and express GFAP- traditional astrocyte marker- while did not express any detectable level of doublecortin- neural progenitor marker- which was common marker ahead of transplantation. Also immunoblotting revealed significant increased expression of GFAP, BDNF, NT-3 (neurotrophin-3) and Bcl2 in injured slices treated with stem cells alone or combination of stem cells and VPA. This study illustrated that EPI-NCSCs transplantation in the ex vivo model of injury can increase neurotrophic and neuroprotective factors which in turn may provide a hospitable context and contribute to promotion of axonal regeneration.

MITOPHAGY, A POTENTIAL RECIPE TO MITIGATE MONOCROTOPHOS INDUCED ALTERATIONS IN MITOCHONDRIAL DYNAMICS AND SUBSEQUENT APOPTOSIS IN NEURAL STEM CELL DERIVED NEURONAL CELLS

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The regulatory dynamics of mitochondria comprises of well orchestrated turnover and selective removal of damaged mitochondria via mitophagy to maintain the mitochondrial and cellular homeostasis. Several pieces of evidence suggest impaired mitochondrial dynamics and its association with the pathogenesis of neurodegenerative disorders. Mitochondrial mediated apoptosis has been strongly implicated in Monocrotophos (MCP) mediated neurotoxicity, which is a widely used Organophosphate pesticide (OP). In the present investigation, MCP induced mitochondrial damage, the role and mechanism of mitophagy was extensively studied using Neural stem cell (NSC) derived neuronal cells. Neuronal cells challenged with MCP revealed mitochondrial potential disruptions mediated by alterations in mitochondrial complex protein levels, cellular ATP depletion and elevated reactive oxygen species generation. In addition, the cells exhibited extensive characteristics of autophagic proteolysis including increased LC3-I to LC3-II conversion and activation of the canonical autophagic pathway as denoted by increase in autophagosome formation and autophagy regulatory elements, including Beclin1, ATG5-ATG12 and AMPK which was further alleviated by pre-treatment with the pharmacological activator of mitophagy, rapamycin and abolished using 3-methyladenine, an inhibitor of mitophagy. We further explored the mechanisms underlying MCP induced mitochondrial dynamics disruption and mitophagy. MCP triggered increased mitochondrial translocation of the dynamin-related protein (Drp1) with a stimulated expression of PINK1 and Parkin facilitating the subsequent degradation of Mitofusin 2, a mediator of mitochondrial fusion. Likewise, mitophagy inhibition increased mitochondrial fission and cell death, whereas stimulation rendered the opposite results, placing mitophagy as a cytoprotective response aimed at eliminating damaged mitochondria. Together, these findings indicate that MCP impairs mitophagy mediated mitochondrial turnover, promoting aberrant mitochondrial dynamics leading to increased oxidative stress, mitochondrial fragmentation, and apoptosis in NSC derived neuronal cells and induction of mitophagy could serve as a curative strategy for retarding OP induced neurological disorders.

HU210 DOSE DEPENDENTLY INDUCES THE DOWNREGULATION OF THE CB1 CANNABINOID RECEPTOR IN RAT RETINA

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Endocannabinoids and the synthetic cannabinoid HU210 have been shown to protect the retina in the *in vivo* model of AMPA excitotoxicity via activation of the CB1 receptor (CB1R) and its downstream PI3K/Akt signaling pathway (Kokona & Thermos, 2015). However, chronic exposure of the synthetic cannabinoid HU-210 has been shown to induce downregulation of the CB1R in several brain regions (Dalton et al., 2009), but to date, there is no evidence to suggest cannabinoid induced CB1R downregulation in the retina. The aim of this study was to investigate whether subchronic or chronic administration of HU-210 a) leads to downregulation of the CB1R in healthy rat retina and b) affects its neuroprotective properties against AMPA excitotoxicity. Sprague-Dawley rats were administered intraperitoneally (i.p) with HU-210 (25, 50 and 100 μg/kg) daily for 4 or 14 days (subchronic or chronic administration) or with vehicle for 13 days and HU-210 on the 14^{th} day (acute administration). Immunohistochemical studies and western blot analysis were performed to examine the downregulation of the CB1R and the phosphorylation of Akt protein. The *in vivo* model of AMPA excitotoxicity was employed to examine whether the subchronic effects of HU-210 on CB1 receptor expression influenced its neuroprotection of retinal amacrine cells. HU-210 (10^{-6} M) was either co-injected with AMPA (8.4 mM) intravitreally or administered i.p (25, 50 μg/kg, 4 days) 24h after AMPA injection. CB1R immunoreactivity (CB1R-IR) was localized in ganglion cells (colocalized with β-tubulin), in the inner plexiform, inner nuclear and outer plexiform layers. HU-210 (25, 50 and 100 μg/kg, i.p, 4 or 14 days) decreased CB1R-IR, in a dose-dependent manner. The attenuation of CB1R expression was also confirmed using western blot analysis. HU-210, at the lowest dose of 25μg/kg, did not affect CB1R expression. Chronic treatment with HU-210 (50 and 100 μg/kg) also led to the reduction of Akt phosphorylation. Subchronic administration of HU-210, at the dose of 25 but not 50 μg/kg, afforded neuroprotection to retinal amacrine cells against AMPA excitotoxicity, in agreement with the neuroprotection observed after its intravitreal co-administration with AMPA. CB1R expression was not affected by AMPA. This study provides novel information regarding the time- and dose-dependent effect of HU-210 on CB1R downregulation in rat retina. In the AMPA excitotoxicity model, only the smaller dose of HU-210 (25μg/mg) afforded neuroprotection, in agreement with its inability to downregulate the CB1 receptor. These results suggest that HU-210, when administered in doses higher than 25μg/mg (i.p) leads to the downregulation of the receptor and abrogates its neuroprotection. Further studies are essential using different a) models of retinopathy and b) routes of administration of HU210 to assess its neuroprotective efficacy in chronic retinal diseases.

SEARCHING FOR SITES AND NEURAL CORRELATES OF CYCLOPEAN PERCEPTION

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The nervous system brings together the independent signals from the two eyes into a single representation of objects in the visual world around us. Helmholtz and Julesz termed this process 'cyclopean perception', referring to the mythical single-eyed giants (Cyclops) of classical Greece. I shall review recent progress towards identifying the neural sites and correlates of cyclopean perception, using data from single-neuron physiology in non-human primates.
Stereoscopic depth is capable of resolving the inherent ambiguity of structure from motion figures. Macaque monkeys were trained to discriminate the direction of rotation of a moving transparent 3-D cylinder by specifying the rotation with a binocular disparity signal. We recorded from single neurons and the neighbouring multi-unit activity in cortical area V5/MT, whilst the animals performed this task. We initially observed that the correlation between the isolated single neuron and its neighbours increased with the choice-related signalling in the cylinder judgment task. On further exploration, we found that with interleaved trials of random motion stimuli, to which the animals did not make any perceptual decision, the levels of interneuronal correlation were similar to those reported in earlier studies of choice-related neuronal signalling of motion discrimination. We further found that interneuronal correlation grows systematically during the 2 second trial of observation of the structure from motion cylinder stimulus and that these correlations are greatest for the most perceptually ambiguous figures.
Whilst there is little evidence to support the idea of a single neuronal site for cyclopean perception, we conclude that correlated activity in small groups of neurons appears to rise around the time of the stereoscopic decision event. Higher interneuronal correlations are associated with more difficult perceptual decisions that are focused within a smaller pool of neurons. These correlations may lead to a tighter linkage between neural firing dynamics and perceptual choice, as predicted by recent computational models of neuronal populations and perceptual decisions.

RAPID SCREENING OF ANTIEPILEPTIC DRUGS IN A GENETIC ABSENCE EPILEPSY MODEL BY BURST-SUPPRESSION EEG REACTIVITY

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Absence seizures are characterized behaviorally by a paroxysmal loss of consciousness of abrupt and sudden onset and offset that is associated with bursts of bilaterally synchronous spike-and-wave discharges (SWD) in the electroencephalogram (EEG). Wistar Albino Glaxo Rijswijk (WAG/Rij) rats are a widely used experimental model of absence epilepsy. EEG recordings in WAG/Rij indicate occasional SWDs consisting of bursts lasting about 6 seconds with a frequency of 9 Hz. The potency of antiepileptic treatments is typically screened in WAG/Rij by monitoring the changes in SWD occurrence. Nevertheless, the reliability of this experimental paradigm critically depends on long telemetric recordings as well as tedious sleep-cycle analysis. Here we aimed to investigate acutely the brain excitability changes in WAG/Rij during standardized deep anesthesia. A burst-suppression (BS) EEG pattern induced by isoflurane at a suppression ratio of 40-80%, abolished the occurrence of SWDs. Nevertheless, BS reactivity, assessed as the reduction in suppression ratio that occurred during intermittent photic stimulation (2 seconds interstimulus interval) for 1 minute, was increased in WAG/Rij rats as compared to age-matched Wistar rat controls. The BS reactivity of WAG/Ri was reduced after ethosuximide and paradoxically increased after carbamazepine at doses that had no effect on controls. Our data suggest that BS reactivity measures could be for used for rapid in vivo screening of antiepileptic drugs as well as for monitoring the epileptic brain excitability in medically induced coma.

This work was supported by grants of the Romanian National Authority for Scientific Research, Project PN-III-P2-2.1-PTE-2016-0114.

DIAZOXIDE EQUALLY REDUCES MICROGLIAL ACTIVATION IN MOTOR NUCLEI WITH DIFFERENT SUSCEPTIBILITY AFTER ACUTE INJURY OF MOTOR AXONS

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Background: In our previous study, by comparative analysis of intracellular calcium of motor neurons with different calcium buffering capacity we showed that axotomy induced higher increase of intracellular calcium in motor neurons with lower buffering capacity. This notion initiated a series of experiments to investigate if motor neurons could be protected by increasing their intracellular calcium buffers. Indeed, by increasing motoneuronal parvalbumin content, intracellular calcium elevation evoked by injury could be prevented, furthermore, the local microglial reaction could be moderately decreased. To investigate if injury induced inflammatory reaction could be further reduced by mutually targeting microglia activation and stabilization of intracellular calcium, in the present study, the effect of diazoxide treatment on microglia activation was tested in axotomy experiments, since besides its anti-inflammatory effect, diazoxide was shown to protect neuronal mitochondria from calcium overload in our previous hypoxia-reperfusion experiments.
Methods: Male Balb/c mice were assigned to three groups (n=10 in each group) according to the type of surgery: unilateral eye enucleation, hypoglossal, or facial nerve axotomy. Five animals in each group were treated with diazoxide, five mice served as controls. Diazoxide (1 mg/kg body weight) was administered daily by intraperitoneal injection for one week before surgery and for four days postoperatively until animals were sacrificed. A combination of photostable diaminobenzidine- and immunofluorescence based immunocytochemistry was developed and used to visualize microglial cells (Iba1) and motor neurons (ChAT). The procedure allows the quantification of area density of microglial cells without jeopardizing the standard efficacy of counting due to unavoidable fading of fluorescent signal, and simultaneous precise delineation of motor nuclei. To determine the relative increase of the density of microglial cells due to axotomy, the unoperated side was used in each case as an internal control. Average increases of microglial density were determined for each animal.
Results: Such injury of motor axons resulted in increase of microglial density in each motor nuclei (expressed as mean±s.e.m.): 26.11±3.14 in Nucl. XII, 37.46±2.90 in Nucl. VII, 15.37±3.69 in Nucl. III. The microglial activation was significantly smaller in the oculomotor nucleus compared to the other nuclei ($p<0.01$). Diazoxide treatment significantly ($p<<0.01$) reduced the lesion induced microglial activation in each motor nuclei resulting in the following density increase values: 3.49±1.78 in Nucl. XII, 0.34±0.28 in Nucl. VII, 4.62±0.93 in Nucl. III.
Conclusions: Photostable labeling of microglial cells allowed quantification of their density in fluorescently marked anatomical regions, which revealed that diazoxide pretreatment equally prevented microglial activation in motor nuclei with different susceptibility to injury.

THE ROLE OF TRANSIENT RECEPTOR POTENTIAL ANKYRIN1 RECEPTOR IN B-AMYLOID1-42-INDUCED ALZHEIMER'S DISEASE

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Background: Alzheimer's disease (AD) is a neurodegenerative condition causing severe dementia. The basal forebrain cholinergic (BFC) neurons play essential roles in memory and learning, they are highly affected in AD. The undegradable β-amyloid oligomer plaques cause neuronal damage in the substantia innominata-nucleus basalis magnocellularis complex (SI-NBM) and somatosensory cortical projection areas of the BFC neurons.
Expression of the Transient Receptor Potential Ankyrin1 (TRPA1) receptors has been demonstrated in nociceptive primary sensory neurons and astrocytes, however their role in degenerative CNS diseases such as AD is still unclear. Our aim was to study the role of TRPA1 in AD. For this purpose we applied β-amyloid$_{1-42}$ (Aβ_{1-42}) peptide into the SI-NBM of TRPA1 receptor-gene deleted mice.
Methods: Real time quantitative PCR and Western blot methods were used to determine the expression of TRPA1 receptor in cortex and SI. The investigation of the presence of TRPA1 receptor on astrocytes was performed with immunhistochemical staining (the astrocyta marker GFAP antibody was used). Aβ_{1-42} (300μM) was injected (0.1μl/min) into SI-NBM of the right hemisphere of adult male wild type (WT) and TRPA1 knock out (TRPA1 KO) mice using stereotaxic microinjection method. The loss of cholinergic fibres was visualized by acetylcholinesterase histochemical staining. The cholinergic cell body loss in the SI was detected with choline-acetyltransferase (ChAT; 1:2000, Merck) immunohistochemistry. Novel object recognition test and radial arm maze test were used to investigate the decline of memory and learning skills. These tests were performed on naive control-, vehicle injected- and Aβ_{1-42} injected groups.
Results: TRPA1 expression was found in the somatosensory cortex and SI both on mRNS and protein level. The immunohistochemical staining showed the colocalization of TRPA1 and astrocytes in cortex and SI region. Cholinergic fibre loss was detected in the ipsilateral somatosensory cortex (IV-V. layers) following Aβ_{1-42} injection into SI-NBM. Loss of cholinergic fibres was 30.56% in WT mice, but it was significantly attenuated, only 1.56% in TRPA1 KO animals. Similar results were observed in cell body loss with ChAT-ir of the SI-NBM. In the *in vivo* studies significant memory loss was detected in WT Aβ_{1-42} injected mice but not in TRPA1 KO Aβ_{1-42} injected group.
Conclusion: Our findings demonstrate that TRPA1 receptors play role in the Aβ_{1-42}-induced cholinergic damage in the SI-NBM and these receptors may be implicated in the pathogenesis of AD.

Support: Richter Gedeon Talentum Foundation, EFOP-3.6.1-16-2016-00004, GINOP-2.3.2-15-2016-00050 - PEPSYS, GINOP-2.3.2-15-2016-00048 - STAY ALIVE Hungarian Brain Research Program KTIA_13_NAP-A-III/4-5, KTIA_NAP_13-2-2014-001, OTKA (#112807), RG-IPI-2016 TP10/042. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

EFFECTS OF CAFFEINE IN A DELAYED MATCHING WORKING MEMORY TASK IN HEALTHY HUMAN VOLUNTEERS

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The prevalence of neurocognitive disorders among elderly people is rapidly increasing as the general population ages. In early stages of dementia there is typically subtle loss of memory abilities, whereas everyday function and quality of life remain relatively intact.
The Cambridge Neuropsychological Test Automated Battery (CANTAB) based tasks are the most validated and widely used computerized measures of cognitive function. With the Delayed Matching to Sample (DMTS) task we can study the short-term visual recognition memory for non-verbal stimulus patterns. Our aim was to examine how caffeine, the most widely used cognitive enhancer substance may affect working memory in the healthy young population. We conducted a double blind placebo-controlled, randomized within-subject study design where participants were given caffeine (3 mg/kg) or placebo in two separate sessions with at least one week interval between the sessions. The DMTS trial had two phases. In the first phase, participants were shown a single complex non-verbal pattern (sample), followed by a given delay. The delay was followed by a multiple choice pattern (second phase, choice). The participant had to select one from the choice patterns (target) which exactly matched the sample. We used two different task variations: in the Delay task, we manipulated the time between the presentation of the sample and the choice: We used short (1-1,9 sec), medium (15-33 sec) and long (40-76 sec) delay intervals. In the Distractor task, the delay was kept constant (5 sec), and we manipulated the number of distracting stimuli in the choice phase (1, 3 or 5 simultaneous distractor patterns). In both task arrangements, we examined the Reaction Time (RT) to the choice pattern and the overall task performance rate.
In the Delay task we found that increasing the interval between the presentation of the sample and the choice patterns has a significant increasing effect on RT ($F(2, 26)=74.402$, $p=.000$). We also find that caffeine has a specific speeding effect in case of long delay intervals ($F(2, 26)=4.949$, $p=.015$). In the Distractor task, we also found that the increase in number of distractors significantly increased RT ($F(2, 32)=347.91$, $p=0.000$) with no specific effect of caffeine treatment. Performance rate was not affected by any task parameters of pharmacological manipulations. Results conclude, that caffeine specifically enhances cognitive performance speed of healthy individuals in task situations when the delay but not the difficulty of memory retention matters.

EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

CHARACTERIZATION OF THE BEHAVIOURAL AND ELECTROPHYSIOLOGICAL EFFECTS OF INTRACEREBROVENTRICULAR DIMETHYL SULFOXIDE INJECTION: A PRELIMINARY STUDY

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Dimethyl sulfoxide (DMSO), an organosulfur compound is often used in medical research as vehicle for water-insoluble drugs. While DMSO is a non-toxic solvent, it might have pleiotropic effects introducing bias in experiments and effects of DMSO might be falsely attributed to the drug. For example, on one hand, an earlier report suggests that DMSO suppresses N-methyl-D-aspartate (NMDA), and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate-induced ion currents and calcium influx in cell cultures. On the other hand, however, multiple reports failed to found effects of intracerebroventricularly (ICV) administered DMSO, even at high doses. Our aim was to further elaborate on this question in vivo by comparing the effects of DMSO and the non-specific, use-dependent NMDA antagonist ketamine using electroencephalography (EEG) and behavioural observations in freely moving rats. ICV injection of ketamine (100µg, 250µg, and 500µg, n = 2) induced dose-dependent behavioural activation, characterized by hyperlocomotion. The EEG effects of ICV ketamine included increased theta (4-12Hz), and gamma (40-100Hz) oscillations. Subcutaneously administered ketamine (5mg/kg, 15mg/kg, 30mg/kg, n = 4) evoked additional dose-dependent effects, including ataxia and increased high-frequency oscillation (160Hz). Contrary to these observations, ICV administered DMSO (100%, 10µl, n = 4) neither evoke behavioural nor EEG effects compared to control (ACSF, n = 4). These observations suggest that DMSO can be used as a vehicle for drug administration in vivo.
Results of this study suggest that ICV administration of DMSO did not have detectable effect on EEG activity.

IGSF9-EGFP MICE ALLOW TARGETED-STIMULATION OF CLIMBING FIBERS

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Climbing fibers (CFs) are neuronal projections of the inferior olivary nuclei (ION) innervating the cerebellum where they form synapses with Purkinje cells (PC) and other neuronal cell types. Typically, CF responses in PCs are identified by their large amplitude, their all-or-none behavior, a step-wise response curve (SRC), and paired-pulse depression (PPD)[1]. Synaptic properties in other neuronal targets of CFs have not been studied so far. Immature PCs are innervated by multiple CFs resulting in small responses, varying levels of PPD, and graded SRCs complicating the stimulation of single CFs. In *Igsf9*-eGFP mice a subset of CFs is labelled by green fluorescent protein (GFP)[2]. GFP labelling and hence visual identification of CFs in *Igsf9*-eGFP mice solves the problem of identifying CFs by allowing targeted stimulation of eGFP-positive fibers.
First we aimed to characterize the origin of GFP-labelled CFs in the ION and to study their innervation pattern in the cerebellum in the course of postnatal development. Second, we tested targeted stimulation of visually-identified GFP-labelled CFs in *Igsf9*-eGFP mice.
Immunohistochemistry showed that all three major divisions of the ION give rise to GFP-labelled afferents. GFP-labelled CFs innervate the cerebellar lobules in a caudal-to-rostral gradient and the ventral part of the dentate deep cerebellar nucleus. Postnatal development is undisturbed in the mouse line. Specifically, GFP-labelled CFs show a normal maturation with 'creeper' and 'nested' stages and a normal CF translocation as well as elimination of all but one CF. Targeted stimulation of visually-identified CFs is possible in immature and mature cerebellum. CF responses in PCs of *Igsf9*-eGFP mice show normal electrophysiological characteristics (PPD, SRC). *Igsf9*-eGFP mice are an ideal tool for studying CFs responses in multiple-innervated PCs as well as in other neuronal targets of CFs.

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ESTIMATION AND FITTING OF HAWKES' SELF-EXCITING POINT PROCESSES TO EEG DATA OF EPILEPTIC PATIENTS

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Objectives: Regarding the inherent stochasticity in brain dynamics and the noisiness of our experiments, theory of stochastic point processes might be a helpful tool when analyzing neural data. On the other hand, it is assumed, that the dynamics of epileptic seizures can be modeled by the interaction of small events that coalesce and lead to macroscopically observable phenomena. Self-exciting point processes introduced by A. G. Hawkes incorporate both features, making it a good candidate to better understand the hidden dynamics of seizure initiation. Our aim is to elaborate a method to estimate the parameters of the process and fit it to the EEG data of epileptic patients.
Materials and Methods: As a primary step, we developed a simulation algorithm of the process based on its dynamic representation. The method of the estimation of the process' three parameters was based on the maximum likelihood estimation (MLE) approach of T. Ozaki. Implementation was carried out using MATLAB (MathWorks Inc.). Simulation and estimation of the processes were done on a wide range of the parameter space. The performance of the MLE was characterized based on the normalized distances of the simulated and estimated parameters.
Result and Discussion: Results of our simulations are consistent with the ones of T. Ozaki and we also achieved a precise estimate of parameters in a large region. However, we received poor estimates in some regions. To reveal its cause, we inspected cross-sections of the cost-function of the MLE in these regions and found that these correspond to degenerate places where the model relies mostly on one parameter instead of three. We also found that this prominent parameter was estimated in these regions with high precision.

GLUCOCORTICOID SIGNALING IN THE CORTEX OF AGED RATS - THE EFFECT OF FOOD RESTRICTION

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Diminished glucocorticoid signaling is associated with an age-related decline in brain functioning. Food restriction is the only intervention known to delay the aging and improve cognitive performance. Although animals subjected to food restriction show elevated serum glucocorticoid level, its influence on glucocorticoid signaling was poorly investigated. We examined the effect of long term intermittent, every other day (EOD) feeding on the glucocorticoid hormone/glucocorticoid receptor (GR) system in the cortex of middle aged and aged rats.
Male Wistar rats were fed ad libitum (AL), or with the 100% of mean daily intake of AL fed animals every other day starting from 6 months of age (EOD). The cortical level of corticosterone and the expression of GR, together with changes in the level of the GR phosphorylated at Ser232 (pGR) and additional proteins involved in glucocorticoid signalling were examined in 18- and 24- month-old animals.
In aged ad libitum-fed rats, no change in the level of total GR and pGR was detected. Conversely, aged rats subjected to EOD feeding showed a higher content of cortical corticosterone and an increase in the level of enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), a major determinant of glucocorticoid access to receptors in the brain. These changes were accompanied by increased Sgk-1 and decreased GFAP transcription, pointing to upregulated transcriptional activity of GR. The most prominent EOD feeding-induced changes were observed in the cortex of 24-month-old animals.
The findings suggest that food restriction can alter the responsiveness of brain cells to glucocorticoids that may contribute to beneficial effects of EOD feeding on neuronal plasticity and survival.

ROLES OF TRPV1 AND TRPA1 ION CHANNELS IN NOXIOUS HEAT RESPONSIVENESS: AN OLD QUESTION WITH NEW ANSWERS

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Noxious heat stimuli are detected by dedicated ion channels expressed in polymodal nociceptors. The first identified member of this channel family is TRPV1 which is activated in transfected cells by temperatures above 43°C. This activation threshold is similar to that of the cell body and peripheral terminals of polymodal nociceptors including cutaneous nociceptors in man, suggesting that the noxious heat threshold is predominantly determined by TRPV1. In accord, the ionic current evoked by noxious heat in cell bodies of primary sensory neurons depended on TRPV1 as revealed in TRPV1 knockout mice. However, the heat threshold of the peripheral terminals of cutaneous polymodal nociceptors was similar in TRPV1 knockout and wild-type mice as studied by single fiber recording. Similarly, in the conventional tests of thermonociception measuring latency of nocifensive reaction evoked by a suprathreshold heat stimulus (hot plate, tail-flick, Hargreaves' plantar test), no latency prolongation was observed in TRPV1 knockout mice or in rats treated with TRPV1 antagonists when lower, near-threshold stimulation intensities were used. However, when heat stimuli of higher intensity were employed, genetic deletion or pharmacological blockade of TRPV1 resulted in prolonged latencies. In human studies, TRPV1 antagonists unexpectedly increased the noxious heat threshold leading to scalding injuries.
In our experiments a novel approach was used to study thermonociception in awake rats and mice that is based on determination of the noxious heat threshold as opposed to latency measured upon suprathreshold stimulation. Using the increasing-temperature hot plate or the increasing-temperature water bath, the stimulation temperature is raised from the innocuous range until a nocifensive reaction occurs. The temperature evoking hind paw licking (hot plate) or withdrawal of the paw or tail (water bath) is considered the noxious heat threshold. Pharmacological blockade and genetic ablation of TRPV1 failed to alter the noxious heat threshold of the rat and mouse hind paw, respectively. On the tail of the mouse, heat threshold was 2 °C higher in TRPV1 knockouts than in wild-types. TRPA1 gene-deficient mice had normal heat thresholds both on the hind paw and tail, but in the Hargreaves test they exhibited prolonged latency upon stimulation with high, but not low, intensity, compared to wild-types. TRPA1 antagonists failed to alter latency in the hot plate and plantar tests in rats or mice.
In conclusion, involvement of TRPV1 in noxious heat responsiveness depends on intraneuronal localization (cell body *versus* peripheral terminal), macroscopic anatomical localization (tail *versus* paw), species (man *versus* mouse and rat), intensity of the heat stimulus (suprathreshold *versus* threshold). TRPA1 is not likely to contribute to setting the noxious heat threshold. Ion channels other than TRPV1 or TRPA1 (TRPM3, anoctamin 1) are also likely to be involved in transduction of noxious heat.

Acknowledgements:
EFOP-3.6.1.-16-2016-00004
Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs The project has been supported by the European Union, co-financed by the European Social Fund. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

EEG ALPHA IS RELATED TO THE COGNITIVE IMPAIRMENT AND „HIDDEN" NEUROPATHOLOGY OF MINOR STROKE

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We aimed this study to investigate the EEG alpha activity at several levels of quantitative EEG analysis, and its relationship to the cognitive functions in the subacute and chronic stages of minor ischemic stroke.
Study included 10 patients with the right middle cerebral artery ischemic stroke and 11 healthy controls. Neurological impairment was measured by National Institute of Health Stroke Scale (NIHSS), whereas the cognitive functions were assessed by Montreal Cognitive Assessment (MoCA) and MoCA memory index (MoCA-MIS). EEG was recorded in resting, awake state with eyes closed using 19 channel EEG system with standard EEG electrode placement. The final 10 minutes artefact-free EEG signals were analyzed in MATLAB R2011a. We have particularly analyzed the EEGs derived from four lateral frontal (F3, F7, F4, F8), and corresponding lateral posterior (P3, P4, T5, T6) electrodes. Quantitative EEG analysis included: the group FFT spectra, the weighted average of alpha frequency (αAVG), the group probability density distributions of all conventional EEG frequency band relative amplitudes (EEG microstructure), the inter-hemispheric and intra-hemispheric coherences, and the topographic distribution of alpha carrier frequency phase potentials (PPs). Statistical analysis was done using the Kruskal-Wallis ANOVA with the post-hoc Mann-Whitney U two-tailed test, and Spearman's correlation.
We have demonstrated the transient cognitive impairment alongside the slower alpha frequency (αAVG) in the subacute stroke patients vs. controls, with no amplitude change, but highly synchronized intra-hemispherically, above the overall ipsi-lesional hemisphere, and inter-hemispherically, above the overall frontal cortex. In addition, the disturbances of EEG alpha activity in subacute stroke patients were expressed as the decrease of alpha PPs over the frontal cortex (indicating a delay of the slower alpha), and the altered "alpha flow", indicating the sustained augmentation of inter-hemispheric interactions.
Although the stroke induced slower alpha was a transient phenomenon, the increased alpha intra- and inter-hemispheric synchronization, the delayed alpha waves, and the newly established inter-hemispheric "alpha flow" within the frontal cortex, remained as a permanent consequence of the minor stroke. This, newly established frontal inter-hemispheric "alpha flow" presented a permanent consequence of the "hidden" stroke neuropathology, despite the cognitive impairment have been returned to the control values. Moreover, all detected permanent changes after minor stroke at EEG level with no cognitive impairment, could be a way for the brain to compensate lesion and restore the lost function.
Our study indicates that slower EEG alpha generation, synchronization and "flow" are related to the cognitive impairment and „hidden" neuropathology of minor stroke, and could present a compensatory post-stroke re-organizational changes, contributing to functional recovery.

INHIBITION OF THE VENTRAL PALLIDAL D2 DOPAMINE RECEPTORS INDUCES PLACE AVERSION

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The ventral pallidum (VP) is innervated by the dopaminergic fibers of the ventral tegmental area. D2 dopamine (DA) receptors can be found both presynaptically and postsynaptically in the VP. It has been shown earlier by our research group that the blockade of the VP D2 DA receptors leads to impairment of spatial learning. In addition, it has been demonstrated that the systemic D2 DA receptor antagonist treatment results in synaptic degeneration in the VP. The goal of the present experiments was to investigate the effect of VP D2 DA inhibition on place conditioning, whether it induces place preference or aversion.
In the first experiment, the potential rewarding or aversive effect of the VP D2 DA receptor inhibition was examined by means of place conditioning paradigm. The apparatus consisted of a circular open field arena, which was divided into four quadrants. In the habituation and test trial animals were placed into the apparatus and had free access to all quadrants. In each conditioning day, rats underwent two conditioning trials: in the morning, a "real" conditioning, while in the afternoon, a pseudo-conditioning was performed. During the conditioning trials rats were confined to the treatment quadrant ("real" conditioning) or the opposite quadrant (pseudo-conditioning) by means of a Plexiglas barrier. The D2 DA receptor antagonist sulpiride was microinjected into the VP in 0.1 µg, 1 µg or 4 µg doses before "real" conditioning trials. Control animals received vehicle. Before pseudo-conditioning, all rats received vehicle. After the conditioning trials the animals were tested.
In the second experiment, using another group of animals, a modified version of place conditioning paradigm was applied to reveal whether the rats associated the effect of the sulpiride with spatial cues. Schedule of this experiment was similar to that of the first experiment. The only difference was that in the second experiment, during the "real" conditioning trials the rats were confined to a rectangle-shaped compartment of the circular pool, while during the pseudo-conditioning rats were restricted to the area of the arena outside the rectangle-shaped compartment.
The results of the present experiments demonstrate that the inhibition of the VP D2 dopamine receptors induces place aversion, and the aversive effect is not associated to the spatial location of the place, but rather proximal environmental cues.

The project has been supported by the European Union, co-financed by the European Social Fund and the ÚNKP-16-4-I New National Excellence Program of the Ministry of Human Capacities, EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs.

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THE INFLUENCE OF ACUTE STRESS ON REINSTATEMENT OF NICOTINE-INDUCED PLACE PREFERENCE IN RATS

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Introductions: Nicotine, as the main component of tobacco smoke, acts through central mechanisms and has influence on mood and emotional tension, and also contributes to the development of physical and mental dependence. Activation of the mesolimbic dopaminergic system by nicotine is associated with its rewarding and reinforcing properties. The rewarding activities of this alkaloid are assessed in many behavioral models using rodents, e.g., conditioned place preference (CPP) paradigm. Additionally, in some experimental animal models, it was demonstrated that chronic or acute stress may aggravate both behavioral as well as neuronal effects caused by administration of nicotine. Stress is a very important factor that precipitates and potentiates addictive effects of different drugs of abuse, including nicotine.
Methods: The experiments were carried out on naive male Wistar rats. We used the CPP version of the reinstatement model to investigate the establishment, extinction, reinstatement and cross-reinstatement of nicotine-induced place conditioning in rats. The CPP-reinstatement paradigm consisted of the following phases: pre-conditioning, conditioning, post-conditioning, extinction and reinstatement. In our studies, immediately before reinstatement, the rats were submitted to nicotine or procedure of an acute stress, i.e., swimming in cold water (10 °C) for 10 min. The present study was designed to investigate the influence of an acute stress on the reinstatement of nicotine-induced CPP as well as the influence of the HPA-related mechanisms of observed activity (e.g., administration of metyrapone, a corticosterone synthesis inhibitor, 50 mg/kg). All agents were administered intraperitoneally in a volume of 5 ml/kg. Control groups received saline injections at the same volume and by the same route.
Results and discussion: Results from present study showed that nicotine produced a place preference to the compartment paired with its injections during conditioning. After 3 day of extinction, both, injection of nicotine and exposition to stressor reinstated nicotine-induced CPP. Our results also demonstrated that metyrapone attenuated the stress-induced reinstatement of nicotine-conditioned response. The results of our studies revealed the mechanisms underlying the stress-induced reinstatement of nicotine place preference and may offer an interesting approach to the relapse-prevention pharmacotherapy of nicotinism.

This research was supported by the statutory activity of the Medical University of Lublin (DS 23).

THE TYRAMINERGIC/OCTOPAMINERGIC SYSTEM OF INSECTS: AN EVOLUTIONARY CONSERVED SYSTEM ORCHESTRATING BEHAVIOR AND PHYSIOLOGICAL PROCESSES

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The nervous systems of all bilaterian animals share many common features ranging from single molecules, genes, intracellular signaling cascades, whole nerve cells to pathways, whole circuits and ensembles of active neurons and, thus, often conserving the behavioural context. The tyraminergic/octopaminergic system of insects is such a conserved system that works in a context of stress (such as hunger or, perhaps, stimulus novelty) and (short-term) energy demanding behaviours. The talk will introduce the neurons which release these biogenic amines, their morphology, their physiology and when during behaviour they are activated. In addition, the different types of neurons have differential roles in the periphery: release of amines is either into the haemolymph like a hormone, or amines are delivered specificaly to different targets which are then modulated. In addition, other neurons act within the CNS and modulate neuronal circuits or central pattern generators. The result of activation of these neurons is a well-coordinated behavioural and physiological response that leads to meaningful „orchestrated" behaviour.

COMMON NEUROTRANSMISSION RECRUITED IN (R,S)-KETAMINE AND (2R,6R)-HYDROXYNORKETAMINE-INDUCED SUSTAINED ANTIDEPRESSANT-LIKE EFFECTS

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Racemic (*R*,*S*)-ketamine, at sub-anesthetic doses, exhibits a rapid and persistent antidepressant (AD) activity in treatment-resistant depressed patients and in preclinical studies in rodents. (*R*,*S*)-ketamine also induces stress resilience. However, the molecular and cellular mechanisms mediating these activities are unknown. (*R*,*S*)-ketamine, a non-competitive antagonist of NMDA receptor, unlikely exerts its AD-like activity solely via a blockade of this ionotropic receptor. We recently reported that (*R,S*)-ketamine-induced increases in presynaptic serotonin (5-HT) release in the medial prefrontal cortex (mPFC) correlated with its AD-like activity in mice. The control exerted by the mPFC is important in regulating stress processing and AD-like activity. However, little is known about the regulation of synaptic excitatory/inhibitory balance and concomitant changes in glutamate/GABA neurotransmission induced by (*R*,*S*)-ketamine or its metabolites in rodent mPFC.
Recently, it was shown that (*R*,*S*)-ketamine metabolism to (2*R*,6*R*)-hydroxynorketamine (HNK) is essential for its AD-like activity, and involves early activation of AMPA receptor in mice hippocampus and mPFC. However, (*R*)-ketamine displays greater potency and longer lasting AD effects than (2*R*,6*R*)-HNK. Brain tissue concentration of (2*R*,6*R*)-HNK is about 25% of that of (*R,S*)-ketamine, but brain regions and neurotransmitter systems supporting (2*R*,6*R*)-HNK effects are unknown.
Here, we compared the sustained AD-like activity and neurotransmitters' changes between (*R,S*)-ketamine and (2*R,*6*R*)-HNK in BALB/cJ mice, using the forced swim test (FST), a model to screen AD-like activity of drug, coupled to *in vivo* microdialysis **in the same mice**. Cortical extracellular 5-HT, GABA, glutamate and glutamine levels were measured by HPLC.
A single dose of (*R,S*)-ketamine and (2*R*,6*R*)-HNK (either 10 mg/kg intraperitoneal, or 1 nmol/side intra-mPFC) administered 24hr prior testing showed comparable AD-like activity in the FST and cortical serotonergic effects. Interestingly, (2*R*,6*R*)-HNK displayed a more glutamatergic, but less GABA-ergic potency than (*R,S*)-ketamine.
Our results confirmed the sustained AD-like activity in mice of both drugs, which required presynaptic cortical release of various excitatory and inhibitory neurotransmitters. We also found that (2*R*,6*R*)-HNK, via AMPA receptor activation, increased excitatory synaptic transmission in the mPFC by enhancing presynaptic glutamate release. We need now to clarify the mechanism of action of (*R,S*)-, (*R*)- or (*S*)-ketamine and (*2R,*6*R*)-HNK by studying the cellular and molecular events modulating their activity in the mPFC, and to focus on top-down excitatory/inhibitory controls, e.g., the mPFC/raphe nuclei circuit on midbrain neurons. Therefore, it is of great interest to make direct comparisons of these compounds in future clinical studies in depressed patients.

"A. L. BYZOV AND THE RUSSIAN RESEARCH ON THE RETINA DURING THE COMMUNIST ERA"

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Alexei Leontyevich Byzov (1926-1998) was a prominent Russian scholar whose scientific work in the field of retina physiology enjoyed an important international reputation. Since 1957 he worked at (and later on headed) the laboratory of visual perception at the USSR Academy of Sciences in Moscow, that was started in the 1950s by mathematician Nikolay Nyberg and physicist Mikhail Bongard, and included personages of the scientific stature of the pioneer of ocular movements studies Alfred Yarbus. In the 1960s, despite the objective difficulties due to the situation of the country, Alexei succeeded in creating a group of brilliant young researchers whose work on the vertebrate retina was at the frontier of the neurophysiological investigation of the time. That period represented a phase of great excitement in retinal studies, prompted by the recent discovery by Gunnar Svaetichin of an atypical intracellular electrical response (called S-potential from its discoverer) consisting of graded, light-induced hyperpolarisations, i. e. signals differing in both polarity and time course from the impulsive action potentials that were generally considered to be the landmark of the electric activity of nerve cells. The work of Byzov and his coworkers (among them, Yu. A.Trifonov, K. T. Golubtzov, T. Shura-Bura, V.V. Maximov, E.M. Maximova, A.V. Minor and L.M. Chailahian) contributed in a significant way to clarify the puzzle of the generation mechanism of these potentials, by showing that, in darkness, photoreceptors are in a condition of relative depolarization and release an excitatory neurotransmitter which keeps depolarized the cells responsible for the S-potentials (i.e. horizontal cells, as it was eventually recognized). A breakthrough of the experimental approach by Byzov and Trifonov was the use of a simple device allowing for extracellular injections of currents between the vitreal and scleral side of the retina. As USSR gradually opened to international exchanges in science, in 1971 Alexei was allowed to visit USA and work for a short period in Mike Fuortes' lab at the NIH in Bethesda. In Bethesda was then working one of us (Cervetto), who started soon afterwards an intense phase of collaboration with Byzov, leading to some important results concerning the two-way synaptic interaction between cone photoreceptors and horizontal cells. The other of us (Piccolino), formerly Cervetto's student in Pisa, had the chance of meeting Byzov in that period, but started collaborating with him much later, in the 1990s, when he realized that an intriguing outcome of his own experiments with divalent cations on retinal synapses could have a significant relation with some previous results obtained by Byzov. Alexei came to work in Piccolino's lab in Ferrara in order to clarify these puzzling issues, and this lead to a series of achievements which overturned some long-established notions on the functioning of retinal synapses and calcium channels. This period was the occasion for Piccolino, as it had been many years before for Cervetto, to enjoy the collaboration and friendship of an energetic and brilliant scientist, rich of humanity and enthusiasm, much to be regretted by the international scientific community, as Alexei Leontyevich Byzov undoubtedly was.

PROTEASOMAL STRESS IN NEURAL CELLS: ASSASIN OR BYSTANDER

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Ischemia-reperfusion injury is associated with delayed neuronal death (DND) of selectively vulnerable neurons. The delay between ischemic insult and DND offers time window for global brain ischemia therapy. But the mechanism of DND is still unclear. Accumulation of polyubiquitinated proteins (APP) after ischemia have been considered as a possible cause of DND. Since the evidence proving their cytotoxicity has not been published yet, the aim of our work was to analyze potential cytotoxicity of APP and to identify the mechanism of cell death after proteasomal stress. Inhibition of 26S proteasome was induced with bortezomib in cell lines SH-SY5Y and T98G. Relative viability after proteasome inhibition was determined via MTT test. Transcriptional changes were quantified using TaqMan Apoptosis panel with RT-qPCR. Changes in relative level of proteins were analyzed using Western blot method. Neuroblastoma cell line SH-SY5Y is more sensitive to proteasomal stress than glioblastoma cell line T98G. However the intensity of proteasomal stress after 4h was in both cell lines similar, after 24h was relative level of APP in T98G cells elevated more. Also cytoprotective mechanism in form of Hsp70 and Hsp90 was responding more intensively in T98G cells than in SH-SY5Y cells. Transcriptional changes in genes coding proteins involved in apoptosis were more complex in SH-SY5Y cells indicating initialization of apoptosis. Not all changes in gene expression were transfered to protein level. Protein Noxa was elevated in transcriptionally independent pathway. Protein Puma was simultaneously upregulated on protein and gene level. Although expression of Bik was significantly upregulated, we determined significant downregulation on protein level. Activation of caspase 3 was documented in both cell lines however in T98G cells the effect was observed later than in SH-SY5Y cells. In T98G cells we also documented increased level of caspase 4 and stabilization of ikappaB-alpha in cytoplasm suggesting inhibition of NFkappaB pathway. Our results does not support the hypothesis about direct cytotoxicity of APP. It seems that disruption of protein homeostasis leads to complex (post)transcriptional changes culminating in initialization of cell death via mitochondrial apoptosis in SH-SY5Y and activated caspase 3 in T98G cells, which might be mediated through caspase 4 or NFkappaB pathway.

HUNTINGTIN AGGREGATION IMPAIRS AUTOPHAGY LEADING TO ARGONAUTE-2 ACCUMULATION AND GLOBAL MICRORNA DYSREGULATION

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Many neurodegenerative disorders, such as Huntington's disease, are characterised by the formation of protein aggregates in the brain. In this project we investigate a potential link from protein aggregation to cell death via autophagy and microRNAs in models of Huntington's disease. To confirm that aggregation of mHTT protein results in impaired autophagy in the brain, we generated neuron specific AAV vectors expressing mHTT with 66 CAG repeats and performed injections in the striatum of adult mice. We found that expression of mHTT resulted in time-dependent aggregation of mHTT that was associated with reduced levels of DARPP32 a marker of medium spiny neurons decreased in HD brains. Animals injected with mHTT displayed an induced autophagy 10 days after injection that were reversed at 3 and 8 weeks when autophagy was impaired. We found significantly more p62 by time coupled with elevated LC3-II and LAMP1 levels suggesting a later autophagic block with autophagic flux impairment. In line with this, we found that mRNA-levels of transcription factors that activate autophagy were up regulated in mice 10 days after injection but at 3 weeks we saw the opposite, all the enhancers were down regulated. We also found an interesting trend for genes involved in lysosomal biogenesis, autophagosome maturation, cargo recruitment and vesicle trafficking: the fold changes of the mHTT/wtHTT mRNA levels were reversed. Furthermore, we investigated a potential link between impaired autophagy and miRNA-machinery in the mHTT mice and found an increased AGO2 protein level. AGO2 plays a central role in RNA silencing processes and it has been previously shown that selective autophagy degrades AGO2 and regulates miRNA activity. We found that the increase in AGO2-levels correlates with an increased level of mature miRNAs, which is coupled with loss of miRNA-activity. We also observed that overexpression of BECN1 reverses mHTT-associated phenotypes by partially preventing aggregation of mHTT, decreasing the number of p62 aggregates and also reducing AGO2 aggregate size and number. Overall, our data shows that mHTT aggregates impair autophagy on a protein and on a transcriptional level. Furthermore, we have shown that mHTT impairs clearance of protein aggregates and also accumulation of AGO2 is associated with global alterations in the miRNA-machinery.

FROM NEURONS TO BEHAVIOUR: COMPLEX CHANGES INDUCED BY ENVIRONMENTAL CONTAMINANTS

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It has been well-known for the last few decades that certain molecules of human origin (e.g. synthetic oral contraceptives), this latter called endocrine disruptors (e. g. progestogens), occurring in the environment can influence the homeostatic state, hormonal functions, and reproduction of non-target living organisms. The presence of a mixture of progestogens at ng/L concentration levels in surface waters is a worldwide problem. Animals living under aquatic conditions are more sensitive to their effects since rivers and lakes often serve as the final sink for these agents.
The catchment area of the largest shallow lake of Central Europe, Lake Balaton, is a habitat of the pond snail (*Lymnaea stagnalis*), where the presence of progestogens (0.23-13.67 ng/L) was published in our previous paper. Therefore, using this invertebrate model species, we investigated the adaptive changes induced by a mixture of progestogens (progesterone, drospirenone, levonorgestrel, gestaden) found in commercially available oral contraceptives. Using treated (1, 10, and 100 ng/L progestogene mixture) and control groups of *Lymnaea*, feeding, respiration and locomotion related behavioural tests were performed. Subsequently, intracellular electrophysiological recordings were carried out from various components of the identified neuronal networks of *Lymnaea* responsible for controlling feeding and respiration.
At the behavioural level, mixture of progestogen treated snails displayed a lower feeding (rasping) activity to sucrose that was used as a feeding stimulus, compared to control animals. Mixture also altered various characteristics of aerial breathing. The start of aerial respiration was significantly delayed in the treatment groups and the number of pneumostome openings also showed a significant decrease. Additionally, the mean pneumostome opening time was prolonged in all treatment groups. In contrast to lower feeding or decreased number of pneumostome openings, the locomotion activity was higher in treated groups. At the cellular level, it was found that hormone treatments decreased the firing frequency of the feeding modulatory interneurons (cerebral giant cells - CGCs) via increasing the amplitude of their Ca^{2+} currents. This effect seemed to be cell type specific, since in the case of the respiratory CPG cells (right pedal-dorsal1 cells - RPeD1) there were no such changes. Feeding motoneurons (buccal cells - B1-B3) also showed decreased activity during fictive feeding inducement, since their responsiveness to external sucrose stimulus was reduced.
Modelling the environmental contaminations, we conclude that mixture of progestogens may disturb regulatory processes of freshwater species, and *Lymnaea* can be indicative for it.

This work was supported by the PD-OTKA grant No. 19099, the National Brain Project No. KTIA_NAP_13-2-2014-0006, and the Bolyai Foundation No. BO/00952/16/8.

BIOMARKERS OF PTSD: PRELIMINARY DATA ON GLYCOMIC BIOMARKERS AND AGING

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Post-traumatic stress disorder (PTSD) is trauma- and stressor-related disorder in which direct exposure to a traumatic experience/stressful event(s) leads to an onset of re-experiencing, avoidance, numbing and hyper-arousal symptoms in some but not all subjects exposed to trauma. Its etiology is far from clear. Alterations in various biological systems (HPA, immune, noradrenergic, serotonergic systems), together with interactions with different psychological, social, environmental, genetic and epigenetic factors, contribute to development of PTSD. At present, there are no specific, sensitive and validated biomarkers for PTSD.
Glycosylation of proteins results in a formation of specific glycans that significantly affect protein structure and function, especially immunoglobulin G (IgG) molecules and is essential in inflammatory cascade. N-glycans are altered in pediatric and adulthood diseases of the CNS. PTSD is frequently complicated with different somatic diseases and characterized by the increased pro-inflammatory state, while N-glycosilation changes are associated with inflammatory processes and aging. Accelerated aging and altered 9 N-glycan structures were reported in a study including a small number of trauma exposed subjects.
Present study evaluated total plasma and IgG N-glycan changes related to PTSD and/or aging in 204 subjects with current and chronic PTSD and 134 control subjects without traumatic experience. Results (39 chromatographic peaks of plasma N-glycans and 24 chromatographic peaks of IgG N-gycans) are presented as total area normalized values (% of each glycan peak /GP/ area in total chromatogram area) and were evaluated using Mann Whitney test.
Results showed 16 increased and 10 decreased plasma N-glycan peaks, and 9 increased and 6 decreased IgG N-glycan peaks in PTSD participants compared to control subjects, confirming significant N-glycome changes in PTSD. As age considerably affects N-glycans, GlycoAge test = log(10) GP1/GP10 revealed significant case-control differences in total (U=8329.00; p=1.24-09), as well as in older (60-77 years) PTSD sample (U=341.00; P=0.022) in plasma N-glycan peaks. Significantly increased plasma GP2 in total PTSD cases and decreased plasma GP11 in older PTSD subjects was related to aging. Moreover, significantly higher G4 and G6 peaks and significantly lower G14 peak in total PTSD and in the oldest PTSD sample versus controls were found for IgG N-glycan associated with aging. These results confirmed N-glycan changes related to aging in PTSD.
PTSD and N-glycans are defined by complex and dynamic interactions between genes and environment. As glycan structures are adaptable and respond to environmental stimuli, changes in specific N-gycans in PTSD subjects, which were correlated with aging, suggest their role in the aging process, might be used as biomarkers, and should be further validated and replicated.

This work was supported by Croatian Science Foundation grant IP-2014-09-4289.

IMMEDIATE AND PERSISTING EFFECT OF TOLUENE CHRONIC INHALATION ON BEHAVIOUR IN MAZE AND ON HIPPOCAMPAL STRUCTURE IN ADULT AND ADOLESCENT RATS

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Toluene and toluene-containing volatile substances are the most widely abused solvents with demonstrative addictive potential in humans. Experimentation with these substances is especially common during adolescence. Besides, because toluene is broadly used as an industrial solvent in manufacturing of chemical pharmaceuticals and multiple house-hold and commercial products, it has the highest potential for abuse for adult workers also. Numerous studies have demonstrated that the exposure to toluene vapors leads to diverse consequences at the level ranging from the cell to whole organism. However relatively few studies have assessed outcomes of toluene chronic misuse in adolescents despite the fact that just the adolescents represent the most numerous group of toluene abusers and a large number of adults who use this substance, also started as adolescents. Another area of uncertainty is related with long-term outcome in toluene abusers. The biggest part of existing research has focused on acute effect of toluene with limited work examining delayed outcome in abusers who abstain from toluene abusers. However to fully understand the nature of toluene addiction, studies need to be done, where both, immediate and persisting effect of toluene misuse in organisms of different age will be compared. In the present research we evaluate immediate and persisting consequences of toluene chronic exposure on behavior in multi branch maze and the structure of hippocampus - the main substrate for learning and memory - in adolescent and adult rats. Adolescent and adult male Wistar rats were used. Each rat was exposed separately to toluene vapor at the concentration 2000 ppm or clean air (control animals) during 40 days (4-5min/day). Immediate effect of toluene chronic exposure was evaluated immediately after the end of toluene inhalation, while persisting effect - 90 days after the end of toluene exposure. The activity of rats in maze was assessed by (i) number of errors made by rats - entrance in deadlocks - while searching for optimal way to nest-box and (ii) exploration period - time, which the rat spent into the maze. To reveal possible alterations in the structure of hippocampus the cell loss was assessed in dentate gyrus and CA1, CA3 areas on Nissl stained sections. The one-way ANOVA of quantitative data was performed separately in adolescents and adults. The results revealed the age-dependent effect of toluene misuse on behavior in maze and hippocampal structure. Thus: according maze test, significant alterations provoked by toluene misuse were observed only immediately after the end of inhalation; after 90 days of withdrawal the behavior of rats in maze was almost the same as observed in control animals. According histological studies, toluene chronic inhalation affects all areas of the hippocampus. The character of alterations depends on the age of animals. Specifically, in all areas of adolescents immediate and persisting effects were almost the same.

NEUROCOGNITIVE CORRELATES OF SPATIAL VISUAL ATTENTION IN THE HUMAN DOT-PROBE PARADIGM

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Threatening stimuli are part of our life, and they are detected in early stages of cognitive information processing in the brain. Previous studies found that humans suffering from anxiety focus more on threatening stimuli compared to healthy individuals. Such biased visual spatial attention to threatening stimuli can be tested with the dot-probe paradigm. The aims of the present study were 1) to test whether biased spatial attention to threatening stimuli may be manifested overtly in reaction time (RT) and/or in covert, EEG based responses, e.g., in the N710 event related potential (ERP) or the N2pc difference potential, 2) to develop a full factorial experimental model using human facial expressions in dot-probe arrangement to test any possible lateralization effects in face processing. Healthy subjects volunteered in the present study and performed several paired blocks of dot-probe tests (neutral vs. angry; angry vs. angry; neutral vs. neutral; neutral vs. scrambled face) with human facial expressions as attentional attractors. In one test two simultaneous facial stimuli (e.g. angry vs. neutral from the same person) appeared on the two sides of the visual field. The duration of the face stimulus was 500 to 550 ms. After a short delay (blank screen), at the position of one of the stimuli a black dot appeared. The spatial position of the dot (left/right) had to be indicated by the participants using reaction time box. Similarly to earlier literature data we did not find any stimulus-related overt biasing effects in the RT (no RT difference between angry and neutral faces). However, we found clear difference between responses to the neutral and angry faces as indexed by the N170 and the N2pc ERP components. Namely, the ERP responses were significantly higher after threatening facial emotions relative to neutral facial stimuli. Our preliminary results support that not only general threatening images, but also standardized threatening faces can effectively bias spatial attention in healthy young individuals. In accordance with the literature we also support the notion that the present adoption of the dot-probe paradigm (neutral vs. angry) will be suitable for detecting spatial attentional biases based on electrophysiological (ERP) measures alone. In addition, lateralization effects of processing facial expression will also be addressed to test the possible advantage of the right hemisphere in the dot-probe paradigm. The paradigm will be further tested using subliminal stimulus presentation durations to disentangle the influence of possible inhibition of return effects on RT or ERP measures. We believe, that the adopted dot-probe paradigm will then be suitable for testing the effects of pharmacological agents which may influence spatial attention.

Support: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

THE INTERLEUKIN 1 (IL-1) RECEPTOR ANTAGONIST ANAKINRA INHIBITS HYPERALGESIA, BUT NOT PERIPHERAL INFLAMMATION IN A PASSIVE-TRANSFER-TRAUMA MOUSE MODEL OF COMPLEX REGIONAL PAIN SYNDROME

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Complex Regional Pain Syndrome (CRPS) is a multifactorial disease characterized by severe, persistent pain accompanied by abnormal hypersensitivity, swelling and autonomic alterations of the skin. Although the ethiology is unknown, immune response against sensory nerve-derived antigens and complex neuro-immune interactions are suggested to be involved in the development of this disease. The therapy is still an unresolved problem, the importance of central versus peripheral sensitization mechanisms and peripheral inflammatory processes is unclear. Therefore, we investigated the effects of the recombinant IL-1 receptor antagonist anakinra and the corticosteroid prednisolone in the novel passive-transfer-trauma CRPS model established and characterized by us. Small plantar skin-muscle incision to mimic the small injury that usually occurs in CRPS patients was performed in female C57Bl/6 mice. They were daily treated with purified serum-IgG from CRPS patients or healthy volunteers (i.p., n=12/group, 6 days). Anakinra (10 mg/kg) or prednisolone (4 mg/kg) was also administered i.p. every day with a 4-h delay to the IgG treatment, saline-treated groups served as controls. The mechanonociceptive threshold of the hindpaws was measured by dynamic plantar aesthesiometry, the volume by plethysmometry, neutrophil/macrophage myeloperoxidase (MPO) activity demonstrating the cellular inflammatory phase by luminescence *in vivo* imaging, inflammatory cytokines by immunoassays, Iba1 microglia and GFAP astrocyte markers by immunochemistry in pain-related brain regions.
CRPS IgG injection significantly increased incision-induced swelling and MPO activity, but more pronounced enhancement and prolongation of hyperalgesia was observed compared to healthy IgG. CRPS IgG did not influence the inflammatory cytokine production, but significantly increased the density of both Iba1 and GFAP immunopositivities in L4-L5 spinal dorsal horn, periaqueductal grey and somatosensory cortex. Anakinra treatment abolished the CRPS IgG-evoked increased hyperalgesia and glia activation, but not the peripheral inflammatory alterations. Prednisolone affected neither edema nor hyperalgesia, and surprisingly did not influence the MPO activity and cytokine production either as compared to the vehicle-treated CRPS IgG-administered group. These results suggest that not peripheral inflammatory processes, but glia-mediated central sensitization mechanisms are likely to mediate the enhanced and prolonged hyperalgesia in CRPS. The ineffectiveness of prednisolone is in agreement with the not sufficient clinical efficacy of glucocorticoids in CRPS patients pointing out the translational relevance of our model. Anakinra is likely to act centrally by inhibiting IL-1-mediated neuroinflammatory pathways and might open new perspectives for its use in CRPS as a novel indication.

KTIA_NAP_13-2014-0022 (PTE-NAP B); Pain Relief Foundation; GINOP-2.3.2-15-2016-00050 - PEPSYS and STAY ALIVE, EFOP-3.6.1-16-2016-00004. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

5-HTTLPR POLYMORPHISM OF THE SEROTONIN TRANSPORTER GENE PREDICTS SENSATION SEEKING BEHAVIOR. THE EEG STUDY

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The goal of the study was to investigate the relations between sensation seeking tendency with genetic and electrophysiological characteristics of the person.
Sensation seeking is a personality trait that is expressed at the behavioral level, the tendency to look for a variety of new and intense sensations and experiences, as well as readiness to take on the physical, social, legal and financial risks (Marvin Zuckerman, 2009). The "Sensation seeking scale" (SSS) consist of 4 subscales: 1) Thrill and Adventure Seeking (TAS), 2) Disinhibition (Dis), 3) Experience Seeking (ES), and 4) Boredom Susceptibility (BS).
Methods: 36 right-handed male (mean age 24+/-6) without any mental and neurological diseases participated in the study.
In current study were compared two groups with different alleles in the 5-HTTLPR polymorphism of SLC6A4 gene, associated with serotonin transporter: «S+» group (genotypes LS and SS - 18 subjects) and «S-» group (genotype LL - 18 subjects) by two EEG parameters: 1) individual alpha frequency (IAF) and 2) index of functional state (IFS).
EEG recording was carried out using a 256-channel electroencephalograph company EGI Electrical Geodesics a sampling rate of 500 Hz and a referent in the vertex. After completing the SSS questionnaire the resting EEG was recorded. IAF and IFS were measured and averaged in five separate regions (frontal, central, temporal, parietal and occipital) for both hemispheres. IAF was calculated as the frequency at which maximum power is observed the alpha rhythm in the range of 7 to 14 Hz (Klimesch W., 1999). IFS was calculated as the ratio of the total power of the alpha and theta rhythms to the total capacity of the beta rhythm (FIS = alpha + theta / beta).
The statistical analysis package Statistica 8 (for Windows, V 8.0, StatSoft) and MatLab (R2007b) were used for data analysis.
Results: Statistical analysis showed significant differences between groups «S+» and «S-». SSS questionnaire have shown that «S+» group was characterized by significantly higher scores compared to «S-» group on the following scales:
- Thrill and Adventure Seeking (TAS), ($p \leq 0,02$);
- Experience Seeking (ES), ($p \leq 0,015$);
- Boredom Susceptibility (BS), ($p \leq 0,034$).

Results of EEG statistics showed, that «S+» group characterized by lower IAF values in all areas of the brain compared to «S -» group, but statistically only in the left central electrodes (9.49 vs 10.04 Hz).
According to the IFS parameter there were obtained significant differences between «S +» and «S-» groups in almost all areas of the brain.
This results means that the «S +» group is characterized by a predominance of slow wave activity in comparison with «S-» group.
So we can suppose that S-allele in the 5-HTTLPR polymorphism of SLC6A4 gene may lead to less activation of the cerebral cortex and slowing of information processes, so behaviorally this people can tend to action to increase this activation (risky sports, travel, change of residence and social environment).

AUTOMATED 3-D COEXPRESSION (A3-DC) ANALYSIS OF CONFOCAL MICROSCOPY IMAGES REVEALS SYNAPSE-SPECIFIC MOLECULAR AND CELLULAR ADAPTIONS IN THE SPINAL NOCICEPTIVE CIRCUITRY OF MONOACYLGLYCEROL LIPASE KNOCKOUT MICE

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Quantitative analysis of the molecular alterations underlying physiological or pathophysiological plasticity processes in complex brain circuits represents a major methodical challenge. While immunofluorescence intensity measurements of confocal microscopy images are widely used to investigate molecular changes in the brain, the observed increase or decrease in fluorescence intensity levels can either directly reflect up-or downregulation of the target protein, or alternatively, it can be indirectly caused by the appearance or disappearance of subcellular profiles harboring the target protein, respectively. Due to the limited resolution and sensitivity of confocal microscopy, reliably distinguishing between these two possibilities in a high yield manner is often difficult and laborious. To circumvent this obstacle, we developed the open-source Automated 3-D Coexpression (A3-DC) plugin for Fiji, which performs unbiased object-based 3-D analysis of protein co-expression within selected cellular compartments on large batches of confocal images thereby facilitating automatized analysis of large sample sizes. We chose to write A3-DC in Python, a language widely in the scientific community, and utilized the plugin "3D Objects counter" for the image processing platform Fiji and ImageJ. In short, the plugin first segments the images, then finds objects in the two channels and determines which objects overlap. A database of properties of objects, such as their volume, intensity, and overlapping ratio is created. Quantitative data can be attained by setting filters for this database. We first demonstrate that A3-DC enables automated evaluation of protein levels. When compared to manual analysis, A3-DC analyzes subcellular structures 200 times faster, produces higher statistical power by high-throughput capacity and excludes observer expectancy selection bias. As a proof-of-principle, we next applied A3-DC to elucidate the molecular and cellular consequences of chronically enhanced cannabinoid signaling in the mouse spinal nociceptive circuitry. It is important, because analgesic tolerance is the major limitation for medical marijuana and for drugs acting on the endocannabinoid system to become exploited in pain management. In mice lacking the endocannabinoid-degrading enzyme monoacylglycerol lipase, A3-DC analysis of 80,989 individual subcellular profiles uncovered 1) widespread and robust CB_1 cannabinoid receptor downregulation; 2) significant loss of excitatory interneuron boutons and nociceptive primary afferents; and 3) reduced pronociceptive substance P neuromodulator levels. These findings demonstrate the exceptional efficiency of A3-DC in identifying three major types of molecular and cellular adaptations: cell-type-independent molecular tolerance, synapse-specific rewiring and compensatory alterations in unrelated signaling pathways that may together underlie the analgesic tolerance to chronically enhanced cannabinoid signaling.

THE NEUROPROTECTIVE EFFECTS OF THE METHANOLIC EXTRACT OF LACTUCA CAPENSIS IN AN Aβ1-42-INDUCED ALZHEIMER'S DISEASE-LIKE RAT MODEL

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Alzheimer's disease (AD) is a neurodegenerative disorder of the central nervous system characterized by progressive cognitive dysfunction and behavioral deficits. Although many studies have been directed to AD, the etiology of the disease is still unknown and a very limited number of treatments are available and efficient regarding the symptom management in AD patients. Currently, much attention has been focused on the potential of using natural herbs as neuroprotective agents. *Lactuca capensis* is a medicinal plant frequently used in traditional medicine to treat various ailments as insomnia, neurosis, anxiety and rheumatic pain. The studies performed on laboratory animals revealed analgesic and anti-inflammatory activities and also antioxidant and anxiolytic proprieties for *Lactuca capensis.* In the present study, the effects of the methanolic extract of *Lactuca capensis* (100 and 200 mg/kg) were assessed on spatial memory performance in an Aβ1-42 rat model of Alzheimer's disease using Y-maze and radial arm-maze tasks. Using a hippocampal tissue, biochemical parameters as superoxide dismutase (SOD), glutation peroxidase (GPX), acetylcholinesterase (AChE), malondialdehyde (MDA) and protein carbonylation level were determined in order to evaluate the oxidative stress levels in AD-treated rats and also the antioxidant capacities of the *Lactuca capensis* methanolic extract. The determination of histone-associated DNA fragments was performed using a Cell Death Detection ELISA kit (Roche) as an indicator of apoptosis. Furthermore, RNA isolation and qRT-PCR techniques were used to assess the absolute expression level of IL1β in the hippocampal tissue. Administration of the methanolic extract in both doses, prevented Aβ1-42-induced cognitive alteration evidenced by increased spontaneous behavior in Y-maze test as well as decreased of working and reference memory errors by performing radial arm maze test. Regarding the biochemical assays, SOD and GPX activities have significantly decreased in Aβ1-42 treated rats. In the same time, it was observed increased AChE activity and MDA and protein carbonyl levels in Aβ1-42 treated rats as compared to control groups. The enzymatic activities of SOD, GPX and AChE and also the MDA and protein carbonyl levels were restored to the normal conditions following the treatment with the methanolic extract. Moreover, the administrated methanolic extract at Aβ1-42 treated rats attenuated the hippocampal apoptosis and the increased IL1β expression in the Aβ1-42 treated rat was significantly decreased by the methanolic extract administration. In summary, our study indicated that the methanolic extract of *Lactuca capensis* could effectively enhance memory processes and restore antioxidant brain status. Therefore, the methanolic extract could be a potential candidate for further preclinical studies aimed at the treatment of cognitive deficits in AD.

IN SILICO STUDIES APPLIED ON ACETHYLCHOLINESTERASE INHIBITORS BASED ON SALVIA OFFICINALIS

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Severe cognitive and behavioral impairments are induced by the imbalance of neurotransmitters which appears in many psychiatric and neurological disorders [1]. Even if the pharmacological therapies are based on several natural and synthetic chemicals as enzymes and membrane receptor ligands, with still unclear specific mechanisms, the symptoms of Alzheimer disorder (AD) remain severe and the progress of the disease is not halted. Under such circumstances, latest strategies in experimental and in silico neuroscience, consider that is critical to identify how already clinically-approved Alzheimer drugs and natural compounds isolated from plants oil are able to improve AD symptomatology [2]. Results of recent studies suggested that acetylcholinesterase and NMDA ligands are able to improve neuroplasticity by themselves but many molecular aspects of these processes are still unclear. There is also very succinct data on how the natural compounds are capable of improving AD symptomatology. Here we present the chemical structures-biological activity relationship (SAR) of these molecules revealed by recent experimental and in silico studies, offering a new perspective on the molecular mechanism.

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AGE-DEPEDENT EFFECT OF MICROWAVE RADIATION ON PROLIFERATION AND CELL DYING IN THE RAT ROSTRAL MIGRATORY STREAM

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Microwave radiation (MWR) from the mobile phone base stations is regarded as having low power, but the output is continual. This radiation is one of the most significant environmental factors and its effect on living organism is a subject of intensive studies. The research of MWR effect is increasingly focused on the brain regions where new neurons are produced in adulthood. It has been shown that both neurogenic areas, the hippocampus and the subventricular zone (SVZ) are vulnerable to adverse factors of an environment and MWR seems to have the most severe impact on adult neurogenesis among exogenous factors studied in this relation.
The aim of our work was to investigate the effect of MWR on proliferation and cell dying in the rat rostral migratory stream (RMS) - a migration route for the SVZ neuroblasts to reach the olfactory bulb. Adult and neonatal (two weeks old) rats were exposed to a pulsed-wave electromagnetic radiation at the frequency of 2.45 GHz and mean power density of 2.8 mW/cm^2, in a purpose-designed exposure chamber for 3 hours daily during 3 weeks. After the exposure to MWR, neonatal rats were allowed to survive till adulthood. Regarding the survival time after the exposure, the adult rats were divided into two groups: without survival and with two weeks survival. Sham-exposed rats of the same ages underwent the same procedures as irradiated animals, except for the MWR exposure. After perfusion fixation, the brains of all rats were processed for morphological analysis. Proliferating cells were immunohistochemically labelled using antibody against the marker of proliferation - Ki-67; dying cells were visualized by Fluoro-Jade C histochemistry.
Quantitative analysis of proliferating cells in the RMS of rats irradiated as adults confirmed the negative effect of MWR on proliferation, which was manifested by the highly significant decrease of the number of dividing cells. After 2 weeks of survival, the proliferation activity slightly increased, but it was still markedly reduced in comparison with control rats. Reduction of dividing cells number was accompanied by non-significant increase of dying cells in both groups of rats irradiated as adults. The MWR impact on proliferation and cell dying in rats that were irradiated as neonatal was manifested differently. The number of proliferating cells in their RMS was also decreased, but this decrease did not show any statistical significance. Interestingly, exposure of neonatal rats to MWR strikingly affected the cells dying. Two months after irradiation, the number of dying cells in the RMS of rats exposed to MWR was about twice as high as in control rats. It suggests that the radiation carried out in neonatal age had long-lasting influence on processes of postnatal neurogenesis, which is more likely manifested by altered cell dying than by changes in proliferation.

Supported by grants VEGA 1/0214/15, 2/0069/15 and APVV-15-0239

WHAT CAN ICA TELL US ABOUT SSVEP SOURCES?

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Steady-state visual evoked potentials (ssVEPs) are EEG responses to cyclic stimuli. Isolating the ssVEP response from other brain activity and from artefacts (e.g. EMG) is a major challenge. Here, we tested the usability of independent component analysis (ICA) for this purpose. We stimulated healthy young human participants (n=11 sessions) by dynamic random dot stimuli consisting of two states alternating at 1.875 Hz. Their EEG was recorded using a 64-channel BrainAmp EEG system at 1 KHz sampling rate. Raw data of at least 1 minute duration were low-pass filtered at 45 Hz. The recording was then decomposed using infomax ICA (implemented in the Matlab EEGLAB toolbox) into 64 independent components. We assessed the strength of stimulus evoked modulation in each ICA component by calculating the Fourier-component corresponding to the frequency of the alternating stimulus. We used T2circ statistics to test if this modulation was statistically significant. We found that significant ($p<0.05$) stimulus evoked modulation was present in 21 to 47 ICA components suggesting that many independent components contained some stimulus evoked response. Therefore, we attempted to reconstruct the ssVEP source by recombining the best k ICA components in decreasing order of their F-values, the test statistics of T2circ-test. We thus started with the most significant component and then added less significant ones one-by-one. The resulting signal was again, assessed by the T2circ-test performed on the signal time course at the Oz electrode position. We found that the combination of k =2 to 16 components (depending on the subject and stimulus) resulted in a signal where F-values reached a maximum. We considered this combination of independent components as an optimal reconstruction of the ssVEP source. Adding more components decreased F-values indicating deterioration of signal quality. The F-value of the optimally reconstructed ssVEP source was 4.09±1.98 times higher (and p-values lower by several magnitudes) than that of the raw signal. We conclude that ICA combined with spectral analysis can greatly improve the detection of ssVEP sources in EEG experiments.

Supported by grants OTKA K108747 and the Hungarian Brain Research Program KTIA_13_NAP-A-I/11, EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

ANTIBIOTICS AS NEW THERAPEUTICALLY STRATEGY IN PARKINSON'S DISEASE : ANTIINFLAMMATORY AND ANTIAGREGANT A-SYNUCLEIN EFFECTS

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Parkinson's disease (PD) is a chronic and progressive disorder that affect dopaminergic (DA) neurons causing gradual motor disability. Although its etiology remains unknown, the pathological role of several factors has been highlighted, namely neuroinflammation, protein misfolding and oxidative stress, in addition to genetic predispositions. The current therapy is mainly symptomatic with L-DOPA aiming to replace dopamine however this drug does not alt the neurodegenerative processes. The tetracycline-derivative doxycycline has been previously shown to be neuroprotective in models of neurodegenerative diseases. In the present study we analyses the effect of doxycycline and the non-tetracycline derivative rifampicin on neuro inflammation induced by LPS or α-synuclein fibers on microglial cells in culture and aggregation of α-synuclein in a cell free model. Results showed that: 1) doxycycline or rifampicin lowered the expression of the microglial activation marker IBA-1 and the release of glutamate induced by either LPS or α-synuclein fibers as well as the production of ROS, NO, and pro inflammatory cytokines (TNF-α and IL-1β); 2) doxycycline interacts with α-synuclein early aggregation intermediates leading to the formation of off-pathway species, with parallel β-sheet content, that do not evolve into fibril formation. These species are neither cytotoxic to dopaminergic cell lines, nor capable of disrupting the integrity of liposomes membrane. Results presented herein place some antibiotics as pleiotropic drugs becoming an attractive therapeutic strategy against PD

TYPE I IFNS ARE REQUIRED TO PROMOTE CNS IMMUNE SURVEILLANCE THROUGH THE RECRUITMENT OF INFLAMMATORY MONOCYTES UPON SYSTEMIC INFLAMMATION

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Brain-resident microglia and peripheral migratory leukocytes play essential roles in shaping the immune response in the central nervous system. These cells activate and migrate in response to chemokines produced during active immune responses and may contribute to the progression of neuroinflammation. Herein, we addressed the participation of type I-II interferons in the innate immune response displayed by microglia and inflammatory monocytes, to comprehend the contribution of these cytokines in the establishment and development of a neuroinflammatory process. Following systemic LPS challenge, we found glial reactivity and an active recruitment of $CD45^{hi}$ leukocytes close to $CD31^{+}$ vascular endothelial cells in circumventricular organs. Isolated $CD11b^{+}$ $CD45^{hi}$ $Ly6C^{hi}$ $Ly6G^{-}$ primed inflammatory monocytes were able of inducing T cell proliferation, unlike $CD11b^{+}$ $CD45^{lo}$ microglia. Moreover, an *ex-vivo* restimulation with LPS exhibited an enhancement of T cell proliferative response promoted by inflammatory monocytes. These myeloid cells also proved to be recruited in a type I interferon-dependent fashion as opposed to neutrophils, unveiling a pivotal role of these cytokines in their trafficking. Together, our results compares the phenotypic and functional features between tissue-resident versus peripheral recruited cells in an inflamed microenvironment, identifying inflammatory monocytes as key sentinels in LPS-induced neuroinflammation.

ROLE OF BDNF IN TYPE 2 DIABETES

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Type 2 Diabetes (T2D), a disease state recognized by impaired insulin sensitivity and hyperglycemia and one of the leading causes of mortality across the globe. Decreased levels of BDNF have been implicated in the insulin resistance. Low levels of BDNF accompany impaired glucose metabolism. Decreased BDNF is a pathogenic factor involved not only in dementia and depression but also with T2D. A substantial body of studies suggested that there is a strong link between depression and T2D. A causal relationship between insulin resistance and BDNF and underlying mechanism has not yet been demonstrated. The present study will show the evidence analyzing the behavioral paradigms and depression like state in type 2 diabetic zebrafish that are affecting BDNF expression and its interplay with insulin as well as glucose homeostasis in T2D. Results showed that type 2 diabetic zebrafish showed symptoms of stress, anxiety and depression. The cognitive abilities like learning and memory were also found to be affected in the type 2 diabetic state of zebrafish. The decreased levels of BDNF has also been found in the case of T2D, thus showing the important role of BDNF in the disease. Furthermore, analysis of epigenetic changes in BDNF, insulin and insulin receptors using histone modification status can also be helpful in this regard in the hope that by such study approach, we can get certain new insight about the T2D as well as new idea about T2D patient care.

Keywords: Type 2 Diabetes, zebrafish, anxiety, depression, BDNF.

EFFECT OF OREXIN B ON NUCLEUS ACCUMBENS MEDIATED EXPLORATORY AND ANXIETY LIKE BEHAVIOUR IN MALE WISTAR RATS

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Background: Reports suggest role for Orexins in alertness, sleep and wakefulness and cognitiveparameters. Therefore, we predicted a role of orexin in exploratory and anxiety like behaviour. Till date there are no specific studies of infusion of Orexin B into Nucleus Accumbens to assess the motor,exploratory and anxiety like behaviour in rats.
Objectives: To elucidate whether Orexin B induced exploratory behaviour is mediated through Catecholamines in male Wistar rats.
Methods: Inbred male Wistar rats (n= 24) were divided into three groups. i. e Control, Treated 1 (orexin B) and Treated 2 (Orexin B antagonist) groups. Using stereotaxic method, guide cannula was set in place bilaterally to reach Nucleus Accumbens. Orexin B and its antagonist, TCS-OX2-29 were infused in separate groups of overnight fasted rats. After four trials of infusion of orexin B, Open field exploration test was conducted. Further, Catecholamines (Dopamine, Adrenaline and Noradrenaline) estimated in brain tissue homogenate by ELISA. Data were expressed as mean±SEM (ANOVA; Student-Newman Keuls test,).$P<0.05$ were considered as statistically significant.
Results: Orexin B increased exploratory behaviour by significantly increasing the number of central and peripheral square entries. Neurotransmitter noradrenaline was significantly increased when compared to controls.TCS-OX2-29 decreased exploratory behaviour and decreased noradrenaline. However Orexin B and its antagonist didn’t alter anxiety like behaviour significantly
Conclusion: These results suggest that Orexin B plays a role in the regulation of exploratory behaviour and it might be mediated through noradrenergic neurotransmission in Nucleus Accumbens.

SINGLE CELL TRANSCRIPTOME ANALYSIS AND ELECTROPHYSIOLOGICAL RECORDING BASED PHENOTYPING OF FRONTAL CORTEX NEURONS

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Frontal cortex neurons attract particular attention because of their involvement in psychiatric diseases and regulation of mood and behavior. Specific targeting of frontal cortex neurons by pharmacological agents would be a step up for causal treatment of psychiatric diseases however it is already done indirectly by influencing VTA monoaminergic neurons projecting to L5 pyramidal cells of the frontal cortex. In the present study we aimed to disclose cell type specific molecular composition of main, physiologically and/or anatomically identifiable types of frontal cortex neurons as L2 and L5 pyramidal cells and fast spiking interneurons. Brain slices of frontal cortical areas were made form adult mice kept in immunologically controlled condition and recorded using step depolarization paradigm in a patch clamp rig. L2 and L5 pyramidal cells were identified by their anatomical position in the frontal cortex, FS cells were selected on the basis of firing pattern and shape of the cell body. The electrophysiological recording was performed as briefly as possible to make minimal changes in RNA expression profile, than the patch clamp pipette was pushed into the cell and the cytoplasm was harvested. Polyadenilated RNA of single cell samples was converted to cDNA, amplified linearly so that a T7 RNA polymerase promoter was inserted into the double strand cDNA and the T7 polymerase produced several strands of antisense chains. In turn, gDNA was not amplified. The method was developed in Eberwine's lab and described elsewhere in details. Samples were deep-sequenced on Illumina NGS platform. Data were normalized and processed by hierarchical clustering. Electrophysiological data was processed by a CED1401 script measuring 12 parameters automatically. Thus far we harvested 110 cells and sequenced a subset of them. RNA expression pattern of electrophysiologically established clusters of neurons resulted L2 and L5 pyramidal cell identifiers and FS cell identifiers, some of them are well targetable cell surface molecules. The RNA expression profiling of data revealed that ion channel composition of cells does not characterize the cell types of different firing patterns suggesting that the same physiology can be performed by different ion channel compositions. Our present study revealed that frontal cortex cells can be targeted directly because of the cell identifier proteins at the cell surface and we established a list of cell biomarker candidates for L2 and L5 pyramidal cells and FS interneurons at mRNA expression level. Our study further shows the importance in coupling of single cell transcriptomics with cellular physiology to better understand the molecular characteristics of neuronal circuit in frontal cortex.

DISTRIBUTION OF THE EXTRACELLULAR MATRIX MOLECULES IN THE PARARUBRAL AREA OF THE RAT BRAINSTEM

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The pararubral area is located in the reticular formation of the mesencephalon, dorsolateral to the caudal level of the red nucleus (RN). This GABAergic group of cells with small to medium sized cell bodies mediates the inhibition of the rubral neurons. The sensorimotor cortical fibers activate the pararubral neurons and through their activation they inhibit the rubrospinal neurons. In this way, the cerebral cortex disynaptically modulates the rubrospinal control over flexor motor execution. Previous studies suggest that the extracellular matrix (ECM) molecules display an area-dependent distribution pattern in the RN, and the molecular and structural heterogeneity of the ECM is correlated with the morphological properties of the neurons. The main components of the ECM are organized into condensed and diffuse forms. The condensed form surrounds the neurons as the perineuronal net (PNN), the diffuse form is deposited in the neuropil. Using histochemical and immunohistochemical methods, in the present study we examined the distribution of the ECM molecules in the pararubral area of the rat brainstem. 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, versican, brevican, neurocan), the tenascin-R (TN-R) and the link protein, HAPLN1 were detected with antibodies. Our results showed, that the ring-like pattern of PNN was visible only in case of the WFA and aggrecan reactions. The versican appeared as stained dots, mainly in the intercellular space, along the axons. We have observed similar dots in case of the brevican, but primarily in the immediate vicinity of the neurons. HAPLN1 was found also around the neurons, but the reaction pattern was variable. In case of the WFA, aggrecan, brevican and HAPLN1 reactions, dendrites were also ensheathed by the labeling. HA, neurocan and TN-R reactions showed postivity only in the neurophil. Intensity of labeling in the neuropil was variable in case of each molecules. According to our findings, on the basis of the distribution of ECM molecules the pararubral area shows high similarity with the parvicellular part of the red nucleus, where the neurons have similar morphological and functional properties. Our results strenghten the recent observations, that the expression pattern of the ECM is correlated with the morphological, physiological and functional properties of neurons.

Support: OTKA K115471 and MTA TKI355.

SPECIAL VOLTAGE-GATED SODIUM CHANNELS GRANT COLD AND HEAT RESISTANCE OF NOCICEPTIVE ENCODING – AN OVERVIEW

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Somatosensory nerve fibers and endings gain their sensitivities by expressing various thermally and/or mechanically activated (or inactivated) ion channels that generate depolarizing receptor potentials (transduction). However, it is the neurons´ capacity to transform depolarization into propagated action potential sequences that decides about transmission of the stimulus-provided information and finally about (conscious) sensation. The voltage-gated sodium channels (NaVs) that provide this capacity are exposed to the same stimulus energies as the transduction channels, and as any macromolecule they are structurally influenced by changes in temperature and mechanical tension. In case of nociceptors that have to encode potentially damaging stimuli these physical energies could incapacitate or even denature the NaV proteins. However, nociceptors are armed with a particular set of NaVs that are rarely expressed by other excitable cell types. A decade ago the TTX-resistant NaV1.8 was identified as a rather frost resistant action potential generator, enabling cold pain perception, while other NaVs succumb to reversible cold block which accounts for the numbness of ice cold fingers. Also heat just 6°C above body temperature can cause a reversible block of nerve conduction, but nociceptors are able to encode temperatures even above 50°C. This capacity depends largely on both, cooperating, TTX-resistant sodium channels NaV1.8 and NaV1.9 whereby the latter - activated already near resting potential - helps to reach the more depolarized voltage activation range of NaV1.8. NaV1.9 surprises with an impressive temperature dependency; its voltage-dependent activation as well as inactivation are enormously accelerated by heating (Q10 > 5) so that at body temperature it alone can generate overshooting action potentials in sensory neurons, whereas at room temperature it appears as the carrier of a persistent sodium current of extremely slow kinetics. The third among the specific NaVs in nociceptors, the TTX-sensitive NaV1.7, normally the fast action potential trigger, only contributes to the basic heat sensitivity of the peripheral nerve endings but is essential at body temperature for the long-distance unmyelinated nerve fiber conduction across branch points and into the sink of the spinal dorsal horn synapse. In consequence, human mutants lacking NaV1.7 are congenitally indifferent to pain (CIP syndrome) which deficit is aggravated in both human and mouse mutants by epigenetic upregulation of opioidergic inhibition. All in all, nociceptor NaVs impress as a splendid example of evolutionary adaptation to harsh environmental conditions.

Supp. by DFG, Pfleger-, Fresenius- and Sander-Stiftung

ALTERED EXPRESSION LEVEL OF GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AND PROLACTIN-RELEASING PEPTIDE IN TYPE 2 DIABETIC PATIENTS

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We reported previously that glucagon-like peptide-1 (GLP-1) of brainstem origin activates neurons located in the dorsomedial hypothalamic nucleus (DM) and contributes to satiation in rats. Focusing on the specific role of the DM in regulation of food intake we now investigated the distribution of the GLP-1 receptor (GLP-1R) and prolactin-releasing peptide (PrRP) in the human DM. A high number of neurons located in this nucleus express GLP-1R in both human and rat brains. In addition, the DM is the only hypothalamic nucleus where PrRP producing neurons are located. We used postmortem human hypothalamic samples from five type 2 Diabetes mellitus (T2DM) patients and five control subjects. We developed in situ hybridization probes for human GLP-1 receptor and PrRP, which resulted in abundant labeling in DM following radioactive in situ hybridization histochemistry. Moreover, the expression pattern of GLP-1 receptor topographically overlapped with that of the PrRP neurons in the DM confirming our data in the rat established with fluorescent double labeling. We found an increased GLP-1R and an in turn reduced PrRP expression in the DM in post mortem hypothalamic samples from T2DM subjects as compared to controls. A previous study reported that in the paraventricular (PVN) and infundibular nuclei (INF), decreased expression of GLP-1R was seen in type 2 diabetic patients. The authors suggested that the decreased expression of GLP-1R in the PVN and in the IFN may be related to the dysregulation of feeding behavior and glucose homeostasis in T2DM. Our findings suggest that GLP-1 and PrRP in the DM have special roles in food intake regulation.

Support was provided by the NAP A program for the Human Brain Tissue Bank Microdissection Laboratory, Semmelweis University and the Hungarian Academy of Sciences KTIA_NAP_13-1-2013-0001 for ER and MP and KTIA_NAP_B_13-2-2014 for AD.

THE TOOL-USE OBSERVATION NETWORK: WATCHING AND USING TOOLS SHARE REASONS

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Action is the means through which we interact with the environment. Because we are social animals, more than often we observe others interacting with the world through their own actions. It has been suggested that the brain areas engaged when observing actions, i.e. the Action Observation Network (AON), could be the core network underlying critical social abilities such as action understanding, imitation and social learning. Most studies on these topics have employed experimental tasks in which stimuli depicting tool-use actions and stimuli depicting non-tool related actions, i.e. very often grasping actions, are employed indistinctively. In parallel, a growing body of evidence shows that tool-use is a defining feature of human species and could be supported by brain areas existing only in non-human primates. Tool-use could be based on cognitive functions including mechanical reasoning on the physical properties ruling the tool-object interaction.
The goal of the present study is to disentangle the Tool-use Observation Network (ToON) from the AON by re-analyzing through a comprehensive meta-analysis all available neuroimaging data on Action Observation.
Areas more consistently involved in the ToON compared with the Grasping network are the left supramarginal gyrus and the left inferior frontal gyrus, previously found as supporting mechanical reasoning. We assessed the difference between observing and performing a tool-use action by comparing the ToON with areas engaged in planning tool-use actions at the 1st person-perspective. The fusiform gyrus was more consistently activated by observing than planning. The same area was found to differ between observing vs imagining a grasping action.
Our results support the view that observing a tool-use action engages the same reasoning functions than planning a tool-use action. In addition, they show that observation and planning differ from a single, possibly generic, brain component.

LIFE AND WORK OF JÁNOS SZENTÁGOTHAI, A PIONEER OF 20TH-CENTURY HUNGARIAN NEUROSCIENCE

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János Szentágothai (né Schimert, 1912-1994) was an emblematic figure and influential actor of Hungarian neuroscience throughout the second half of the the 20th century. Besides being an eminent scientist and serving as a role model for many young researchers, he was also an active scholar. As the Head of the Departments of Anatomy at the universities of Pécs and Budapest, he taught generations of medical students. Later in his life he held several leadership positions, he was the 14th president of the Hungarian Academy of Sciences, and became a member of the Hungarian parliament between 1990 and 1994. While fulfilling his official responsibilities, he was also an enthusisatic father, grandfather, gardener and literature lover. In my lecture I will try to summarize his life and work both from a scientific and a personal angle.

EFFECT OF THE ENDOGENOUS PACAP ON THE PROTEOME OF MICE BRAIN AREAS

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Pituitary adenylate cyclase-activating peptide (PACAP) is a widespread neuropeptide, which has diverse effects. It has remarkable neuroprotective and neurotrophic properties, already described in animal models of Parkinson's disease, Alzheimer's disease and Huntington chorea. The complex effect of PACAP on the proteome has not yet been fully described. To examine the effect of endogenous PACAP on the proteome of the brain with good resolution, we used imaging mass spectrometry (IMS) on brains of PACAP knock out and wild type mice.
The most widespread IMS scanning technique is matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS) imaging (MALDI Imaging). Because MALDI Imaging is a relatively new technique with various factors influencing the measured intensities, we needed to confirm the results with other methods. We used polyacrylamide gel electrophoresis (PAGE) and after that liquid chromatography mass spectrometry (LC-MS) to analyze the samples from the neocortex and mesencephalon of PACAP knock-out and wild type mice and confirmed the results of MALDI Imaging.Our findings with MALDI imaging suggested differences in the expression of numerous proteins in PACAP knock-out mice. After the densitometry analysis of identified proteins, we found significantly lower expression in the levels of beta-synuclein in PACAP knock-out mice. Beta-synuclein is a presynaptic protein that can block the oligomerization of alfa-synuclein. Since alpha-synuclein plays crucial role in the pathomechanism of Parkinson's disease, beta-synuclein-related pathways could have protective effects in neurodegenerative diseases.Polyacrylamyde gel electrophoresis and liquid chromatography mass spectrometry measures verified our results of MALDI Imaging.In conclusion, our finding suggests that the altered beta-synuclein levels might be responsible for the known vulnerability of PACAP knock-out mice in neurodegenerative diseases and against neurotoxic agents.

EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, GINOP-2.3.2-15-2016-00050 "PEPSYS, MTA-TKI, KTIA_13_NAP-A-III/5, the National Research, Development and Innovation Fund K119759
The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

RHYTHMIC PERSISTENT FIRING OF NEUROGLIAFORM INTERNEURONS IN THE HUMAN AND RODENT NEOCORTEX

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Persistent firing is a form of activity-induced ectopic action potential generation which has been recorded in several GABAergic interneuron classes from the hippocampus and the neocortex of rodents in vitro and in vivo. In order to assess potential species specific aspects of persistent firing, we performed whole cell patch clamp recordings of layer 1 interneurons in rat and human neocortical slices in submerged recording chamber. Interneurons in layer 1 of the human neocortex were capable of establishing persistent firing induced by the same paradigm used in rodents. In addition, we detected episodes of persistent firing alternating with silent periods rhythmically in the frequency range of slow oscillations (0.5 - 2 Hz) in identified human neurogliaform cells. This bistable persistent firing state was absent in human and rodent non-neurogliaform interneurons remaining silent following a single persistent firing episode. Furthermore, we show that rodent neurogliaform cells are also capable of rhythmic persistent firing but only in dual superfusion recording chambers, presumably due to a more physiological environment. We hypothesize that rhythmic persistent firing in neurogliaform cells might contribute to the generation of slow oscillations in the rodent and human neocortex.

MORPHOLOGICAL, IMMUNOCYTOCHEMICAL AND TRANSCRIPTOMIC FINGERPRINTS DURING EMBRYONIC DEVELOPMENT OF *EISENIA ANDREI* (ANNELIDA, CLITELLATA)

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Development of a multicellular organism is mediated by both spatial and temporal coordination of different processes, like cell division and migration, cell attachment and differentiation, tissue- and organogenesis. In earthworm, similarly to other invertebrates, the formation of the central nervous system (CNS) and its anteroposterior patterning are determining steps of the embryogenesis. Probably secretion activity of the CNS has a strong influence on cell and tissue morphogenesis. By means of conventional light microscopy, electron microscopy, GABA immunocytochemistry and real time polymerase chain reaction (PCR) tissue and organ morphogenesis were investigated during the embryogenesis of *Eisenia andrei*.
GABA, as early putative signal molecule, was synthesised by several neurons and the number of GABAerg cells increased parallel with both gangliogenesis and the formation of the body segments.The expression of Neuromacin mRNA increased till the hatching when all tissues and organs were developed. Neuromacin was identified first in the CNS of the adult earthworms and thought to be a key-player of cell morphogenesis. Our results suggest that GABA is a key player during both the early events of tissue and organ development (cell proliferation, migration and differentiation) while Neuromacin is possibly more involved in later stages of tissue morphogenesis (axonogenesis, development of anchoring cell junctions, electric and chemical synapses).

THE EFFECT OF CHRONIC STRESS AND CONCOMITANT CANNABIS EXPOSURE ON BEHAVIOR AND ADULT HIPPOCAMPAL NEUROGENESIS IN MICE.

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Cannabis is a widely used recreational drug, but it has an ambiguous effect on the emotional response and cognitive functioning of the user. Advocates argue that cannabis can be beneficial in treating anxiety and depression while others claim that it can actually cause anxiety and may even trigger psychotic episodes.Our aim here was to mimic real life situation of cannabis exposure in experimental animals. We exposed adult male mice to stress and concomitantly tomarijuana and investigated the consequences on behavior and adult hippocampal neurogenesis. Adult male NMRI male mice (n=36) were subjected to 2-months of daily chronic restraint stress (CRS) and marijuana smoke exposure. The animals were divided into fourexperimental groups: Control, CRS, Marijuana, CRS + Marijuana groups. We used the restraint stress paradigm as a stressor and animals were restrained for 6-hours / day for 5-days / week. The animals were exposed to marijuana smoke with the help of a manual smoking system (Teague Enterprise) for 6-hours / day, 5-days / week. During the 2-months experimental period the animals were subjected to behavioral tests to analyze anxiety and cognitive performances. To examine the effect of long-term cannabis smoke exposure on pulmonary function we used a whole body plethysmograph analysis. To investigate cell proliferation, the exogenous thymidine analog BrdU was administered on the last experimental day and animals were sacrificed 24-hours later. Animals were transcardiallyperfused with a fixative at the end of the experiment and 50-µm brain slices were cut and stained for anti-BrdU and anti-doublecortin immunohistochemistry.Doublecortinis a marker for immature neurons. Currently, we analyze the collected data. Preliminary results will be presented on the poster.

Support: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

EXPRESSION OF COMT AND TH GENES AND PREPULSE INHIBITION IN RATS WITH GENETIC CATATONIA

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The experiment was performed on the rat strain with **g**enetics **c**atatonia (GC), which are prone to passive defensive reactions of cataleptic freezing.
Aim: Investigation of mRNA level of tyrosine hydroxylase (*Th*) and catechol-O-methyltransferase (*Comt*) genes, prepulse inhibition (PPI) and startle reflex in the GC rats.
Methods: *RT-qPCR*, investigation of PPI (prepulse inhibition) and startle reflex in TSE Startle Response System (Germany).
Results: For the first time a decrease in PPI in inbred GC rats was observed, as compared to the control rats of Wistar Albino Glaxo (WAG) strain. A low level of PPI was shown at 75 dB prestimul force (Mann-Whitney U=24.0, $p < 0.001$), as well as at 85 dB (Mann-Whitney U=25.0, $p < 0.001$). The study of startle reflex amplitude indicated elevated values ??in GC rats, which conforms with the previous data (Popova et al., 2000); increase of startle reflex amplitude is also well known as typical feature of animals with a high level of anxious behavior. Habituation of startle reflex in GC rats did not differ from control group.
We examined the mRNA level of the *Comt* and *Th* genes in the frontal cortex, hypothalamus, amygdala, striatum, midbrain, medulla and in adrenal gland in GC and WAG rats. The mRNA level of the *Th* gene did not differ in the rat structures. The expression of the *Comt* mRNA revealed difference in the frontal cortex only. It was increased ($p < 0.05$) in GC frontal lobe.
Conclusion: The PPI reduction in animals, caused by various exposures, serves as an experimental model of schizophrenia (Martinez et al., 2000); in addition, changes in the activity of catechol-O-methyltransferase enzyme in the frontal cortex are associated with schizophrenia and bipolar disorder (Abdolmaleky et al., 2006). Thus, according to the data obtained, reduced level of PPI and changes in the *Comt* expression count in favor of functional similarity of GC line (as a genetic model of psychopathology) with the prototype (psychopathology itself).

The research was supported by the Russian Foundation for Basic Research (grant no. 16-04-01325).

PHYSIOLOGICAL EXPRESSION OF OLFACTORY DISCRIMINATION RULE LEARNING BALANCES WHOLE-POPULATION MODULATION AND CIRCUIT STABILITY IN THE PIRIFORM CORTEX NETWORK.

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Once trained, rats are able to execute particularly difficult olfactory discrimination tasks with exceptional accuracy. Such skill acquisition, termed "rule learning", is accompanied by a series of long-lasting modifications to three cellular properties which modulate pyramidal neuron activity in piriform cortex; intrinsic excitability, synaptic excitation, and synaptic inhibition. Here, we explore how these changes, which are seemingly contradictory at the single-cell level in terms of their effect on neuronal excitation, are manifested within the piriform cortical neuronal network to store the memory of the rule, while maintaining network stability. To this end, we monitored network activity via multisite extracellular recordings of field postsynaptic potentials (fPSPS) and with single-cell recordings of miniature inhibitory and excitatory synaptic events in piriform cortex slices. We show that although 5 days after rule learning the cortical network maintains its basic activity patterns, synaptic connectivity is strengthened specifically between spatially proximal cells. Moreover, while the enhancement of inhibitory and excitatory synaptic connectivity is nearly identical, strengthening of synaptic inhibition is equally distributed between neurons while synaptic excitation is particularly strengthened within a specific subgroup of cells. We suggest that memory for the acquired rule is stored mainly by strengthening excitatory synaptic connection between close pyramidal neurons and runaway synaptic activity arising from this change is prevented by a nonspecific enhancement of synaptic inhibition.

OXYTOCIN INTRANASAL ADMINISTRATION AFFECTS NEURAL NETWORKS UPSTREAM OF GNRH NEURONS

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The last decade has witnessed a surge in research investigating the trial application of intranasal oxytocin as a method of enhancing social interaction in human, while all aspects of its effects are not well understood. Since oxytocin is involved in the regulation of hypothalamic-pituitary-gonadal axis by affecting gonadotropin-releasing hormone (GnRH) system, we hypothesized that GnRH neurons might also be affected by the treatment. Accordingly, we evaluated effects of different levels of oxytocin intranasal administration on GnRH expression in the male rat hypothalamus. In addition, we assessed expression of two excitatory (kisspeptin and neurokinin B) and two inhibitory (dynorphin and RFamide related peptide-3) neuropeptides upstream of GnRH neurons as a possible route to relay oxytocin information. Here, twenty four adult male rats received 20, 40 or 80 µg oxytocin intranasally for 10 days and then posterior (PH) and anterior (AH) hypothalamus dissected for evaluation of target genes. Finding results revealed the increased level of Gnrh mRNA in the both PH and AH; however, only the highest dose of the treatment was effective to induce a significant elevation of the gene expression. Also, we found strong kisspeptin expression in the AH at the highest oxytocin dose; whereas the treatments, dose dependently decreased Kiss1 mRNA in the PH. Moreover, the basal level of Nkb mRNA was increased following oxytocin treatments. Furthermore, although intranasally applied oxytocin decreased hypothalamic Rfrp mRNA level, the Dyn mRNA neither in the PH nor the AH was statistically affected. Therefore, intranasal administration of oxytocin could affect GnRH system and neural networks upstream of GnRH neurons.

CUCURBITACIN B MITIGATES EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS BY INHIBITION OF IL-17/IL-23 IMMUNE AXIS

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Pharmacological approaches to inhibit brain acute inflammation may represent important strategies for the control of autoimmune diseases. Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating and autoimmune disease of the central nervous system (CNS). Cucurbitacin B (CuB), an oxygenated tetracyclic triterpenoid compound extracted from Cucurbitaceae plant species, is a bioactive agent by disruption of microtubule polymerization and inhibition of JAK/STAT signaling. However, there has been little information about impact of CuB on MS treatment. In this research, for the first time we examine effects of CuB (specific STAT3 blocker), in experimental autoimmune encephalomyelitis (EAE) mouse model of MS. EAE was induced by subcutaneous immunization of MOG35-55 in 8-week-old C57BL/6 mice. CuB was administered at different doses (0.25, 0.5 and 1 mg/kg body weight/day/i.p) from the first day of the experiment. Inflammatory responses were examined using qRT-PCR, western blot and immunohistochemistry (IHC) analysis of specific markers such as p-STAT3, IL-17A, IL-23A, CD11b and CD45. CuB reduced STAT3 activation, leukocyte trafficking, and also IL-17/IL-23 immune axis in this model. Treated mice with lower doses of CuB exhibited a considerable depletion in the EAE clinical score which correlated with decreased expression of IL-17, IL-23 and infiltration of CD11b+ and CD45+ cells into the CNS. Our in vivo results suggest that STAT3 inhibition by CuB will be an effective and new approach for the treatment of neuro-inflammatory disease such as MS.

THE EFFECT OF ACUTE EXERCICE ON WORKING MEMORY PERFORMANCE

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Objectives: The purpose of this study was to compare the influence of acute moderate intensity aerobic exercise on the speed and accuracy of working memory tasks.
Methods: Participants (N=30, 20 male, 10 female; Age: mean=22.3 years, range=21-28 years) completed a dual n-back working memory task before the start, immediately after and 5 min after an intervention of exercise. In this task, ten pictures (viz. ball, book, car, cake, cat, fish, heart shape, pencil, boot, and spoon) appeared on the screen randomly and subjects had to click on the target box when the current picture was a repeat of what they had seen or picked 2 pictures ago. Therefore, the target had to click if the same picture was repeated in the third place. It constituted of 30 trials. After completion of the 30 trials, results were displayed. Three minute step test was used for acute moderate intensity aerobic exercise. The data obtained were compared using paired t test.
Results: Findings revealed a significant benificial influence ($t=2.55$, $p \leq 0.05$) of acute moderate intensity exercise on the accuracy of working memory of subjects after a rest of 5 min after exercise in comparision to working memory before start of exercise. In contrary, no significant relation between the speed of working memory performance and exercise was found. Likewise, independent evaluation of speed and accuracy of working memory after exercise between male and female did not show any significant difference.
Conclusion: This work indicates that acute moderate intensity aerobic exercise is effective in improving working memory function and may be beneficial for healthy adults whose cognitive performance is relatively low.

COMPARTMENTAL ORGANIZATION IN THE MOUSE CEREBELLAR CORTEX AND NUCLEI, AND INFERIOR OLIVE BASED ON MOLECULAR EXPRESSION PATTERNS AND TOPOGRAPHY OF PURKINJE CELL AXONAL PROJECTIONS

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Introduction: The adult cerebellum is organized into longitudinal compartments that are revealed by specific axonal projections, and thus are presumably involved in different motor and cognitive functions. Heterogeneous Purkinje cell (PC) subsets with different molecular compositions are distributed into transverse zones and parasagittal stripes in the cerebellar cortex. In this study, we compared the differences in expression pattern of other heterogeneously-expressed molecules, including Protocadherin 10 (Pcdh10), with aldolase C (=zebrin II), which has been the most extensively used marker for compartmentalizing the cerebellum, to identify parasagittal stripes of PC subsets finer than those identified solely by aldolase C.
Method: Pcdh10 gene expression was visualized by the reporter molecule beta-galactosidase using heterozygote samples of the knock-in mouse strain OL-KO, and the expression patterns analyzed in the cerebellar cortex and nuclei, and inferior olive. The positional relationship of their striped expression patterns was analyzed with serial section alignment analysis (SSAA) in coronal and horizontal sections. In addition, we investigated PC axonal projections of cortical subpopulations using fluorescent tracer injections.
Results: β-gal histochemical procedure revealed that Pcdh10 can selectively label PCs within the cerebellar cortex by identifying distinct compartments and sub-stripes in areas where aldolase C does not show clear patterning. Pcdh10 was also expressed in topographically matching subareas in the cerebellar nuclei and inferior olive. Each injection of tracer labeled neurons or axonal terminals in a subarea in a single sub-nucleus of the inferior olive or cerebellar nucleus, indicating presence of fine compartmentalization.
Conclusion: The heterogeneous expression of Pcdh10 in PCs may be linked with topographic axonal projections and the formation of neuronal circuits. In the future, Pcdh10 could be used as a potential therapeutic target for interventions in neurological diseases such as autism, which is known to affect information processing in the brain, by altering how nerve cells and their synapses connect.

EFFECT OF CHRONIC TREATMENT WITH RESVERATROL ON SIRT1 IN SEVERAL BRAIN REGIONS OF AGED RATS

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Aim: Resveratrol is an antioxidant compound which exhibit neuroprotective effects in aged rats. Some of their effects may be mediated by SIRT1, which is a crucial component of multiple interconnected regulatory networks that modulate neuronal plasticity, inflammation and cognition. As it is affected by aging, we have studied the levels of SIRT1 and its 75 kDa fragment in several brain regions of young and aged rats, and after resveratrol chronic treatment in aged rats.
Methods: Aged (20 month-old) Wistar male rats were treated with resveratrol (20 mg/kg, i.p., during 28 days), and their corresponding control (20 month-old) and a group of young rats (3 month-old) were treated with corn oil (1 ml/kg, i.p., during 28 days). The levels of SIRT1 (110 and 75 kDa) were quantified in hippocampus, striatum, frontal cortex and cerebral cortex by Western immunoblot with a specific antibody.
Results: It was observed an age-related decline in SIRT1 protein levels in rat hippocampus (110 kDa: 35%; 75 kDa: 14%), striatum (110 kDa: 47%; 75 kDa: 22%) and frontal cortex (110 kDa: 29%; 75 kDa: 11%), with an increased in SIRT1 fragmentation index. In contrast, no effects of aging on SIRT1 levels were found in cerebral cortex. Resveratrol prevented the deleterious effect of aging on SIRT1 levels, recovering the content with values close to those of young rats in hippocampus (110 kDa: 90%; 75 kDa: 105%), striatum (110 kDa: 78%; 75 kDa: 114%) and frontal cortex (110 kDa: 90%; 75 kDa: 96%), reducing also the fragmentation index.
Conclusions: Aging leads to an important reduction in SIRT1 levels and increases its fragmentation index in brain regions related to cognition. In contrast, resveratrol has the ability to reverse these aging effects, suggesting beneficial effects of resveratrol against brain aging.

INVESTIGATING NOVEL SMALL MOLECULE ENZYME INHIBITORS FOR TREATING COGNITIVE DECLINE AND DEMENTIA.

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Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by progressive loss of memory and deterioration of cognitive function. Currently, there is no effective treatment for AD. Ghrelin is a 28-amino acid hormone that is produced in response to calorie restriction (CR) and is generated in a wide variety of tissues. Ghrelin exists in two distinct forms, the ‘active' acylated ghrelin (AG) and the ‘inactive' unacylated ghrelin (UAG). AG can be enzymatically de-acylated to UAG by acyl-protein thioesterase 1 (APT1). As CR is associated with neuroprotection and improved memory, we have investigated whether ghrelin mediates these effects. Indeed, we have shown that CR enhances adult hippocampal neurogenesis (AHN) and memory in an AG-dependent manner and that AG is essential for the neuroprotective effect of CR. Thus, increasing the bio-availability of circulating AG, by preventing APT1 mediated de-acylation, may prevent the damage caused by neurodegeneration. We show that PalmostatinB, an APT1 inhibitor, increases levels of AG. Therefore, PalmostatinB, as other APT1 inhibitors, may be considered a possible therapeutic agent for the treatment of dementia. Based on these findings, the goal of my study is to establish whether the inhibition of APT1 can indeed promote the bio-availability of AG and lead to neuroprotection and improved cognition. Firstly, I have identified several drug-like APT1 (and APT2, a close homolog of APT1) inhibitors and carried out assays of cell viability and toxicity in different cell lines, including RAW267.4 (macrophages) and differentiated ReNcell VM (Human Neural Progenitors) that express endogenous APT1. Preliminary data shows that the mitotoxicity and cytotoxicity are unaltered and that these compounds do not affect the size and the growth of dendrites. Studies are ongoing to identify novel APT1 inhibitors. Selected lead compounds will be tested in pre-clinical models to assess potential toxicity, as well as its effects on AHN, visuo-spatial memory deficits. The ultimate aim of this work is to generate APT1 inhibitors to treat neurodegeneration in vivo.

HIGHER SUSCEPTIBILITY OF SOMATOSTATIN 4 RECEPTOR GENE-DELETED MICE TO CHRONIC STRESS-INDUCED BEHAVIORAL AND NEUROENDOCRINE ALTERATIONS

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The somatostatin 4 receptor (sst4) is widely expressed in stress-related brain areas (e.g. hippocampus, amygdala) and regulates the emotional behavior in acute situations. Since its importance in chronic stress-induced complex pathophysiological alterations is unknown, we investigated the involvement of sst4 in the responsiveness to chronic variable stress (CVS). Sstr4 gene-deficient ($Sstr4^{-/-}$) mice and their wildtype counterparts ($Sstr4^{+/+}$) were used to examine the behavioral and neuroendocrine alterations as well as chronic neuronal activity (FosB expression) changes in response to CVS. In $Sstr4^{+/+}$ mice, there was no behavioral response to the applied CVS paradigm. In contrast, immobility time in the tail suspension test increased after the CVS in the knockouts. In the forced swim test, $Sstr4^{-/-}$ animals showed increased baseline immobility and then it decreased after the CVS. Light-dark box and open field test behaviors and sucrose preference did not respond to the stress in the knockouts. Adrenal weights increased and thymus weights decreased in both $Sstr4^{+/+}$ and $Sstr4^{-/-}$ mice demonstrating the effect of chronic stress. The relative adrenal weight of stressed knockouts increased to a greater extent, while relative thymus and body weights decreased only in the $Sstr4^{-/-}$ mice. Basal plasma corticosterone concentrations did not change after the CVS in either genotype. FosB immunopositivity in the central and basolateral amygdaloid nuclei was enhanced in stressed knockouts, but not in wild types. This is the first evidence that sst4 activation is involved in the behavioral and neuroendocrine alterations induced by chronic stress with a crucial role of plastic changes in the amygdala.

Supported by: KTIA_NAP_13-2014-0022, GINOP-2.3.2-15-2016-00050, GINOP-2.3.2-15-2016-0004. EFOP-3.6.1.-16-2016-00004
Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs
The project has been supported by the European Union, co-financed by the European Social Fund
The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

LAMINAR DISTRIBUTION OF CALRETININ NEURONS IN THE MONKEY PREFRONTAL CORTEX

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Previous studies in primate associative cortex have shown that calretinin neurons represent 12% of the total neuronal pool whereas in rodents their proportion does not exceed 3%. It is still unclear if such an increase is reflected in laminar distribution and present in entire cortex. In this study, we have compared phylogenetically and functionally different areas of the macaque monkey prefrontal cortex (Brodmann areas 24, 32 and 9).
Stereological analysis of serially cut sections, immunohistochemically stained for the neuronal nuclear marker (NeuN) and calretinin (CR), revealed that calretinin neurons are mostly located (80%) in the upper cortical layers. Across all analysed areas, upper cortico cortical layers have a higher proportion of calretinin neurons: in layer III 20%, in layer II 30% and in layer I almost 50% of neurons express calretinin. The increased number of calretinin neurons in the lower part of the layer I of the primate prefrontal cortex might be the reason for the increased cellularity (3% of total neuronal number) of this layer.
Our data showed that in the primate prefrontal areas, GABAergic neurons are overrepresented in upper layers, implying more complex network organization and information processing. Absence of differences between prefrontal areas in macaque monkeys confirms that principal neocortical input and output cell types are a conserved feature of the dorsal telencephalon.
Comparison of orbito-medial prefontal cortex (Džaja et al. 2014, Society for neuroscience 446.19) with lower densities in dorso-medial parts suggests that during primate evolution increase in neuron complexity and connections precedes in dorsal areas of prefrontal cortex.

This work was supported by the Croatian Science Foundation project No. 5943

EFFECTS OF DARK EXPOSURE ON MOUSE VISUAL CORTEX PLASTICITY

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Changes in the excitatory-inhibitory (E/I) balance, mediated by maturation of parvalbumin positive (PV) GABAergic interneurons, have been identified as a key factor controlling the critical period of experience-dependent plasticity. Interventions that slow PV cell maturation, such as dark rearing, also delay the time course of the critical period. In both rats and cats brief dark exposure (DE) later in life can restore plasticity and enable recovery of vision through a previously deprived eye.
We studied the effects of DE on ocular dominance and single-cell responses in the binocular zone of mouse V1, during recovery from long-term monocular deprivation (MD). We employed chronic intrinsic signal imaging and two-photon calcium imaging. We also examined the density of PV neurons and of perineuronal nets (PNNs), a known structural brake on plasticity. After reopening of the deprived eye one group was placed in a dark room for 7 days and subsequently transferred to standard cages in a 12h day/night cycle while the other group was placed immediately in standard cages. Animals were imaged 5 times at weekly intervals. In terms of ocular dominance, mice that had experienced DE exhibited more rapid recovery (within 1 week) than those that had not, but the end points were not significantly different. Calcium imaging showed no significant difference in the recovery of orientation selectivity of excitatory neurons between the two groups. PV interneurons exhibited a smaller ocular dominance shift during MD but no differences in subsequent recovery. The proportion of PV cells surrounded by PNNs was smaller in mice that had experienced DE.
Our results show that DE causes a moderate enhancement of mouse visual cortex plasticity.

ON THE MECHANISMS OF ACTION OF NOBILETIN ON BRAIN MITOCHONDRIA

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Citrus flavonoid nobiletin has anticancer, antiviral, neuroprotective, anti-inflammatory activities and depending on the cell types exhibits both pro- or anti-apoptotic properties that suggest the effect of this compound on the central metabolic systems, such as mitochondrial bioenergetics.
The aim of our study was to investigate the influence of nobiletin on brain mitochondrial bioenergetics and determine the target molecules, involving in nobiletin-induced alterations.
Methods: Analysis of mitochondrial enzymes' activities and estimation of respiration rate, mitochondrial membrane potential, ROS production were performed in bovine brain mitochondria to evaluate the direct effect of nobiletin on the mitochondrial bioenergetics. Additionally, we made an HPLC determination for detection of nobiletin's metabolism in isolated mitochondria.
Results: We have found that nobiletin decreases oxygen consumption in the presence of glutamate and malate and increases in the presence of succinate. In parallel, nobiletin increases NADH oxidation, alpha-ketoglutarate dehydrogenase activities and alpha-ketoglutarate-dependent production of ATP. Additionally, nobiletin reduces the production of peroxides in the presence of complex I substrates and does not change succinate-driven peroxide formation. Besides, nobiletin induces transient elevation of membrane potential followed by mild depolarization. Also, HPLC measurement detected that nobiletin is not metabolized by isolated mitochondria, this underlines nobiletin's direct effect on isolated mitochondria.
Conclusion : We propose that nobiletin may act as a mild "uncoupler", which through activation of a-KGDH-complex and acceleration of matrix substrate-level phosphorylation maintain membrane potential at a normal level. This switch in mitochondrial metabolism could elevate succinate-driven oxygen consumption that may underlay both pro- and anti-apoptotic effects of nobiletin.

NEUROTOXIC EFFECTS OF PRENATAL HYPERHOMOCYSTEINEMIA IN RATS

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Adverse impacts on the maternal organism during pregnancy can lead to serious consequences on embryogenesis and postnatal development of the offspring, and their nervous system is the most vulnerable to the harmful factors. Increased serum levels of homocysteine (HC) are a risk factor for neurodegenerative diseases. It has been shown that accumulation of the products of methionine metabolism including HC in the organism is accompanied by oxidative stress and impaired catecholamine metabolism. Due to the ability to pass through the placental barrier HC may have an adverse effect on developing embryos. The content of nerve growth factors in the serum and brain structures can serve as a marker of neurodevelopmental disorders. The present work was designed to analyze the changes in the content of a neurotrophic factor NRG1 and the levels of biogenic amines in the brain of rats during embryonic and postnatal periods as well as formation of different types of memory in adult female rats subjected to prenatal hyperhomocysteinemia (PHHC). Our results demonstrate that PHHC has resulted in increased HC levels in blood serum of newborn rats and in a significant increase of NRG1 in the brain of fetuses at E20. However, despite the fact that HC content in the blood and NRG1 level in the hippocampus of female rats subjected to PHHC returned to control values by 2-2.5 months after birth, the negative effects of PHHC could still be observed when they were tested using novel object recognition and elevated 8-arm maze tests which revealed disruption of different types of memory. There was also a decrease of noradrenaline and serotonin content in the hippocampus of these rats which can underlie dysregulation of functions associated with biogenic amine transmission in the brain.

Supported by RFBR 14-04-00776 and 16-04-00694.

VISUAL CLASSICAL CONDITIONING REDUCES SPONTANEOUS FIRING OF MOUSE EXCITATORY CORTICAL NEURONS IN LAYER 4 - EX VIVO STUDY

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Sensory experience induces plastic, structural and functional, changes in neuronal network of the brain. Our earlier experiments on mice showed that classical conditioning in which monocular visual stimulation was paired with an electric shock to the tail enhanced GABA immunoreactivity within layer 4 of the monocular part of the primary visual area (V1), contralaterally to the stimulated eye. In the present study we investigated whether the same classical conditioning paradigm induces changes of neuronal excitability in this cortical area. Two experimental groups were used: mice that underwent 7-day monocular visual classical conditioning and controls. Patch-clamp whole-cell recordings were performed from *ex vivo* slices of mouse V1. The slices were perfused with the modified artificial cerebrospinal fluid, the composition of which better mimics the brain interstitial fluid *in situ* and induces reliable spontaneous activity. The neuronal excitability was characterized by frequency of spontaneous action potentials. We found that layer 4 star pyramidal cells located in the monocular representation of the "conditioned" eye in the V1 had lower frequency of spontaneous activity in comparison with neurons from the same cortical region of control animals. Weaker spontaneous firing indicates decreased general excitability of star pyramidal neurons within layer 4 of the monocular representation of the "conditioned" eye in V1. Such effect could result from enhanced inhibitory processes accompanying plastic changes in this cortical area induced by classical conditioning.

Supported by the Polish National Science Center grant Symfonia 1 (2013/08/W/NZ4/00691) and funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 665735.

NEUROPEPTIDE PACAP PREVENTS OXIDATIVE DAMAGES AND APOPTOSIS DURING GESTATION IN FETAL ALCOHOL SYNDROME (FAS) MOUSE MODEL

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Prenatal exposure to ethanol exerts teratogenic effects inducing oxidative stress as well as a massive wave of apoptosis in the developing brain. Pituitary adenylate cylase-activating polypeptide (PACAP) exerts antioxidative and neuroprotective activities on neuronal cells and prevents ethanol neurotoxicity. The present study focuses on the ability of PACAP to protect brain from alcohol induced oxidative damage and toxicity during development in a fetal alcohol syndrome (FAS) mouse model.
Methods: Pregnant mice were divided randomly into 4 groups, *i.e.* a control group (saline 0.9% solution), an ethanol group (1.5g/kg; intraperitoneal injection), a PACAP group (5 µg/10µl; intra-uterine injection) and an ethanol+ PACAP group. Injections were started on day 7 of gestation (G) an repeated daily until G16 or G18. On day G16, some fetuses were collected while some other animals were allowed to deliver their offspring naturally, which remain with their mother until weaning. At the age of 4 weeks (P30), these animals were subjected to battery of various behavioral tests.
Results: Gestational exposure to ethanol provoked reduction of body weight and cerebral atrophy associated with an enhancement of caspase-3 activity in G16brain. Co-treatment with PACAP significantly prevented alcohol-induced body weight reduction and brain atrophy and reduced apoptotic cell death. Production of reactive oxygen species (ROS), a major cause of ethanol neurotoxicity, was enhanced in ethanol-exposed fetuses while the activity of the antioxidant enzymes superoxide dismutase (SODs) and catalase was reduced. In fetuses exposed *in utero* to ethanol, administration of PACAP inhibited ROS overproduction, prevented the decrease of SODs and catalase activities, and blocked oxidative damage of cell molecules *i.e*. formation and accumulation of lipid oxidation products, malondialdehydes and protein carbonyl compounds induced by this toxin. Behavioral tests conducted on offspring at the age P30, revealed that in alcohol-exposed mice motor activity in the open field test as well as exploration and increased anxiety in the elevated maze test were decreased. However, PACAP significantly attenuated these behavioral impairments.
Conclusion: This study demonstrates that the neuropeptide PACAP exerts a potent neuroprotective effects in a FAS model at early stages of brain development, and indicates that PACAP and/or PACAP analogs might be useful as therapeutic drugs against alcohol intoxication during pregnancy.

Supported by research Unit UR11ES09, INSERM U1239, IRIB and a France-Tunisia exchange programs PHC-Utique num 16G 0820

EEG SPECTRAL POWER CHANGES DURING LISTENING TO THE ROCK-MUSIC WITH MODIFIED FREQUENCY RANGE

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People listen to music due to its ability to elicit strong emotions and pleasure. And experienced musicians and sound engineers, while mixing and mastering, consider the fact, that depending on signals level of certain frequencies music can elicit different emotions and affect on its strength. In this study we investigate an influence of low-frequency component in rock-music on music perception and emotional experience.
30 healthy volunteers (17 women and 13 men) - students aged 18 to 22 years with no prior musical education participated in this study. Four stimuli were presented: white noise (40 seconds), song of nightingales (60 sec), instrumental rock-composition (75 seconds) and the same rock-composition with reduced low-frequency level (60 Hz, 150Hz, 400Hz). In order to estimate spectral power EEG changes we compared every audio session sample with preceding rest state sample. During EEG registration volunteers were sitting in a comfortable chair in a darkened, soundproof room with closed eyes. After EEG registration participants were asked to estimate audio stimulus using valence and arousal emotional scales from "-5" to "+5".
During listening to the sounds and music the most significant EEG changes were observed mainly in beta and gamma-bands, which related to emotional and cognitive processes. Listening to the white noise caused frontal activation in theta1-, theta2-, alpha3-, beta1- and beta2-subbands basically in right hemisphere and general activation in gamma-band. The right frontal activation could be related to a negative emotional response to a stimulus. In contrast, there was increase of SP in theta1- and gamma- in left hemisphere during listening to the song of birds, which related to positive emotional response and relaxation. There was an increase of SP in beta2-subband in left temporal, parietal, occipital and in right frontal lobe, and general activation in gamma-band during listening to the rock-composition with normal frequency range. Whereas there was increase of SP in gamma-band only in left posterior areas and in right frontal area, and in alpha3- and beta2-bands in occipital and right frontal lobes during listening to the rock-composition with reduced low-frequency signal level. The less activation in gamma-band during listening to the second rock-composition could be related to the lower level of emotional activation.
The right frontal activation in alpha3- and beta2-subbands may also indicate on emotional processes. However, we don't exclude a possibility that changes in beta-and gamma-activity are related to such cognitive procesess as music component processing and memory.

MODES AND MECHANISM OF MEMANTINE ACTION ON ASIC1A CHANNELS

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Proton-gated ion channels (ASICs) are widely distributed in central and peripheral nervous systems of vertebrates. They're thought to be involved in the modulation of synaptic signal transmission and have been shown to be implicated in various acute and chronic states, making ASICs properties and pharmacology an important target for further investigation. It has been recently shown that various monoamines, including NMDAR channel blockers, may also affect ASICs. In this work we carried out a detailed analysis of the action mechanisms of memantine, known ASIC1a inhibitor. The study was performed using the whole-cell patch clamp recording. CHO cells were transfected with the rat ASIC1a along with GFP for cell identification.
First, the concentration dependence of memantine at holding potential of -80mV was measured; fitting of concentration curve with Hill equation resulting in the IC50 value of 4537±1013 μM. We also found that memantine action was strongly voltage-dependent, with effect of 1mM of memantine ranging from 21±12% potentiation at +20mV (n=8) to 47±15% inhibition at -140mV (n=10). The drug action did not however display a pH-dependence, as inhibition of responses evoked by pH drops from 7.4 to 6.5 and to 5.0 were not significantly different.
Notably, influence of memantine on the decay time constant was much more pronounced in comparison to its effect on response amplitude. The decay time constant was reduced by 71±11% at -140mV and 1mM concentration, with IC50 of 678±72 μM. The action on amplitude and decay time constant was also shown to be strongly correlated. Thus, the main effect of memantine is its increase of decay speed, which in turn leads to the amplitude reduction as a result of an earlier peak cutoff. Such effect may be due to ligand affecting the speed of channel desensitization or due to open-channel block with the pronounced voltage dependence of action implying the latter possibility.
Surprisingly, when the conditioning pH was changed to 7.1 and memantine was present only between the applications of the acidic activating solution, it caused a marked increase of 141±38% (n=5) in response amplitude. This effect is distinct from memantine inhibition because the amplitude increase was not accompanied by changes in kinetics of the response and was virtually independent of the membrane voltage.
Since up to 70% of ASIC1a channels are desensitized at pH 7.1, it is possible that memantine prevents steady-state desensitization when present in conditioning solution. However, at higher concentration of 3mM the potentiating effect reached 640±104% (n=5), which is beyond the scope of desensitization compensation and implies an additional mechanism of action.
In conclusion, our work demonstrated complex mechanism of action for memantine, which likely includes open-channel block and modulation of activation/desensitization properties of ASIC1a. Comparison with other data suggests that the same may also be true for other ASIC ligands.

MORPHOLOGICAL EXAMINATION OF BLOOD VESSELS IN THE CORTICAL WHITE MATTER OF PATIENTS WITH TEMPORAL LOBE EPILEPSY

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The temporal lobe epilepsy (TLE) is the most common form of the focal epilepsies and it mainly caused by hippocampal sclerosis. In addition to neuronal death and alterations in synaptic connections, change in the morphology of blood vessels in the hippocampus was also reported in TLE. In the present study, we examined the morphology of the blood vessels in the white matter of the temporal neocortex in TLE patients.
Tissue samples contained the temporal cerebral cortex including white matter were used. The samples originated from patients with drug-resistant TLE who had a temporal lobectomy and the control samples were from patients operated with brain tumors. The tissues were embedded in paraffin, and the vessels were visualized on 10 µm thin sections with periodic acid-Schiff (PAS) staining. Vasculogenesis and endothelial cell proliferation was detected with vascular endothelial growth factor (VEGF) and Ki-67 antibodies. Anti-human IgG was used to reveal the leakage of the blood-brain-barrier (BBB). Sections stained with PAS and immunohistochemistry were examined with light microscope, and the ultrastructure of BBB was examined with transmission electron microscope (TEM). Results of quantification of the area occupied by blood vessels, their number, and the density of Ki-67-, VEGF-immunoreactice cells as well as PAS-positive profiles were correlated with the clinical data of patients. The majority of the vessels in the white matter made were small capillaries and venules in both group, but we noticed many abnormal, glomerulus-like vessels in the epileptic samples. The density of blood vessels, as well as the area occupied by them was significantly larger in the epileptic cortical white matter than in that of the controls. In contrast to it, we did not find increased endothelial cells proliferation in the epileptic white matter. Both in epileptic patients and in the control the number of Ki-67-immunoreactive endothelial cells was low. VEGF expression was observed in astrocytes, and density of VEGF-immunoreactive astrocytes was significantly higher in TLE than in controls. Degeneration of basal membrane (BM) was indicated by the presence of PAS-positive patches in the epileptic tissue, their density correlated with the duration of disease. TEM revealed splitting and degeneration of the BM around the vessels, and the presence of IgG in the brain parenchyma support leakage of the BBB.
Our results indicate that blood vessels in the white matter of the temporal neocortex are significantly affected in TLE. The morphological changes and the leakage of the BBB may contribute to epileptogenesis and the progress of the disease.

Our work was supported by the NAP (KTIA_13_NAP-AII / 11), and by the EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. L. Váncza was a visiting student sponsored by Erasmus Fellowship. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

POSSIBLE NEUROPROTECTIVE POTENTIAL OF SOYA-LECITHIN (CHOLINE PRECURSOR) AND ITS INTERACTION WITH GALANTAMINE (ACETYLCHOLINESTERASE INHIBITOR) AGAINST ICV-STZ MODEL OF SPORADIC DEMENTIA OF ALZHEIMER'S TYPE

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Background: Galantamine, an acetylcholinesterase (AChEs) inhibitor, a well known drug used for the treatment of Alzheimer's disease. Soya-lecithin (phosphatidylcholine analogue/ choline precursor) is a good source of choline whose role has been very well documented in cognitive performance.
Objective: Present study investigates the possible neuroprotective potential of soya-lecithin and its influence on galantamine against intracerebroventricular streptozotocin (ICV-STZ) induced memory impairment in a rat model of sporadic dementia of Alzheimer's type.
Methods: Animals received single bilateral ICV-STZ (3 mg/kg). Drugs galantamine (2 mg/kg), soya-lecithin (100, 200 & 400 mg/kg) and their combinations were administered for a period of 21 days.
Results: ICV-STZ administration significantly impaired cognitive performance as indicated by MWM test, oxidative damage (raised lipid peroxidation, nitrite concentration, reduced glutathione, catalase activity), raised AChEs level, TNF-α level and alterations in neurotransmitter levels, mitochondrial dysfunction and histopathological alterations as compared to sham treatment. Chronic treatment with soya-lecithin (100, 200 & 400 mg/kg) and galantamine (2 mg/kg) for 21days significantly improved the cognitive performance on MWM task, reduced AChEs activity, oxidative damage (reduced lipid peroxidation levels, nitrite level, restored glutathione levels), TNF-α levels, restored neurotransmitter levels, mitochondrial enzymes and histopathological alterations as compared to ICV-STZ treated animals. Further, the combination of galantamine (2 mg/kg) with soya-lecithin (100 & 200 mg/kg) significantly attenuated spatial learning and memory (MWM performance), AChEs level, oxidative damage, TNF-α level, restored neurotransmitter levels, mitochondrial functions and reduced histopathological alterations in ICV-STZ treated rats as compared to their effect alone.
Conclusion: The present study suggests that co-administration of galantamine with soya-lecithin significantly improves cognitive performance in ICV-STZ treated rats as compared to their effect alone.

Keywords: ICV-STZ, soya-lecithin, galantamine, cholinergic dysfunction, memory impairment

PERINATAL ASPHYXIA INDUCES ADHD-LIKE BEHAVIORAL DEFICITS IN CONJUCTION WITH ACUTE NEUROINFLAMMATION AND LASTING DYSFUNCTION OF THE GLUTAMATERGIC SYSTEM

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Perinatal asphyxia is one of the primary causes of neonatal mortality, accounting for around 700.000 neonatal deaths each year. Among survivors, neonatal asphyxia contributes to a wide range of severe life-long disabilities including cerebral palsy, epilepsy, mental retardation, psychiatric disturbances and cognitive impairments. Several studies suggest that even milder forms of perinatal asphyxia without acute encephalopathy and major sensory motor damages may associate with lasting behavioral deficits, however, the underlying mechanisms are not well known. Here we aimed to characterize a recently developed translational rodent model of moderate perinatal asphyxia1 which does not include any surgical intervention, in order to investigate putative mechanisms through which asphyxia may contribute to the subsequent behavioral alterations.
Wistar rat pups on postnatal day 7 were exposed to a gas mixture containing 4% O2, 20% CO2 and 76% N2, or to room air for 15 min at 37oC. Rats were subjected to comprehensive behavioral assessment from 24h post-asphyxia into adulthood. Brain perfusion and microglial activation were investigated with SPECT and MRI in vivo. Inflammation and neuronal injury were studied by using cytometric bead array, immunofluorescence and confocal/super-resolution microscopy.
Brain perfusion changes, microglial activation and neuronal apoptosis were detected 24h after asphyxia. In adult rats, changes in vesicular glutamate transporter (VGLUT-1 and VGLUT-2) levels in synaptic terminals were found in the CA1-CA3 regions of the hippocampus and in the prefrontal cortex, whereas no signs of focal neuronal loss or white matter injury were observed. Neonatal asphyxia did not cause significant sensory-motor deficits in pups or in adult rats. However, long-term functional testing identified increased anxiety of asphyxiated rats in the elevated plus maze, and spatial memory deficits were found in the Morris water maze test. Moreover, changes in emotional and memory functions in adult post-asphyxia rats were accompanied by increased impulsivity-like behavior and attention deficits in the Delay Discounting paradigm and in the 5-Choice Serial Reaction Time test.
In summary, neonatal asphyxia evoked acute neuroinflammation in the absence of major neuronal injury and resulted in dysfunction of glutamatergic terminals in conjunction with ADHD-like behavioral symptoms in adulthood. The present model may provide clinically relevant insight into mechanisms of the diverse psychiatric disturbances in humans who have suffered from moderate birth asphyxia.

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MOLECULAR PROFILING OF COLONIC SENSORY NEURONS BY SINGLE-CELL RNA SEQUENCING AND CA2+-IMAGING

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Dysregulation of visceral sensation can lead to chronic abdominal pain, which is challenging to treat and a significant source of morbidity. Modulation of visceral nociceptor excitability is a key approach to the development of novel visceral analgesics. However, sensory neurones projecting to visceral organs represent <10% of dorsal root ganglia (DRG) neurones, differentially express genes and are developmentally regulated by different transcription factors to somatic nociceptors. Classical nociceptive markers, such as TRPV1, poorly define such populations in the viscera and whilst visceral neurones are predominantly peptidergic, they are capable of encoding both innocuous and noxious stimuli. The aim of this study is to comprehensively characterise visceral neurones, by whole transcriptome RNA sequencing of colonic neurons at the single-cell level. Colonic sensory neurones were retrogradely labelled using Fast Blue and individually harvested from primary cultures of both thoracolumbar (T10-L1) and lumbosacral (L5-S1) DRG by pulled glass pipette. For RNA sequencing, full-length cDNA from polyadenylated RNA of single-cells was generated by Smartseq2 and libraries prepared using Nextera XT (Illumina). Samples were 96-way multiplexed and sequenced on an Illumina NextSeq500, achieving ~4.9M reads/cell. Using gene expression of individual cells and performing unsupervised clustering analysis of ~28,000 genes/cell, colonic neurones could be split into 7 distinct subsets. These subsets were validated firstly through single-cell qRT-PCR of isolated retrograde labelled colonic neurones and secondly at the protein level by conducting immunohistochemistry on DRG sections. Lastly, we performed Ca^{2+}-imaging on isolated retrograde labelled colonic neurones, followed by single-cell qRT-PCR, and were able to demonstrate functional diversity in parity with the subgroups predicted by RNA-sequencing.

SINGLE NEURON CORRELATES OF MULTIMODAL MISMATCH DETECTION

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Mismatch detection as a cognitive process may involve latent learning, prediction and error signal generation in the cortex. The neural correlates of this phenomenon have been extensively investigated in the auditory domain and in human EEG studies examining event related potentials. However, much less is known about the cellular level representation of mismatch detection especially in multimodal contexts. The goal or our study was to determine single neuron correlates of mismatch negativity during multimodal processing in different cortical areas in mice. Extracellular potentials were recorded simultaneously in primary visual cortex (V1) and higher order area AL by means of two 32 channel microelectrode arrays in a multimodal mismatch negativity task combining specific auditory (pitch) and visual (orientation) stimuli. Unit activity was determined by using spike sorting techniques. Different auditory, visual and multimodal mismatch related single unit responses were identified. Interestingly, a new type of neuronal response called conditional mismatch was revealed, which showed significant firing rate increase for auditory mismatch only when preferred visual stimuli were presented. In order to reveal the laminar distribution of the synaptic inputs corresponding to different forms of mismatch detection, the single cell current source density distribution was calculated. These preliminary findings suggest highly specific single neuron correlates of the mismatch processing depending on the specific stimulus context in a complex multimodal task.

This research was supported by the grant ERA-NET FLAG-ERA, Human Brain Project, NKFIH NN-118902: CANON project and National Research, Development and Innovation Office NKFIH under grant no. K-113147 and National Brain Research Program grant no. KTIA_13_NAP-A-IV/1,2,3,4,6.

INFERENCE OF INTRA AND INTER HIPPOCAMPAL DIRECTED CAUSAL RELATIONSHIPS BASED ON FORAMEN OVALE RECORDINGS

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The traditional method for seizure onset zone determination is based on the temporal order in the appearance of pathological waveforms. However, this method assumes, that the well observable pathological waveforms are really the first signs of those processes, which play important role in the seizure generation. Our objective is to develop and apply new mathematical methods to identify the causal relations between brain areas during epileptic activity.
The Convergent Cross Mapping method of Sugihara was modified to reveal temporal dynamics of causal connections, and applied to current source density distribution data calculated from multichannel foramen ovale electrode recordings, near the hippocampi billaterally.
The causality analysis revealed directed connections between the two hippocampi and specific changes in intra and inter-hippocampal connections comparing interictal and ictal periods: intrahippocampal connections get stronger and spread away spatially, while inter-hippocampal connections show more complex temporal development patterns during the seizures: the functional connectivity decreases during initial phase of the seizures in some cases, while increases during the full blown seizures. Onset of the tonic phases accompanied by temporal increase of the causal connections between the two hippocampi as well.
New mathematical analysis method, such as Convergent Cross Mapping raise the possibility of objective determination the causal relationships at the initiation of the seizure, without specific assumptions about the waveforms. Causality analysis can support more precise identification of seizure onset zones, thus results in better surgical outcome.

This research was supported by the grant ERA-NET FLAG-ERA, Human Brain Project, NKFIH NN-118902: CANON project and National Research, Development and Innovation Office NKFIH under grant no. K-113147 and National Brain Research Program grant no. KTIA_13_NAP-A-IV/1,2,3,4,6.

THE EFFECTS OF EXERCISE ON L-DOPA-INDUCED DYSKINESIA

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Introduction: L-DOPA alleviates the motor symptoms of Parkinson's disease, but its long-term use is associated with undesirable dyskinesia. We now tested whether exercise can attenuate this L-DOPA-induced dyskinesia (LID). We tested the effects of exercise on LID in 6-hydroxydopamine hydrochloride-hemiparkinsonian mice. Animals were treated with L-DOPA/benserazide
Objective: Test the effects of exercise in Induced by L-dopa-Induced Dyskinesia in Swiss mice.
Methods: Adult Swiss mice (male, 8–10 weeks old, weighted 40–45 g). 6-hydroxydopamine hydrochloride (6-OHDA, 10µl in 1 lL of 0.1% sodium metabisulfite diluted in 0.9% NaCl) was injected using steotaxic surgery (AP: +0.5 mm; ML: +2.0 mm; DV: -3.0). First, the hemiparkinsonism was evaluated through R(-)-apomorphine challenge (0.5 mg;kg, s.c.),(1) cylinder task and rotarod test. After 4 weeks of recovery, selected animals were daily treated with L-DOPA plus Benserazide (50/25 mg/kg, i.p.) Swiss mice submitted to 4 weeks of high-intensity exercise. LIDs, or Abnormal Involuntary Movements (AIMs) were evaluated for 120 min during 4 weeks, after we test with amantadine (60 mg/kg, i.p) in both groups treadmill and untreadmill.
Results: In baseline, in cylinder task the animals showed decreased use of the contralateral paw after surgery and after the end the treadmill they recovery the use the contralateral paw (2). In rotarod test the treadmill showed an increased in of falls latency (3). Exercise drastically prevented the development of LID after second week and remained until the end of training (4). When we adminitrade amantadine in exercise group they do not showed more reduction in LID score, bot untreadmill they have decrease in LID. (5). In western blot analysis, we can observe the treadmill group showed lower levels of TH (Tirosine Hidrosilase) and DAT(Dopamine Transporter). In GDNF analysis the exercise group showed an increase levels in this protein, can be involved in restauration in dopaminergic neurons (6).
Conclusion: Our results indicate that exercise can partially prevent the development of LID more than amantadine.

CHEMICAL STIMULATION OF DURA MATER WITH INFLAMMATORY SOUP CAUSES CHANGES IN CGRP LEVELS IN THE CAUDAL TRIGEMINAL NUCLEUS

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Introduction: Migraine is one of the primary headaches with a not clearly understood pathomechanism. However, based on previous data sterile neurogenic inflammation in the dura mater and the activation and sensitization of the trigeminal system play a crutial role in the attack. Topical inflammatory soup (IS) can be used to model the inflammation of the dura mater. The application of IS causes hypersensitivity to mechanical and thermal stimulation and activates the primary and secondary trigeminal neurons.
Calcitonin gene-related peptid (CGRP) plays an important role in the dural inflammation and muigraine pathogenesis.
Thus we investigated the effect of IS induced dural inflammation to the CGRP levels in the caudal trigeminal nucleus (TNC) and whether the pretreatment with sumatriptan or kynurenic acid has effect on the IS induced changes.
Material and methods: Adult male Sprague-Dawley rats were divided into six groups (n=6 per group). The animals in the first group, called the placebo group, received only synthetic interstitial fluid (SIF), while in the second group we applied IS onto on side of the dural surface. In the third and fourth groups, the animals received subcutaneous sumatriptan, while in the fifth and sixth group recieved kynurenic acid pretreatment 10 minutes before the SIF or IS treatment. 140 minutes after the SIF or IS injection, the TNC was processed for immunohistochemistry.
Results: Our results show that IS is able to increase the amount of CGRP immunoreactive fibers in the TNC compared to the placebo group, but no difference was observed between the treated and the untreated side in either groups. The sumatriptan and kynurenic acid pretreatments were able to attenuate this effect.
Conclusion: The application of IS onto the dural surface of rats increases the CGRP levels in the central terminals of primary nociceptors suggesting enhanced synthesis after activation. The bilateral effect might be relataed to the diffusion of IS to the contralateral side.
The modulatory effect of sumatriptan and kynurenic acid suggests the involvement 5HT1B/1D and NMDA receptors during the neurogenic inflammation of the dura and possibly during the migraine attack too.

ANALYSIS OF THE CORTICAL COLUMN ACTIVITY USING CURRENT SOURCE DENSITY REPRESENTATION AND MODULAR CONNECTIVITY FACTORIZATION METHOD

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Introduction: Extracellular potentials, such as the Local Field Potentials (LFPs), are routinely measured in numerous electrophysiological experiments. Apart from basic preparation, such as band pass filtering and artifact removal, many analytic pipelines have been proposed. One approach is to translate recorded potentials into current source density representation in brain tissue. Here we discuss a recently developed inverse method for current source density estimation - kernel Current Source Density (kCSD). Moreover, using the Modular Connectivity Factorization method we estimate and compare functional connectivity in the cortical columns network. We test and apply such analytic tools both on simulated data and on recordings from mice brains.
Aim of the study: The main goal of the study is the comparison of the effective connectivity and the structure of sinks and sources in cortical columns during slow oscillations. We also search for improvements for analytic methods (MCF and kCSD) using simulated data from cortical column model.
Methods: Analytic methods: kernel Current Source Density and Modular Connectivity Factorization (MCF) applied to LFP recordings.
Simulated data: Electrophysiology simulation for cortical column model has been performed in NEURON simulator.
Experimental methods: Multielectrode in vivo recordings from cortical column of the wild type (WT) and fragile X mental retardation protein (FMRP) knock-out mice (KO).
Results: Preliminary analysis shows different distribution of the current sources in WT and KO mice during slow oscillations. Modular Connectivity Factorization method applied to LFP recordings separates cortical column layers into interpretable modules both in simulation and experimental data. Comparison of the functional network organization between studied mice groups shows different connectivity pattern in superficial layers.
Conclusions: KCSD and MCF methods significantly differentiate the Fmr1 knock-out from wild type mice. Physiological interpretation of the kCSD method has been validated on the cortical column model but MCF results need to be further checked on the simulated data.

HIGH-RESOLUTION RETINOTOPIC MAP REVEALED BY INTRINSIC SIGNAL OPTICAL IMAGING IN THE VISUAL CORTEX OF THE CAT (AREA18)

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Retinotopy is the orderly representation of visual field locations on the retinal surface which is also present in the visual cortex called, retinotopic map. The aim of the present study was to obtain a high-resolution retinotopic map of area 18 of the cat visual cortex, i.e. to determine the map of visual field polar coordinates for each cortical location.
Anaesthetized and paralysed cats were prepared for in vivo intrinsic signal optical imaging (ISOI) according to institutional guidelines and the update of EU Directive 86/609/EEC, regarding the protection of animals used for experimental and other scientific purposes (2010/63/EU)). Monocular visual stimuli were presented on a computer monitor 57 cm in front of the animal's eye. The stimulation paradigm consisted of a sequence of stimulus windows (40x1,5 deg vertical or 1,5x30 deg horizontal rectangle) shifted at 1.5 deg steps in vertical (for elevation) or 3,0 deg steps in horizontal (for azimuth) directions. The location of each stimulus was registered with respect to vertical and horizontal meridian as determined by tapetal back-projection from the retina on the stimulation screen. The stimulus windows contained an oriented square wave luminance grating (4 cardinal orientations) drifting along two possible opposite directions. Data acquisition was carried out for 4,5 sec for each stimulus condition including a blank period and repeated 15 times. Signal to noise ratio of ISOI was improved by applying image analysis methods, such as first frame analysis, normalisation by cocktail blank, low-pass filtering (boxcar filter) on ROIs which excluded non-brain tissue components and major blood vessels. Then, the patchy activity resulted by the particular stimulus was smoothed using an image thresholding method, i.e. the median value of pixel positions representing the highest activity was localised and smoothed using a built-in function of Matlab ('rlowess'). In this way, a raw map of retinotopy could be generated at a spatial resolution of 1.5 deg for elevation and 3.0 deg for azimuth. A high-resolution retinotopic map was computed by linear interpolation of the raw-retinotopic map. Finally, as a control, the precision of the retinotopic maps obtained with ISOI was validated using electrophysiological recordings of single- and multi-unit activity. Receptive field positions detected with the latter method did not differ significantly from retinotopic polar coordinate values of corresponding ISOI pixels (elevation: $p = 0,4113$; azimuth: $p = 0,346$).
These results show that ISOI is a useful tool for determining retinotopic representation at a high spatial resolution for over 10 mm anteroposterior extent in cat visual cortex. Future applications can use retinotopy as an important attribute for exploring structure-function relations, e.g. bouton distribution of single cortical cells and their representation in the visual field.

Supported by MTA-DE Neuroscience Research Group and KTIA13_NAP-A-I/7 to Z.K.

INNER RETINAL ABNORMALITIES ACCOMPANYING PHOTORECEPTOR DEATH IN A RHODOPSIN MUTANT MOUSE

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In Retinitis Pigmentosa (RP), a mutation in a retinal specific gene triggers rod-to-cone degeneration, in turn driving regressive remodeling of inner neurons. With different kinetics, bipolar and horizontal cells (HCs) undergo dendritic atrophy, loss of synaptic receptors, formation of ectopic synapses and secondary death. Studies on developmental rodent models of RP (i.e. rd10 mice) showed that rod bipolar cells (RBCs) and HCs are among the first neurons affected by rod death, limiting the possibility of therapeutic intervention relying on inner retinal preservation. However, in humans, photoreceptor death does not overlap with development and available information on remodeling might not conform to what happens in human RP. Also, changes in the innermost retinal layers following photoreceptor death have not been investigated in detail. To address these issues, we performed remodeling studies on the retina of a novel RP mouse model, the Tvrm4 strain, carrying a dominant, single nucleotide mutation in the rhodopsin gene. The mutation can be activated at the protein level upon brief exposures to strong, white light. We hypothesize that remodeling in Tvrm4 mice induced in adulthood conforms to stereotyped features previously described in developmental models of RP.

Tvrm4 mice aged 3-5 months and wild type (WT) littermates were exposed to 12k lux, white-neon light for 2 minutes and harvested 3, 6 or 9 weeks afterwards. Retinal tissue was processed for immunocytochemistry to reveal RBCs and HCs, along with synaptic ribbons and gap junctions, which were studied and quantified by confocal microscopy and image analysis using Metamorph routines for manual cell counting and density measurements.

No variation in the density of RBCs was found in Tvrm4 mice compared to WT controls at all the ages tested. However, we observed dendritic retraction, axonal arborization shrinkage and loss of spatial order in RBCs. HCs showed similar axonal and dendritic retraction and a 37% density drop in the central retina, 6 weeks after photo-induction.

Synaptic ribbons showed a significant increase in the area occupied in the inner plexiform layer (IPL) 3 weeks post-induction; however, 6 and 9 weeks after induction, no significant difference was found compared to WT controls. Image thresholding analysis demonstrated a significant increase in the density of the IPL area occupied by gap junctions in all 3 experimental groups with respect to the WT samples.

Major survival of RBCs despite regression of dendritic and axonal arbors have been described in various RP mutants including the present and are therefore hallmarks of RP. These processes are not influenced by the age of disease onset and the causative mutation. In spite of the fact that rod bipolar axonal endings are shrinking, inner retinal synaptic contacts are still present. Further investigations are still to be carried out to identify the functional effects of ribbon and gap junction remodeling described here.

ADAPTATION OF SPONTANEOUS ACTIVITY IN V1 TO NOVEL STIMULUS STATISTICS

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The visual system is hypothesized to construct an internal model of the visual environment and use this model to make inferences about upcoming stimuli. The internal model adapts to the statistics of the environment throughout the lifetime of the animal, a process that can be captured in the shaping of the statistics of spontaneous activity (SA). In particular, statistics of the SA was shown to be matched to that of the (average) evoked activity (EA) in the mature, but not in the juvenile primary visual cortex. However, it is not known whether and how these principles apply when the mature animal is exposed to a novel, albeit limited, set of stimuli for a limited time. We investigated how the activity statistics of V1 neurons in behaving monkeys is shaped when a set of artificial stimuli are shown over a single session of training. We used a latent variable model of neuronal activity to assess the statistics of EA and SA under different conditions. Using this model the population activity could be characterized on a trial-by-trial basis, as opposed to traditional methods that can only address trial-averaged responses. Dissimilarity of response statistics was assessed by comparing latent states inferred in multiple trials under SA and EA using Kullback-Leibler divergence. Analysis of SA before, during, and after exposure to the sequence of stimuli reveals a gradual transformation of the statistics of SA during the adaptation process. Statistics of neural responses to stimuli is undergoing similar changes: while EA early during exposure is more similar to SA before exposure, it becomes more similar to SA after exposure as adaptation to stimuli progresses. Changes are not due to less selective neural responses at later phases of training since the fidelity of stimulus encoding is left intact by the transformations. Crucially, we show that adaptation-induced joint transformation of SA and EA is such that they become more similar. Our results demonstrate that during a limited exposure to a novel stimulus set, principles of statistical learning theory can be identified in the transformations of population activity during EA and SA. However, the results of such short exposures does not saturate as adaptation occurs in repeated sessions too.

This work was supported by an MTA Lendület Fellowship and an award from the National Brain Research Program of Hungary (NAP-B, KTIA_NAP_12-2-201).

THE EFFECT OF TREATMENT WITH RHO-KINASE INHIBITOR AND CHITOSAN SCAFFOLD ON TISSUE SPARING, AXON REGENERATION AND FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY

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Introduction: Currently, a large number of therapeutic approaches was developed to promote axonal regeneration across the spinal cord injury lession. The aim of this study was to reduce the development of cytoskeletal changes in neuronal cells by blocking the Rho signalling pathway after SCI using Rho-kinase (Y-27632) inhibitor, and to bridge the lesion site with chitosan porous scaffold (ChPS).
Materials and methods: Adult Wistar albino rats (n = 15) were used in the experiment. The animals were divided into 3 groups:
1. compression of the spinal cord at the Th9 level, causing the paralysis of hind limbs
2. spinal cord compression followed by intrathecal administration of Y-27632, (Rho-kinase inhibitor; 40µg/rat/day; for 2 weeks) - (Y-27632)
3. combined treatment: porous chitosan scaffold implanted into the lesion 14 days after compression and Y-27632 - (Y-27632/ChPS)
The neurological score was evaluated weekly for 8 weeks of survival using the Basso-Beattie-Bresnahan evaluation scale. The extent of lesion was monitored in rostro-caudal direction from the epicenter (20 mm in 2 mm blocks) by histological staining (Luxol Fast Blue). Immunohistochemical staining (GFAP - astrocytes, SMI312 - neurofilaments, GAP43 -outhgrowing axons) was used for visualisation of glial scar and overgrowth of damaged axons.
Results: Treatments with Y-27632 or Y-27632/ChPS prevented the number of neurofilaments and significantly increased the axonal regeneration in spinal cord after Th9 compression and 8 weeks of survival. Application of myelin associated Rho-kinase inhibitor (Y-27632) markedly enhanced the expression of GAP43 protein at the site of injury and in its vicinity. Number of SMI-312 labeled axons was increased in rostro-caudal direction almost in all regions after treatment, except for lateral funiculus in rostral segments (Y-27632 group) and the dorsal funiculus in the caudal segments (Y-27632/ChPS group). Comparing with SCI, we noted extensive improvement in the white matter preservation in all monitored areas. There was no difference between the groups regarding the formation of glial scar. Although progress in motor function was significant in both treated groups during the first two weeks after trauma, better recovery rate of the hindlimbs was not seen at the end of survival in Y-27632/ChPS group.
Conclusion: Our results show that Rho-kinase inhibitor enhanced the plasticity and effectively promoted the overgrowth of damaged axons at the lesion site. The chitosan scaffold bridged the lesion, but outgrowing axons did not penetrate through the biodegradable material.

The work was supported by the VEGA project 2/0173/14 and the project APVV-15-0766.

EFFECTS OF RILUZOLE AND MEMANTINE ON ACQUISITION LEARNING OF A SPATIAL TASK IN AN ANIMAL MODEL OF OBSESSIVE-COMPULSIVE DISORDER

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The objective of this study was to test effects of treatment with drugs decreasing glutamatergic activity in an animal model of obsessive-compulsive disorder (OCD) induced by chronic sensitization with quinpirole, a dopamine D2-like receptor agonist. Adult male Long-Evans rats were sensitized by ten injections of quinpirole spaced by one-day interval on a circular open-field arena. The dose of the drug was 0.25 mg/kg. Animals were tested for acquisition in a place avoidance task on a rotating arena (Carousel) and during testing memantine (1 mg /kg and 5 mg/kg) and riluzole (1 mg /kg and 5 mg/kg) were co-administered with quinpirole during behavioral testing. Quinpirol at the tested dose induced a deficit in acquisition of the active place avoidance task, marked by inability to avoid a hidden sector and hyperlocomotion. Higher dose of the drugs (5 mg/kg) exacerbated this deficit, whilst lower dose (1 mg/kg) did not change it significantly, although a trend of improvement was seen. We conclude that quinpirole-sensitization model of OCD exhibits relatively low reactivity to the drugs interfering with glutamatergic neurotransmission. Further studies on these influences are underway in our laboratory.

The study was supported by GACR grant 17-04047S, AZV grant 15-34524A. Institutional support for IPHYS was provided by RVO: 67985823.

SPATIAL MEMORY AND COGNITIVE CONTROL AND FLEXIBILITY DEFICITS IN ANIMAL MODELS OF SCHIZOPHRENIA AND OBSESSIVE-COMPULSIVE DISORDER

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Navigation is a ubiquitous mechanism for localizing spatial goals in the environment. Cognitive control refers to the ability to select relevant and appropriate sensory stimuli and behavioral actions. Animals and humans face abundance of incoming information, some relevant or important, and other useless or even distracting. Based on experience, subjects must attend to the former and ignore the latter in order to choose the appropriate behavior. Both spatial navigation and cognitive control are significantly impaired in several disorders of the central nervous system. This lecture will discussed deficits in these processes in schizophrenia and obsessive-compulsive disorder and show a convergence of preclinical studies done in rodents and clinical studies with patients and healthy controls.

This work was supported by AZV grants 15-34524A and 17-30833A, GACR grant 17-04047S and GACR center of Excellence P304/12/G069. Institutional support for IPHYS was provided by RVO: 67985823. Institutional support for NIMH-CZ was provided by the project "Sustainability for the National Institute of Mental Health", under grant number LO1611, with a financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the NPU I program

EXPRESSION OF GLUCOCORTICOID AND MINERALOCORTICOID RECEPTORS IN THE GASTRIC MUCOSA UNDER SHORT-TERM AND LONG-TERM DEXAMETHASONE ACTION

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Glucocorticoids may have dual action on the stomach: gastroprotective and proulcerogenic one. The previous study was designed in our laboratory to investigate how physiological gastroprotective action of glucocorticoids can be transformed to pathological ulcerogenic effect. The results obtained demonstrate that single injection of dexamethasone at a dose of 1 mg/kg may attenuate or aggravate indomethacin-induced gastric erosions depending on the time of the injection before indomethacin (prolongation of dexamethasone action).
To elucidate of the mechanisms of transformation of gastroprotective action of dexamethasone into ulcerogenic one we tested the hypothesis that disturbance of the balance between the glucocorticoid and mineralocorticoid receptors can contribute to this transformation.
To test the hypothesis, dexamethasone model (1 mg / kg, i.p.) was used: 2 time points after dexamethasone administration were selected - 1 h (when gastroprotective action was observed) and 24 h (when proulcerogenic effect was observed).
The expression of glucocorticoid and mineralocorticoid receptors was detected by an immunohistochemical staining technique using α-glucocorticoid (ab3580; 1:500. Abcam) and mineralcorticoid (ab41912; 1:500. Abcam) receptor antibodies. The results of the staining were assessed by a morphometric study of microscopic images in five fields of view using the ImajeJ software. A distribution of cytoplasmic staining was estimated with relative stained area (%) in gastric glands and smooth muscle cells of muscularis mucosae.
The results of work on the evaluation of glucocorticoid (GR) and mineralocorticoid receptors (MR) suggest that short-term dexamethasone effect (1 h) does not cause significant changes in the GR levels, while its long-term effect (24 h) increases the GR levels in stomach tissues. Acute dexamethasone effect (1 h) did not cause significant changes in the MR levels but its long-term effect (24 h) resulted in a significant reduction in MR level in stomach tissues. It has been revealed that MR are prevailed in stomach tissues under the short-term dexamethasone effect but GR - under its long-term effect. The results suggest on the imbalance between GR and MR in the stomach under dexamethasone-induced ulcerogenic effect and raise a lot of new questions.

The study was supported by grant of Russian Science Foundation (RSF) #14-15-00790.

THE EFFECT OF DOPAMINE FOR HAND FUNCTION IN INFARCTION OF STRIATUM

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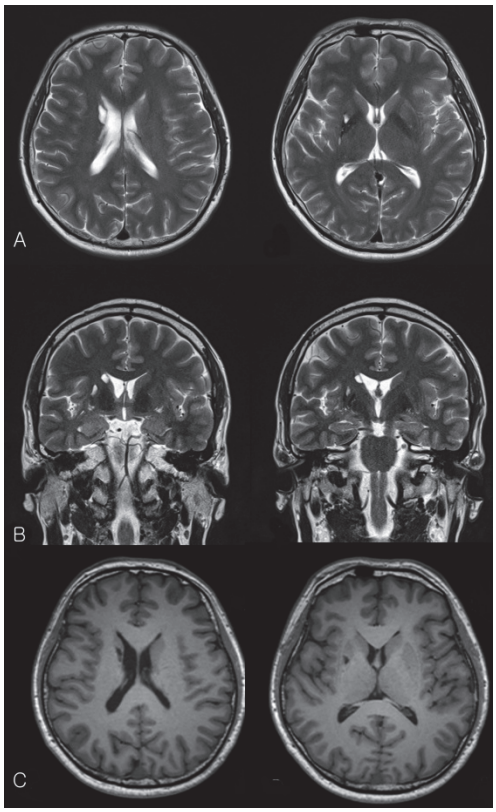
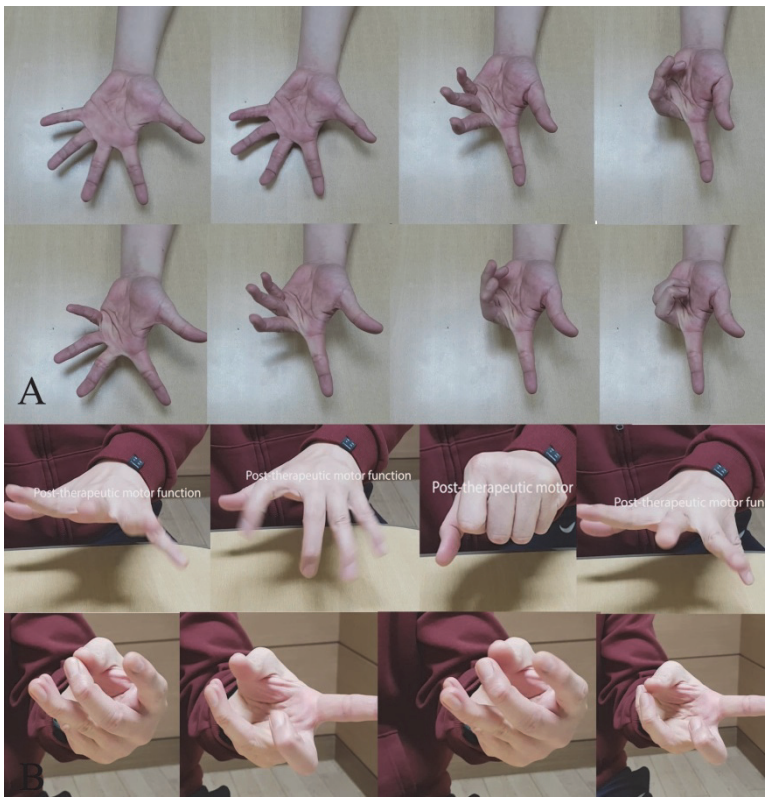
A 42-year-old, right-handed man presented to a local clinic with mild dysarthria and clumsiness of his left hand. He was diagnosed with a small infarction in right basal ganglia. He did not have any other medical history, nor had he any history of trauma or surgery. Clumsiness of his left hand exacerbated over the next five months, and he was referred to the department of rehabilitation by his attending neurologist. The T2-weighted magnetic resonance imaging (MRI) of his brain revealed high signal in right head of caudate nucleus and ventral putamen without any hint of further progression compared to five months ago (Fig. 1). The manual muscle testing revealed a muscle power of grade 4 in his left hand, and grade 5 in all other limbs. The hand function test showed longer processing time in his left side for single-hand activities including moving objects and flipping cards. He showed frustration for not being able to cope with simple grasp-release activities (Fig. 2A). With assumption of the striatum lesion being the cause for the impairment of motor function of his contralateral hand, the patient was prescribed with 0.5mg of pramipexole (Mirapex; a dopamine agonist of the non-ergoline class) twice a day. After two weeks, the patient returned to the clinic with substantial improvement in his left hand function. He was able to better manage grasp-release motions and opposition of thumb and fingers (Fig. 2B).





The striatum is one of the nuclei in the subcortical basal ganglia responsible for the initiation of goal-directed behavior apart from cerebral cortex. Reduced dopamine in the striatum may lead to hypokinesia or difficulty in initiating different motor patterns. Previous studies demonstrated prominent activation of contralateral putamen and caudate nucleus with hand movements. It has been also revealed that the ventral striatum and caudate nucleus have a critical role in recovery and isolated functional movements of upper limb. Thus, supplement of dopamine in striatal infarction could be crucial for restoration of hand function.
Although such case is not common, physicians are encouraged to consider trial of dopamine in patients with hand clumsiness caused by striatal injury, for a small dosage of dopamine is relatively safe and effective without a serious complication.

NEUREXIN 1α COOPERATES WITH α2δ SUBUNITS OF VOLTAGE-GATED CALCIUM CHANNEL TO REGULATE CA2+ INFLUX

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Synapses constitute cellular interfaces between neurons that modulate the flow of information via specialized pre- and postsynaptic membrane domains. Voltage-gated calcium channels(VGCCs) are essential for this process because they trigger Ca^{2+}-dependent neurotransmitter release from vesicles at the presynaptic membrane. We reported earlier that the Ca^{2+}-dependent release is impaired when the presynaptic cell adhesion molecules α-neurexins (αNrxn) are deleted, pointing to an intricate link between cell-cell recognition/adhesion and neurotransmission. Most members of the VGCCs family (Ca_V) consist of three major subunits: a pore-forming, ion-conducting α1 subunit that classifies Ca^{2+}currents, and at least two auxiliary subunits, an intracellular β subunit and a predominantly extracellular α2δ subunit. Auxiliary subunits are necessary for trafficking, kinetics and molecular partner interactions of VGCCs. It remained unclear, however, how the assembly, abundance and activity of VGCCs are regulated at synapses. Here, we report that neurexin 1α is able to regulate VGCCs by cooperating with distinct α2δ subunits.
Using tsA201 cells as a heterologous expression system, we transfected different VGCCs pore-forming α1 subunits along with the auxiliary β3 and α2δ1-3 subunits. We observed that calcium currents through recombinant P/Q-type channels ($α1_A$/β3/α2δ1 or α2δ2) were strongly facilitated in presence of neurexin 1α. In contrast, co-expression of neurexin 1α with N-type calcium channels ($α1_B$/β3/α2δ3) significantly reduced the calcium currents. While the current densities were altered under these conditions, the kinetic, half-activation voltage, reversal potential and inactivation time constants were not changed significantly by the presence of neurexin 1α. Remarkably, neurexin 1α did not affect the calcium influx when other α2δ subunits combinations ($α1_B$/β3/α2δ1 or α2δ2, $α1_A$/β3/α2δ3) or L-type channel ($α1_C$) combinations were probed. Moreover, other adhesion molecules such as SynCAM failed to alter calcium currents in all combinations tested. Thus, our findings suggest that neurexin 1α promotes the function of P/Q-type channels with α2δ1/α2δ2, and suppresses Ca^{2+} currents through N-type channels in combination with α2δ3. This unexpected regulation represents a novel and specific pattern how neurexins can impact VGCCs, and, thereby, Ca^{2+}-dependent synaptic vesicle release.

SEROTONIN TRANSPORTER POLYMORPHISM, PLATELET SEROTONIN CONCENTRATION AND SLEEP DISTURBANCES IN VETERANS WITH POST-TRAUMATIC STRESS DISORDER

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Post-traumatic stress disorder (PTSD) is a complex psychiatric disorder, which develops after exposure to traumatic experience(s). Sleep disorders, such as nightmares and insomnia, are hallmarks of PTSD. Although the neurobiological basis of PTSD is not fully elucidated, this disorder most likely results from complex interactions between genetic and environmental factors. It has been suggested that serotonin (5-HT) system, due to its important role in controlling mood, arousal, and sleep, is involved in the development of PTSD symptoms. Several studies have investigated the association of functional polymorphism located in the 5-HT transporter gene (5-HT transporter linked polymorphic region, 5-HTTLPR) with sleep disturbances, however the findings are contradictory. The basic function of 5-HT transporter is re-uptake of 5-HT into presynaptic neuron or platelets, and 5-HTTLPR modulates the transcriptional activity of 5-HT transporter gene, influencing mRNA and protein levels. Since platelets and 5-HT neurons share similar biochemical processes, such as intake, storage, release and degradation of 5-HT, as well as the expression of 5-HT transporter and some 5-HT receptors, the concentration of platelet 5-HT may be used as a peripheral marker of certain PTSD symptoms. Therefore, the aim of this study was to determine the possible association of 5-HTTLPR and platelet 5-HT concentrations with sleep disorders in Croatian war veterans with PTSD. Croatian male, medication-free war veterans with PTSD (N=393), with or without comorbid depression and various sleep disturbances, were evaluated using the Structured Clinical Interview (SCID) based on DSM-IV criteria, the Hamilton Rating Scale for Anxiety (HAMA), the Hamilton Depression Scale (HDRS) and the Clinician Administered PTSD Scale (CAPS). Veterans with PTSD were subdivided according to sleep disturbances in those with or without insomnia and other sleep disturbances (nightmares, interrupted sleep). Genomic DNA was extracted from peripheral blood using a salting out method. Genotyping was performed using polymerase chain reaction (PCR) and DNA fragments were separated on a 2% agarose gel and visualized. 5-HT concentration was determined spectrofluorimetrically in platelets isolated from platelet rich plasma by series of centrifugations. All data were evaluated using GraphPad Prism version 4.00. Significantly higher frequency of the LL genotype of the 5-HTTLPR compared to S carriers and significantly higher platelet 5-HT concentrations were detected in veterans with PTSD with early insomnia compared to veterans with PTSD without early insomnia. Other sleep disturbances were not associated with 5-HTTLPR genotypes or platelet 5-HT concentration in veterans with PTSD with or without comorbid depression. Further studies with larger groups should clarify the association of 5-HTTLPR and platelet 5-HT with sleep disturbances in PTSD.

This work was supported by Croatian Science Foundation grant IP-2014-09-4289.

CONSTRUCTION OF A USER-FRIENDLY KINETIC MODELING ENVIRONMENT FOR TESTING HYPOTHESES REGARDING THE MODE OF ACTION OF SODIUM CHANNEL INHIBITORS

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Sodium channel inhibitors are important therapeutic agents as local anesthetics, antiepileptics, antiarrhythmics, analgesics, neuroprotective agents, *etc*. They exert their effect both by channel block and by channel modulation, *i.e.*, stabilization of non-conducting states. There are several different types of sodium channel inhibitors depending on their exact mechanism of action, and this mode of action also determines their therapeutic profile. While state-dependent affinity is generally acknowledged as the essence of sodium channel inhibitor mechanism, not much is known about the contribution of channel block *vs.* channel modulation, the contribution of state-dependent affinity *vs.* state-dependent accessibility, and on the exact rates of access/egress as well as binding/unbinding. We believe that the diversity of sodium channel inhibitor mechanisms is much underestimated, and we see this as a major obstacle to successful drug development. Therefore, we consider it essential to understand inhibition mechanisms, and to understand how a specific inhibition mechanism leads to a specific therapeutic effect.
Drugs acting on ion channels exert their effects in the context of a complex network of conformational transitions which make the ion channel operable; their binding necessarily must affect these transition rates. In order to understand the interactions of drug binding/unbinding and gating transitions, kinetic modeling is an essential tool. It allows prediction of effects of specific inhibition mechanisms on experimental results, and verification of hypothetical mechanisms of inhibition. Proper usage of kinetic modeling softwares requires some special expertise, and acquiring this may require months or even years of practice. Our aim was to create a user-friendly modeling environment that simplifies kinetic modeling to the extreme without compromising the complexity of drug modes of action. In this modeling environment anyone familiar with ion channels can create his/her model in a simple, intuitive and foolproof way, and can use it instantly for testing drug mechanisms of action. The process does not require writing of differential equations, or equations describing the voltage dependence of rate constants. Users are able to choose from models from the literature, which we have supplemented with drug-bound states in order to allow simulations of drug effects. Simulations of different modes of action are done by simple sliders, and the results are promptly shown.
The current state of development for this kinetic modeling environment is presented on the poster.

LONG-TERM SURVIVAL OF THE POST MORTEM ADULT HUMAN RETINA IN ORGANOTYPIC CULTURE

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Purpose: Corneal transplantation replaces diseased or damaged tissue with healthy tissue from an organ donor. While the surgery to harvest transplantable corneas from recently deceased donors removes the whole eye, the rest of the tissue is typically disposed of. We developed a culture technique which maintains retinas from those eyes in excellent condition for more than three months, enabling research on human retinal tissue that was previously impossible.
Methods: Human eyes were collected through scheduled multi-organ donations. Following enucleation and dissection of the eyeball, organotypic retina cultures were prepared within 1-2 hours after the circulation arrest in the donor. Isolated pieces of the full-thickness neural retina or complex retinas with attached retinal pigment epithelium (RPE) and choroid were cultured on a polycarbonate membrane in a specific, serum-free, chemically defined medium which has been optimized for the human retina. The cultures were kept for up to 100 days. After fixation, they were processed and analyzed as histological sections or whole mounts by immunohistochemistry using cell type-specific antibodies.
Results: Both isolated neural retina cultures and complex retina-RPE-choroid co-cultures were astonishingly well preserved morphologically with low inter-sample variability. Every major cell type survived and all retinal layers were maintained even after ten or more weeks. Although rods and cones with preserved outer segments could be detected in high numbers in both cultures, the quality and the density of outer segments were superior in retina-RPE-choroid co-cultures. Cones did not undergo severe apoptosis in culture and a mean density of 3500-4000 cones/mm^2 was measured even in long-term cultures. Subpopulations of bipolar, horizontal and amacrine cells showed close to normal morphology. The stratification of the inner plexiform layer remained recognizable. Only the number of surviving ganglion cells showed a significant decrease in long-term cultures, but ganglion cells were still present even after 70 days. Synaptic structures showed close to normal morphology on samples stained against synapthophysin, while prominent telodendria on cone pedicles indicated intact gap junctions.
Conclusions: Our results show that the post mortem adult human retina can be maintained in an appropriate culture system for at least three months. By long-term culturing, both acute and chronic effects of pharmacological compounds could be tested directly on human tissue in a cost- and time-effective manner. Further, the long culture time allows the administration of viral vectors and opens new strategies for developing and testing gene therapeutic approaches. We strongly believe that our unique technology could reduce or in many cases even replace the use of animals both in academic and industrial research.

INVESTIGATION OF THE ELECTROPHYSIOLOGICAL PROPERTIES OF HIPPOCAMPAL SYNCHRONOUS POPULATION ACTIVITY, IN VITRO

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Population activities like hippocampal sharp wave-ripples (SPW-Rs) and dentate spikes described for freely moving rats appear during slow wave sleep and behavioral immobility and are thought to play a leading role in memory processes. Previous studies showed that potentials from the dentate gyrus can be recorded, in vitro. These “dentate waves” composed of depolarizing currents and appeared to be locally generated in the granular cell layer. However in vivo SPW-Rs were never observed within 200 msec after dentate spikes in simultaneous recordings from DG and CA1 pyramidal cell layer, spontaneous dentate waves of slices were always followed by SPW-Rs in CA3 region. In vitro population events are usually originated in the hippocampal CA3 area and spread to the dentate gyrus, CA1 region and subiculum. We investigated the cellular and network properties of these activities with laminar multielectrode in rat hippocampal slice model, using physiological bathing medium. We combined extracellular electrophysiological measurements with two-photon imaging. The electrode array was positioned on the surface of the hippocampal slice, perpendicularly to the granule and pyramidal cell layers. Spontaneous population activities were generated in the dentate gyrus and CA3 region of slices prepared from the temporal hippocampus of young rats, in vitro. These events were characterized by a local field potential gradient (LFPg) transient, increased fast oscillatory activity and increased multiple unit activity (MUA). Events were apparently similar to synchronous population bursts previously recorded in rodent hippocampal slices, considered as in vitro models of SPW-Rs. The synchronous population activity recorded in the dentate gyrus was termed dentate wave in rat. CSD analysis, which estimates transmembrane currents in the local neuronal population confirmed that SPW-Rs were locally generated in each of the DG and CA3 region with conserved intrahippocampal connections. Simultaneous recordings showed that the waves were often synchronized in the DG and CA3, even though data showed that propagation was not unidirectional, it proceeded from both the CA3 region and DG as well.

PROTECTIVE EFFECT OF OLAPARIB AGAINST HYPOXIA-INDUCED RETINAL DEGENERATION

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Retinal hypoxia is a potentially blinding mechanism underlying a number of sight-threatening disorders including central retinal artery occlusion, ischemic central retinal vein thrombosis, complications of diabetic eye disease and some types of glaucoma. Therefore, it is important to examine the promising retinoprotective agents such as PARP inhibitors in rat models of different retinal diseases. Poly (ADP-ribose) polymerase (PARP) is a family of proteins involved in a number of cellular processes such as DNA repair, genomic stability, and programmed cell death. Olaparib, a PARP inhibitor, may involve inhibition of PARP enzymatic activity and increased formation of PARP-DNA complex, resulting in disruption of cellular homeostasis and cell death.
Therefore, the aim of the present study was to demonstrate the activation of PARP, and to evaluate the effect of PARP inhibition (by Olaparib) in retinal ischemic retinal degeneration by assessing morphological and cytokines changes in the retina.
We used four different animal groups for the experiments: (i) a group of Wistar rats were kept in room air, (ii) room air + Olaparib (in water; 4mg/kg daily), (iii) rats were exposed 10 % oxygen concentration for 14 days, (iv) hypoxia + Olaparib (in water; 4mg/kg). Retinas were isolated four weeks after treatments. Statistical comparisons were made using Kolmogorov-Smirnov normality test followed by two-way ANOVA and Fisher LSD's post hoc analysis.
Olaparib treatment caused significant protection in the thickness of different retinal layers, such as INL, ONL, and IPL, which resulted in better-preserved whole retinal distance in the hypoxic group. Moreover, the cytokine levels, which were increased under ischemic conditions such as CNTF, fraktalkine and VEGF, also showed a decreased activation after Olaparib treatment in the retina.
In the present study we provided evidence for the retinoprotective effects of Olaparib in hypoxia-induced retinal degeneration.

Acknowledgement: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, GINOP-2.3.2-15- 2016-00050 “PEPSYS”, PTE AOK Research Grant KA-2017-15, Supported by the ÚNKP-16-4 new national excellence program of the ministry of human capacities, Bolyai Scholarship, MTA-TKI PACAP Research Group. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

DESCRIPTION OF CENTRAL AMYLIN AND ITS POTENTIAL IMPACT ON MATERNAL BEHAVIOR IN RODENTS

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Amylin, a 37-AA peptide previously known to be released from the pancreas, was found to be expressed in the preoptic area of mother rats in our previous study. A significant increase in amylin expression was found immediately after parturition, and remain elevated as long as the pups are not removed from the dams. Amylin expression was also induced in virgin but maternally behaving non-lactating but not in females who did not become maternal despite the sensitization procedure. Immunohistochemistry verified the increased amylin peptide expression in maternally behaving rats and demonstrated the same expression pattern in dams as *in situ* hybridization. Furthermore, most amylin neurons were activated in response to pup exposure suggesting their activation in dams. Since our previous studies suggested that suckling effect on maternal motivation may be mediated by posterior thalamic neurons expressing tuberoinfundibular peptide of 39 residues (TIP39), we examined the relationship of amylin and TIP39. Fiber terminals containing TIP39 and the parathyroid hormone 2 receptor (PTH2 receptor; the receptor of TIP39) have similar distribution as amylin neurons in the preoptic area. TIP39 terminals closely apposed amylin neurons suggesting their innervation by TIP39 neurons. The maternal induction of amylin was markedly reduced in mice lacking the PTH2 receptor suggesting a functional relationship between TIP39 and the maternal induction of amylin.
To assess maternal motivation, a place preference test was performed. Mother and maternally sensitized female mice preferred the pup-associated cage while the virgin and ovariectomized mice did not show such preference. In the forced swimming test used to examine depression-like behavior, mothers spent more time with active behavior than sensitized and virgin femal mice did. In the absence of amylin, however, mice showed depression-like behavior in the forced swimming test. The unique induction, time course and distribution pattern of amylin mRNA in mother rats and mice, its relationship with the TIP39-PTH2R neuromodulator system, and the elevated depression-like behavior in amylin KO mice implicate that amylin is a neuropeptide with specific maternal function.

Acknowledgements: OTKA K100319 research grant and KTIA_NAP_B_13-2-2014-0004 Program.

THE ROLE OF MEDIAL ORBITOFRONTAL CORTICAL GLUCOSE-MONITORING NEURONS IN THE MAINTENANCE OF HOMEOSTASIS

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The medial orbitofrontal cortex (mOFC), part of the forebrain limbic circuitry, plays important role in the central regulation of feeding and metabolism associated motivated behaviors. Special chemosensory cells, the glucose-monitoring (GM) neurons exist in the mOFC which have already been shown to be involved in these functions.
In the present study, electrophysiological, metabolic and behavioral experiments were conducted to reveal multiple functional attributes of these GM cells and to determine their role in the maintenance of homeostasis.
In electrophysiological experiments, extracellular single neuron activity was recorded in anesthetized rats by means of tungsten wire multibarreled glass microelectrodes, during microelectrophoretic administration of chemicals and intraoral gustatory stimulations. Metabolic and behavioral tests were performed in awake rats after destruction of the GM cells by streptozotocin (STZ) microinjection into the mOFC. Metabolism was evaluated by the glucose tolerance test, and we also measured plasma concentration of various metabolites. For behavioral examinations, the conditioned taste avoidance and the taste reactivity tests were performed.
Fifteen percent of the mOFC neurons responded to D-glucose. Acetylcholine altered neuronal activity in 61%, whereas noradrenaline did so in 23%, and dopamine in 30% of all neurons. GABA inhibited 42% of the examined cells. Sweet taste stimulation changed the activity of one fourth of neurons, whereas one third did so in case of the sour taste. The other primary taste qualities and orange juice influenced the firing rate of appx. 60% of all neurons.
In the acute glucose tolerance test, the peak of blood glucose concentration of the STZ treated rats appeared to be higher, and the dynamics of changes slower: 30 and 60 minutes after the glucose load, the blood glucose concentration in the STZ-treated rats was significantly higher than that in the control animals. When measuring plasma metabolites following STZ microinjection into the mOFC, the triglyceride concentration was found significantly higher in the STZ-treated compared to the control rats. In the plasma concentrations of other metabolites no significant difference was observed.
The ability to acquire conditioned taste avoidance was preserved in both groups of animals. In the taste reactivity tests, however, the STZ microinjected animals apparently felt both pleasant and unpleasant tastes more pleasant compared to the control rats.
Our data indicate that glucose-monitoring neurons of the medial orbitofrontal cortex are of distinguished significance in the integration of signals arising from the internal and external environments to maintain the healthy homeostatic balance.

Supported by: PTE AOK KA 2013/34039/1; EFOP-3.6.1-16-2016-00004 The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

ORGANIZATION OF ACTIN-BINDING PROTEINS IN PURKINJE CELL SPINES

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Dendritic spines are the primary recipient of excitatory input in the mammalian brain. It is generally accepted that forebrain excitatory synapses show activity-dependent morphological changes. The underlying actin cytoskeleton and its regulatory enzymes serve as a dynamic scaffold for such spine-morphing. However, dendritic spines of the cerebellar Purkinje cells (PCs) differ from those of the hippocampus. PC spines are almost exclusively mushroom-shaped and are generally larger than hippocampal CA1 spines. In addition, cerebellar spines are rich in endomembranes, which express high levels of the inositol trisphosphate receptor (IP_3R). In contrast, hippocampal spines in the adult brain contain only occasional endosomes. Unlike hippocampal spines, synapses on PC spines appear to lack NMDARs, as do have high levels of mGluR1. In this two different brain regions, spines also exhibit different developmental principals: the formation of PC spines is an intrinsic phenomenon, independent of their presynaptic partner. Formation of hippocampal spines requires an active presynaptic axon terminal. Thus, the apparent resemblance may be misleading, though spines in both regions play a role in biochemical compartmentalization. As an example, our previous studies have indicated that in CA1 spines cortactin resides in the spine core, while other enzymes are restricted to the shell. In addition, the mechanism of various forms of synaptic plasticity (e.g. LTD and LTP) significantly differs between forebrain and PC synapses. Therefore we aimed to study actin-binding proteins (ABPs), in order to see if there are any differences between their subcellular distribution within dendritic spines of hippocampal and PC spines. We examined the localization of cortactin in the spinoplasm from both brain regions using preembedding labeling techniques combined with quantitative immunoelectron-microscopy. Our results confirmed that cortactin was situated in the vicinity of the PSD and was associated with the non-synaptic spine membrane (shell). Interestingly, this organization was notably opposite to that of the CA1 spines. As cortactin stabilizes the branch points along F-actin filaments it may create a stable spine shell in PC spine, which may underlie the differences in synaptic plasticity between forebrain and cerebellum.

This research was supported by the 11475-4/2016/FEKUT grant of the Hungarian Ministry of Human Resources. B.R. is also supported by the János Bolyai Research fellowship of the Hungarian Academy of Sciences.

IMPAIRMENT OF NEURAL COORDINATION IN HIPPOCAMPAL NEURONAL ENSEMBLES AFTER PSYCHOTOMIMETIC DOSE OF DIZOCILPINE

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According to cognitive coordination hypothesis of schizophrenia discoordination of activity within neuronal networks is underlying pathological mechanism behind some of the symptoms, including cognitive symptoms of the disease. NMDA receptor antagonists induce psychotic symptoms in humans, and are used as a model of psychosis in experimental animals. Within the framework of cognitive coordination hypothesis we tested the effect of MK-801 (NMDA-receptor antagonist) on activity of hippocampal neurons. We hypothesized that MK-801 would not affect firing rates of the neurons, but would alter coordination of timing of neuronal firing. Six male Long-Evans rats (250-350g) were anesthetized with urethane (1.2g/kg i.p.) and implanted with NiChrome wire (25umø) tetrodes into the pyramidal cell layer of CA1 field of the dorsal hippocampus (AP=-4, L= 2.5, DV=2.5mm). Local field potential was recorded from epidural electrodes over contralateral hippocampus. After the stability of the signal was confirmed, signals were recorded for 60 minutes before and 120 minutes after MK-801 injection (0.15mg/kg i.p.). Activity of 56 neurons was recorded, 47 complex spike cells (CSCs) and 9 theta cells (TCs). MK-801 did not cause significant changes in average firing rates in either group of neurons, but it increased the number of co-active cell pairs. Further analysis, revealed that MK-801 increased the level of co-activation in originally anti-correlated cell pairs. Analysis of neuronal activity in entire ensembles (9-18 neurons) indicated highly similar activity during intervals before MK-801 and much lower similarity between pre-injection and post-injection intervals. LFP analysis revealed that after MK-801 injection, the number of the CSCs modulated by theta waves increased. Yet, the effect of MK-801 on theta phase preference for CSCs and TCs, or on strength of theta modulation, was insignificant. Present results show that effect of MK801 was primarily manifested in changes of coordinated activity between hippocampal cells and support the hypothesis that increased coordination of firing between these neurons might be responsible for cognitive discoordination in psychosis.

This research was funded by the Czech Science Foundation, grant nr. 14-03627S, Ministry of Health of the Czech Republic, grant nr.15-34524A and project Nr. LO1611 with a financial support from the MEYS under the NPU I program.

ALPHA-SYNUCLEIN SEQUESTERS THE CHAPERONIN HSP10 IN THE CYTOPLASM AND REDUCES ITS ACTIVITY IN THE MITOCHONDRIA

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Aims: Mitochondrial dysfunction is characteristic in the substantia nigra in both familial and inherited forms of Parkinson's disease (PD). However, the precise molecular mechanisms involved are still unclear. In addition, increased levels of α-synuclein are associated with familial forms of the PD and are critical for disease development. Here, we hypothesized that mitochondria in the striatal presynaptic terminals are more vulnerable to increased α-synuclein expression than in other cellular compartments. Our aim was to identify new mechanisms underlying α-synuclein-mediated mitochondrial dysfunctions.
Methods: In order to assess mitochondrial function, we measured oxygen consumption rate, mitochondrial membrane potential, and reactive oxygen species *in vivo*, in synaptosomes, and *in vitro*, in primary neuronal cultures overexpressing of α-synuclein. Using mass spectrometry, we identified α-synuclein-interacting proteins from striatal synaptosomes of α-synuclein transgenic mice.
Results: Overexpression of α-synuclein reduced spare mitochondrial respiratory capacity, handling of reactive oxygen species, and mitochondrial membrane potential in striatal synaptosomes of young α-synuclein transgenic mice. We identified HSP10 as a strong α-synuclein-interacting partner, and, interestingly, we found that its presence in the cytoplasmic fraction increased. In addition, we found decreased levels of mitochondrial MnSOD2. By overexpressing HSP10 *in vitro*, we partially reversed α-synuclein-induced dysfunctions.
Conclusion: Increased levels of α-synuclein may sequester HSP10 in the cytoplasm, reducing mitochondrial chaperonin function. In total, our study identified a new mechanism by which α-synuclein regulates mitochondrial functions.

ALTERED BEHAVIOR, LEARNING AND MEMORY FUNCTIONS IN SOMATOSTATIN RECEPTOR SUBTYPE 4 GENE-DEFICIENT MICE

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Somatostatin is an inhibitory neuropeptide regulating a variety of functions both in the peripheral and central nervous systems. Our team has previously discovered that among its five Gi-protein-coupled receptors, somatostatin receptor subtype 4 (sst4) mediates anti-inflammatory, analgesic and antidepressant effects without endocrine actions. Recent papers reported that a synthetic sst4 agonist improved the cognitive functions in a mouse model of Alzheimer's disease. Therefore, the aim of our study was to examine the role of the sst4 receptor in behaviors related to learning and memory processes using gene-deficient mice.
Sst4 gene-deficient (sst4-/-) male and female mice, as well as their wild-type counterparts (sst4+ /+) (12 weeks old, 18-25 g) were observed in the spontaneous alternation Y-maze test. Spontaneous locomotor activity and anxiety were measured in an Open Field Test (OFT). Short and long-term learning was examined with the Radial-Arm Maze (RAM) by determining the numbers of repetitive entries to a respective arm and the found reward food pellets. Novelty detection and recognition memory was investigated by the Novel Object Recognition Test (NOR). The Noldus system and the EthoVision XT software were used for the evaluation.
Female sst4+/+ mice time spent significantly longer time in the middle of the OFT than their male wild-type counterparts, but the number of visited arms and arm combinations in the Y-maze was not different. Furthermore, they visited, repeated and missed significantly more arms, but found the same amount of rewards in the RAM, and they also spent significantly longer time by exploring the novel object in NOR compared to male wild-types.
Female sst4-/- mice visited and repeated less arms in both mazes, but they found the same amount of rewards and spent more time to explore the novel object in NOR compared to their wildtypes. In contrast, male sst4-/- mice found significantly less rewards in the RAM and they were less interested to explore both objects in the NOR compared to their wild-types, but there were no statistical differences in any other investigated parameters.
Based on these data, female mice showed greater spontaneous locomotor activity, less anxiety, worse long-term learning ability and exploration skills than males. Sst4-deficiecy resulted in faster short-term learning in females, but worse exploratory behaviour in both sexes. These results suggest that sst4 is an interesing complex regulator of behavior and cognitive functions under healthy conditions, but further investigations are needed to determine its role in memory deficits during aging or other pathological conditions.

Support: EFOP-3.6.1-16-2016-00004, KTIA_NAP_13-2014-0022, GINOP-2.3.2-15-2016-00048. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

ALTERATION OF HIPPOCAMPAL LOCAL INHIBITORY NEUROCIRCUITS IN TEMPORAL LOBE EPILEPSY

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Epilepsy is one of the most common neurological diseases, affecting approximately 0.5-1.5% of the world's population. About 60% of all focal epilepsies are represented by the temporal lobe epilepsy (TLE).
The most salient feature of all epilepsy types is the abnormal, excessive and/or synchronous neuronal activity in the brain. Excitability of neural networks is largely controlled by inhibitory interneurons, which provide both general inhibition and temporal regulation of principal cell activity. Distinct interneuron types act in discrete time windows, and interact with excitatory inputs in a domain specific manner.
The hippocampus has a major role in TLE. Our aim was to study the timing of the morphofunctional alterations during epileptogenesis, therefore we quantified, using male Wistar rats, the changes in the main interneuron populations of the CA1 region of the hippocampus using the status epilepticus (SE) pilocarpine model of TLE. The animals were continuously video monitored in order to establish seizure pattern and behavior. Part of the animals was sacrificed after the occurrence of spontaneous seizures, another part two days after SE in order to study the initial impact of the epileptogenic insult. We studied the density of basket cells that provide perisomatic inhibition, axo-axonic cells that control the hippocampal output of principal cells, bistratified cells that essentially modulate Schaffer collateral input, oriens-lacunosum moleculare (O-LM) cells that modulate entorhinal cortical input and ivy cells that provide an overall control of excitation. The cell types were identified by triple immunohistochemical staining. We used parvalbumin, neuropeptide Y, somatostatine and neuronal nitric oxide synthase proteins as markers. Hippocampal sclerosis was assessed by Nissl staining. Spontaneous recurrent seizures developed in average after 14 days. We did not find correlation between the degree of hippocampal sclerosis and interneuronal alterations.
Parvalbumin positive basket and axo-axonic cells were lumped together as perisomatic interneurons because our immunohistochemical method did not allow their differentiation. In the group sacrificed after the occurrence of spontaneous seizures the density of perisomatic inhibitory cells was maintained, just like the density of bistratified cells. The density of O-LM cells and ivy cells was reduced. In the animals sacrificed shortly after SE the density of all detected interneuron subtypes was drastically dropped. Because in the later stage of epileptogenesis the observed density of all interneurons was higher than in the early period, we assume that the early drop is not indicating cellular death, but rather weakening of immunodetectability which could be the consequence of reduced expression or conformational changes of the labeled proteins.
Further studies are necessary to elucidate the functional state of the not detectable interneurons and also to establish the length of this state.

N-(PROP-2-YNYL)-CARBOXAMIDO STEROIDS INHIBIT THE OPENING PROPERTIES OF TRP ION CHANNELS BY MODULATION OF LIPID RAFTS

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Background: Transient Receptor Potential (TRP) cation as the TRP Vanilloid 1 and TRP Ankyrin repeat domain 1 channels (TRPV1 and TRPA1) are nocisensors playing important role to trigger pain. Two „melastatin" TRP receptors as TRPM8 and TRPM3 are also expressed in subgroups of primary sensory neurons. All these cation channels serve as thermosensors and are suitable to be activated also by several exogenous and endogenous chemical ligands. We provided evidence that the disruption of the plasma membrane microdomains of lipid rafts influenced the activation mechanisms of TRP channels. Lipid raft disruption was made by in vitro pretreatment with sphingomyelinase (SMase), depletion of cholesterol with methyl □-cyclodextrin (MCD) and gangliosides breakdown by myriocin. We described also that a N-(prop-2-ynyl)-carboxamido-steroid compound (C1) had an inhibitory effect on TRPV1 activation. Our aim was to investigate whether this compound exerted its effect through the lipid raft disruption. We examined whether the steroid ring itself (C3) or the position of carboxamido group of the compound (C2: unnatural backone, C3: natural backbone) was essential for the development of the inhibitory action.
Methods: The effects of steroid compounds on TRP ion channels were analysed on isolated trigeminal (TG) neurons by measuring agonists-induced Ca^{2+} transients with ratiometric technique and on TRPV1- and TRPA1-expressing CHO cells with calcium-uptake experiment. Measure of cholesterol-depletion by filipin staining and changes in membrane polarisation by fuorescence spectroscopy with laurdan dye were performed.
Results: It has been revealed that intracellular Ca^{2+} enhancement evoked by capsaicin (TRPV1) was inhibited after C1 and C3 incubation, but the responses after C2 administration remained unaltered. C1 and C3 treatment diminished the percentage of responsive cells and the magnitude of responses. The effect of the most efficient C1 was investigated on the activation of allyl isothiocyanate, formaldehyde (TRPA1), pregnenolon sulphate (TRPM3) and icilin (TRPM8). C1 compound reduced the percentage of responsive cells and the magnitude of responses. We proved by filipin staining that all of the three compounds induced cholester depletion in CHO cells. C1 induced significant changes in membrane polarisation. C1 caused a shift in the excitation and emission spectra of laurdan in the treated TG neuron membrane fractions, which was the clear indication of the C1-caused cholesterol-depletion.
Conclusion: Our results revealed that the presence and the position of the carboxamido group in the N-(prop-2-ynyl)-carboxamido-steroid compounds essential for the development of the inhibitory effect of the compounds. We suggest that the hydrophobic interactions between the TRP channel and lipid raft interfaces modulate the opening properties of these channels and therfore, targeting this interaction might be a promising tool for drug developmental puposes.

Az Emberi Erőforrások Minisztériuma ÚNKP-16-4-I. Kódszámú Új Nemzeti Kiválóság Programjának támogatásával készült, Richter Gedeon Talentum Foundation, EFOP-3.6.1-16-2016-00004, GINOP-2.3.2-15-2016-00050 – PEPSYS, GINOP-2.3.2-15-2016-00048 – STAY ALIVE, Hungarian Brain Research Program KTIA_NAP_13-2-2014-0022 MTA-PTE NAP B Chronic Pain Research Group. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

SUBTHRESHOLD VS. SPIKING RESONANCE INVESTIGATED BY COMPUTATIONAL MODELING AND IN DYNAMIC CLAMP

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Intrinsic excitability of nerve cells allows them to transform synaptic inputs into action potentials. This process reflects a complex interplay between the synaptic inputs and the voltage-dependent membrane currents of the postsynaptic neuron. While neurons in natural conditions mostly fire under the action of intense and temporally complex synaptic inputs, conventional techniques to characterize intrinsic excitability mainly utilize static means of stimulation. Recently we have shown that voltage-gated membrane currents regulate the firing responses under current step stimulation and under physiologically more realistic inputs in a differential manner. At the same time, a multitude of neuron types have been shown to exhibit some form of sub-threshold resonance that potentially allows them to respond to synaptic inputs in a frequency-selective manner. In the present study we developed computational models and performed dynamic clamp experiments to examine how specific voltage-gated currents regulate neuronal excitability under simulated frequency-modulated synaptic inputs. The model simulations revealed that the impact of voltage-gated currents in regulating the firing output is strongly frequency-dependent and mostly affecting the synaptic integration at theta-frequencies. Notably, robust frequency-dependent regulation of intrinsic excitability can be found even when the particular neuron model phenotype exhibits no sub-threshold membrane resonance. We validated the model predictions using simulated synaptic bombardment and concurrent biophysical manipulation of cultured hippocampal pyramidal neurons using the dynamic clamp technique. These experiments, in agreement with the model, revealed that the insertion of a computer-generated inwardly rectifying K-current reduced the intrinsic excitability of hippocampal neurons most effectively under theta frequencies. Our findings show that resonant-type regulation of intrinsic excitability is more common and more elaborate than anticipated from conventional analysis of subthreshold membrane resonance.

This work was supported by the Hungarian Brain Research Program (KTIA_NAP_13-2014-0018) and by the National Research, Development and Innovation Office (NVKP_16-1-2016-0016).

THE EFFECTS OF EARLY ENVIRONMENTAL ENRICHMENT AND PACAP IN AGING RAT MODEL OF PARKINSON'S DISEASE

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The causative therapy of Parkinson's disease (PD) is still under investigation. One of the well-studied effects of enriched environment and pituitary adenylate cyclase-activating polypeptide (PACAP) is the strong neuroprotective effect. We have previously described the neuroprotective effects of PACAP and postnatal enriched environment in Parkinson's disease in young animals. The aim of our present study is to investigate the protective effects of these factors in aging (12-18-months-old) rats after unilateral 6-OHDA-induced lesion of the substantia nigra (s.n.).
Wistar rats were used in our experiment (n=35). Animals were divided into standard (n=17) and enriched groups (n=18).Animals of the standard group were placed under regular conditions. For environmental enrichment, during the first five postnatal weeks we placed pups in larger cages supplemented with toys, objects, running tunnels and rotating rods of different shape, size and material.In aging animals PD was induced by unilateral injections of 6-OHDA (2 µl,5 µg/µl) into the left substantia nigra, control animals received 2 µl physiological saline Following the 6-OHDA injections half of the animals received 2 µl (1µg/µl) PACAP treatment into the s.n.. On the 7th postoperative day brain of the animals were removed and samples of the substantia nigra were collected. Dopamine levels and DJ-1 protein content of the substantia nigra were measured by HPLC-Q Exactive orbitrap MS system and ELISA method, respectively.
The substantia nigra of the 6-OHDA-treated standard and enriched animals showed significantly lower DA levels compared to the saline-treated animals of the same groups. Consistent with our previous studies in young animals the PACAP treatment could also increase the DA levels after 6-OHDA-induced lesion in aging rats. Also, the DJ-1 protein content of the substantia nigra was significantly higher in groups receiving PACAP treatment following the lesion. However, early environmental enrichment did not have any protective effects in this experiment.
Although the protective effect of early postnatal environmental enrichment is described in young animals, we could not prove it in aging animals. However, similarly to younger animals PACAP could restore the decrease of DA and DJ-1 protein levels, which could play a role in its neuroprotective effect in Parkinson's disease.

This work was supported by: National Scientific Research Fund (OTKA K104984); the National Research, Development and Innovation Fund K119759; GINOP-2.3.2-15-2016-00050; PTE AOK KA Research Grant; National Brain Research Program (KTIA_13_NAP-A-III/5), TAMOP 4.2.4.A/2-11-1-2012-0001 National Excellence Program; UNKP-16-4-IV, New National Excellence Program of the Ministry of Human Capacities, MTA TKI 14016 Program, Bolyai Scolarship, EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

THE EFFECTS OF PACAP IN DIFFERENT ANIMAL MODELS OF PARKINSON'S DISEASE

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It is well known that pituitary adenylate cyclase-activating polypeptide (PACAP) has neuroptorective effects in different neuronal injuries. Our research group has previously shown the neuroprotective effect of PACAP in a rat model of Parkinson's disease, where the peptide was able to reduce the motor deficits and the dopaminergic cell loss after unilateral 6-OHDA lesion of the substantia nigra. The aim of our work was to examine the detailed mechanisms of this protective effect using different animal models of Parkinson's disease.
In the first part of the study we examined the changes in monoamine (DA and serotonin), metabolic enzyme (S-COMT, MB-COMT and MAO-B) and PARK7 protein concentrations after PACAP treatment in unilateral 6-OHDA lesion of the substantia nigra in rats. In case of PACAP co-treatment we found significantly increased DA and PARK7 protein levels and decreased MB-COMT concentration in the substantia nigra both in young and aging rats, but it had no effect on serotonin, S-COMT and MAO-B levels.
In the second part of our work we used similar methodology completed with behavior studies to examine the effect of PACAP treatment in rotenone-induced Parkinson's model in snails. Similarly to rats, PACAP improved behavioral deficits, increased DA and decreased MB-COMT levels, but it had no effect on serotonin, S-COMT and PARK7 levels. Earlier we have proven that PACAP deficient (KO) mice have higher vulnerability in several pathological conditions. To examine the effects of endogenous PACAP in neurodegenerative disorders we analyzed the morphological and molecular alterations of the brain between wild type and PACAP KO mice. With SDS-PAGE, mass spectrometric-based proteomic analysis and volume imaging techniques we found several differences in the diencephalon and mesencephalon leading to altered biochemical processes in mice lacking endogenous PACAP. PACAP KO animals showed more severe behavioral alterations and dopaminergic cell loss after unilateral 6-OHDA lesion of the substantia nigra compared to wild animals. These results provided evidence for the protective effect of endogenous PACAP in this Parkinson's disease model.
We also evaluated the changes in Pac1 receptor expression in basal ganglia related to Parkinson's disease in MPTP-induced macaque model. Striatum, pallidum and cortex were evaluated for Pac1R immunostaining. We found downregulation of PACAP receptor, which suggests that the PACAP/Pac1R system may play an important role in the development/progression of the disease.
With these results we showed that the neuroprotective effects of PACAP in Parkinson's disease in different animal species are well correlated to each other on the level of neurotransmitters, enzymes, proteins and receptors.

Supports: KTIA_13_NAP-A-III/5, GINOP- 2.3.2-15- 2016-00050 "PEPSYSFIS PI13 01293; ISCIII Plan Estatal de I+D+I 2013-2016), Fundacion Seneca (FS/19540/PI/14, IMIB OTKA K10498, NKFIH K119759, PTE AOK Research Grant No. KA-2017-01, TAMOP 4.2.4.A/2-11-1-2012-0001 „National Excellence Program", UNKP-16-4-IV New National Excellence Program, EFOP-3.6.1.-16-2016-00004, MTA TKI 14016 Program, Bolyai Scholarship. The present scientific contribution is dedicated to the 650th anniversary of the foundation of the University of Pecs, Hungary. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

SIGNALING EVENTS INDUCED BY THE PROTEASOME INHIBITOR MG-132 IN PC12 (RAT PHEOCHROMOCYTOMA) CELLS

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The rat pheochromocytoma (PC12) cell line is a popular model system to study neuronal differentiation, survival and apoptosis. Upon prolonged nerve growth factor (NGF) exposure these tumor cells stop to divide, become polygonal, grow projections and start to look and behave like sympathetic neurons. Differentiation of PC12 cells can also be induced by MG-132, which is a cell permeable, potent and selective, aldehyde type proteasome inhibitor, that inhibits the chymotrypsin-like activity of the proteasome in a reversible manner.
The Ubiquitin-Proteasome System (UPS) plays a crucial role in the breakdown of unneeded, misfolded or damaged proteins, and it regulates the activation or inactivation of several signaling molecules involved in cell cycle control, inflammation, apoptosis or differentiation. It also shows the importance of this system, that around 80% of intracellular proteins are degraded this way in a well-regulated manner. Poly-ubiquitinated proteins are recognized and broken down by a cylindrical multicatalytic proteinase complex, called proteasome. The disfunction of the UPS has been shown to be involved in many pathologic conditions, such as inflammation, tumors and neurological diseases. There are proteasome inhibitors already approved for the treatment of some hematological tumors and their field of application is getting broader.
Treatment of PC12 cells with 2,5μM MG-132 results first in process formation, but after longer incubation with the agent the cells undergo apoptosis. The signal transduction events behind these processes are not fully understood. Besides the activation of stress signaling pathways (p38-, JNK-pathways) we could observe sustained phosphorylation and nuclear translocation of ERK1/2 which are well documented underlying conditions of PC12 cell differentiation. MG-132 induced prolonged ERK1/2 activation, nuclear translocation and neuritogenesis required the intact function of Src, TrkA, Ras and MEK since the inhibition of either one of these molecules results in the reduction of the above phenomena. Based on our observations we concluded and have shown for the first time that intact Src function is a necessary requirement of TrkA phosphorylation, followed by Ras- and MEK-dependent sustained ERK1/2 activation with nuclear translocation of the latter, and neuritogenesis during MG-132 treatment of PC12 cells. On the other hand, the amount of apoptosis-inducing stress signaling molecules also increased in the cells and when the compensating ERK activation declined the signs of apoptosis were observed in the cultures. Both the differentiation- and apoptosis-inducing effects are beneficial in terms of tumor therapy, thus, proteasome inhibitors are promising anti-tumor agents in various tissues.

This work was supported by EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

RECOVERIN IMMUNOPOSITIVE BIPOLAR CELLS OF THE CAT RETINA

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Introduction: Bipolar cells of the retina are starting points of parallel visual processing pathways. The retinae of rodents and primates feature 10 main morphological bipolar cell types. Many bipolar cell types of these species have been characterized by neurochemical markers. Cats are important model organisms in studying visual pathways but their bipolar cell types are poorly described. In a search for new bipolar cell markers, we performed recoverin immunohistochemistry in the cat retina. Recoverin, a phototransduction protein, is a marker of photoreceptors and a single type of OFF-cone-bipolar cell in primates and rodents. Here we describe the recoverin immunopositive bipolar cell type of the cat retina.
Methods: Retinas were obtained from adult cats used in terminal electrophysiological experiments. Eyes were removed following an overdose of anesthetics, posterior eyecups were prepared on ice and fixed for 45 min with 4% PFA in 0.1 M PBS (pH 7.5) solution at 4^{o}C. The isolated retinae were cut into four quadrants and stored in a cryoprotecting solution at -20 ^{o}C. Cross-sections of 20 um thickness were cut on a cryostat. In addition, whole mounts were prepared from each quadrant. Anti-recoverin immunohistochemical staining was performed using 10% normal donkey serum as blocking agent, rabbit anti-recoverin antibody (Millipore) at 1:1000 dilution as primary antibody and Texas-Red conjugated goat-anti-rabbit antibody (Sigma, dilution 1:200) as secondary antibody. At the end of the staining, the sections or whole mounts were mounted onto gelatinized slides and coverslipped with Aqua PolyMount. The labeling was examined and the microphotographs were taken using the Zeiss LSM 710 confocal laser scanning microscope.
Results: The appearance of photoreceptors of the cat retina was similar to that of other species with strong recoverin positive labeling of photoreceptor outer segments, cell bodies and axons. A sparse population of recoverin-positive cell bodies was seen at the upper margin of the inner nuclear layer. Their short dendrites were seen to enter the outer plexiform layer. The axons of these cells ramified in the deepest layers (layer 4 and 5) of the inner plexiform layer (IPL). Based on their low density and ramification pattern, these cells can be identified as an ON-cone-bipolar cell. Recoverin immunpositive bipolar cells have not been described so far in the cat retina. Compared with earlier descriptions of cat bipolar cell types, the recoverin immunopositive bipolar cell is most likely to correspond to type cb8, the putative blue-cone bipolar cell.

Supported by grants from the Medical School, University of Pécs, OTKA K108747 and the Hungarian Brain Research Program KTIA_13_NAP-A-I/11, EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

RETINAL GANGLION CELL RESPONSES IMPOSE A POSTDICTIVE PROCESSING OF VISUAL SIGNALS IN THE BRAIN

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All information about the world is detected by sensory organ receptors (hearing, seeing, touch etc.) and then transmitted to the brain by nerves. To detect the oneness of signals reflecting to the same object, various sensory signals are processed together in the brain by a mechanism called feature-binding. This multisensory perception is performed by different neural pathways at different speeds and thus the brain collects information about an object in an extended time window and binds that together in a retrospective/postdictive fashion. Similar binding mechanism must take place when various visual features of an object are processed through parallel retinal pathways. In this study we examine the time frame in which various retinal pathways carry information towards the brain by recording retinal ganglion cell light responses evoked by full-field photopic light stimuli. We find that ganglion cells signal the brain with different delays in a subtype dependent manner. The discrepancy in signal speed can be as much as 100ms. A subtype dependent trial-to-trial variability in response delay was also found as certain ganglion cells displayed responses with a rather consistent delay, whereas responses of other ganglion cells showed a considerable delay variability. Therefore, the collected data indicate that the observed variability in ganglion cell signal speed alone imposes a 100-150 ms long postdictive processing of visual signals in the brain.

Support: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

A NOVEL, ENHANCED PASSIVE-TRANSFER-TRAUMA MOUSE MODEL FOR COMPLEX REGIONAL PAIN SYNDROME: ROLES FOR NON-INFLAMMATORY AUTOANTIBODIES AND CENTRAL GLIA-CELL ACTIVATION

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Immune response against sensory nerve-derived antigens and complex neuro-immune interactions are suggested responsible for pain and autonomic signs in some CRPS, but the pathophysiological mechanisms are unclear. We established and characterized an enhanced passive-transfer translational mouse model for CRPS. Small plantar skin-muscle incision was performed in female C57Bl/6 mice daily treated i.p. with purified serum-IgG from CRPS patients or healthy volunteers (n=4-4; 6 mice/group) for 1-13 days. Hindpaw mechanonociceptive threshold was measured with aesthesiometry, paw volume with plethysmometry, myeloperoxidase activity with luminescence in vivo imaging, sensory neuropeptides and inflammatory cytokines with immunoassays, glia markers in pain-related brain regions with immunochemistry. CRPS IgG significantly increased and prolonged swelling, and induced stable hyperalgesia of the incised paw compared to healthy IgG. The strongest hyperalgesic effect was observed towards the end of the study, whereas in the control group mechanical hyperalgesia-, and in all groups swelling and post-incision paw-inflammation had fully resolved by that time. CRPS IgG treatment significantly increased the density of astrocyte-related glial fibrillary acidic protein (GFAP) and microglia-staining Iba1 in L4-L5 spinal dorsal horn, periaquaductal gray and somatosensory cortex compared to controls. In an enhanced passive-transfer-trauma model for CRPS, daily serum-IgG injection induces stable, strictly unilateral hyperalgesia in rodents over at least two weeks, but this effect does not appear to be related to peripheral inflammation. Astrocyte and microglia activation accompany this process, and might contribute to sustain the enhanced hyperalgesia alongside the presumed regional non-inflammatory autoantibody activity.

Support: KTIA_NAP_13-2014-0022(MTA PTE NAP B Fájdalom Kutatócsoport, 888819); Pain Relief Foundation; TÁMOP-4.2.4.A/ 2-11/1-2012-0001; EFOP-3.6.1-16-2016-00004. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

NEURAL CIRCUITS AND FUNCTIONAL CONNECTIVITY OF FEAR MEMORY EXTINCTION IN αCAMKII AUTOPHOSPHORYLATION-DEFICIENT MICE

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Understanding how activity in neural circuits drives behavior is a fundamental problem in neuroscience. Here we presents a functional connectome of 24 brain regions underlying a specific behavior of extinction of contextual fear in WT and αCaMKII autophosphorylation-deficient heterozygous mice (T286A +/-) after short (1 day) or long (30 days) delay after training. αCaMKII-T286A mutants were chosen, because it has been shown previously that αCaMKII autophosphorylation plays a pivotal role in synaptic plasticity as well as memory formation and extinction. To identify neuronal network involved in extinction of contextual fear and it is represented by network interactions between brain regions we applied immediate early gene c-Fos immunostaining to determine activity of brain regions and graph theoretical concepts to calculate inter-regional correlation (network). Based on the analysis of c-Fos expression pattern, we claim that when autophosphorylation of αCaMKII is disrupted, extinction of fear memory engages different neuronal circuits than in WT mice. Network analysis showed that T286A +/- mice have also different functional connectome (i.e. nodes distribution, vertex density) and their network disassembly, creating smaller nets after remote memory extinction. Moreover correlation of these two measures in mutant mice results in poor remote memory extinction, suggesting an important role of αCaMKII autophosphorylation in this phenomenon.

GRAPHENE QUANTUM DOTS SHOW ANTI-INFLAMMATORY EFFECT ON ANIMAL MODEL OF NEUROINFLAMMATION

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Background: Multiple sclerosis (MS) is an inflammatory demyelinating disorder of central nervous system. Different immunomodulatory and antiinflammatory drugs were tested for the therapy of MS and its corresponding animal model, experimental autoimmune encephalomyelitis (EAE). Graphene quantum dots (GQD) are oval two-dimensional sheets of graphite with a diameter <100 nm and a thickness of one carbon atom (1 nm), with potential applications in biomedicine. It is shown that large GQD alleviate immune-mediated liver damage. Up to now, there are no data about the potential anti-inflammatory effect of GQD in the CNS tissue.
Objective: To examine the potential anti-inflammatory effect of GQD on a model of neuroinflammation, EAE
Methods: Female DA rats were immunized with spinal cord homogenate and Freund's complete adjuvant. GQD were administrated intraperitoneally (10 mg / kg body weight) at different stages of disease (from the day of immunization until the 7th or 28th day post immunization (d.p.i.), and from 7th until the 28th d.p.i. Clinical score of the disease was monitored during next 28 days. For the analysis of inflammatory infiltration and demyelination of the CNS, appropriate histo-chemical methods were used. Quantitative PCR method and flow cytometry were used to examine the expression of proinflammatory cytokines and specific transcription factors. For data analysis Mann Whitney test was used and p value less than 0.05 ($p<0.05$) was considered as statistical significant difference.
Results: GQD administration, in all phases of EAE, significantly reduced clinical score of the disease. Clinical improvement correlates with a decrease of inflammatory infiltrates and demyelination in the spinal cord tissue. Expression of the TH1 cytokine, IFN-γ and its transcription factor (Tbet) was significantly reduced in an infiltrated spinal coard T cells.
Conclusion: GQD reduce neuroinflammatory damage probably by reduction of inflammatory infiltration and inhibition of TH1 responses in the CNS tissue.

METABOLIC ASYMMETRY OF THE HYPOTHALAMUS IN MALE AND FEMALE RATS

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In modern life, a considerable part of human population is affected by certain kinds of reproductive or metabolic disorders. The hypothalamus, as the main integrator of peripheral and central reproductive and metabolic signals, plays a crucial role in keeping the physiological homeostasis that, if disturbed, results in health disorders. It has been established for some time that the left and right sides of the central nervous system are specialized to the regulation of certain specific, but distinct functions. Along with these findings, asymmetry of the neuroendocrine hypothalamus has also been indicated by a limited number of studies, still, the hypothalamus is considered as an unpaired midline structure, in which the two morphologically symmetric sides regulate the exact same biological functions. Hypothalamus-driven homeostatic functions are considerably energy-dependent and therefore rely on mitochondrial ATP-production. The regulated mitochondrial respiration correlating with the actual cellular energy consumption offers the method of measuring mitochondrial respiration rates to directly indicate the intensity (and changes in intensity) of overall functions in hypothalamic regions that are involved in the regulation of homeostatic processes.
We analyzed the metabolic asymmetry between the left and right hypothalamic sides of male and female rats by measuring mitochondrial respiration rates. In all experimental animals, mitochondrial fractions were obtained from the separated left and right hypothalamic sides, then mitochondrial oxygen-consumption was measured. Results separately gained form the left and right hypothalamic sides of individuals were compared to each other. After comparison, we also identified the more active side of the individual (i.e. left or right sided metabolic dominance).
Our results demonstrated that the like-named nuclei on the left and right sides of the hypothalamus might have different roles, as it has been discovered and accepted long ago with regard to higher brain areas. It seems that the left and right hypothalamic sides, even though they are able to regulate the same functions, might act on different activity levels to react to homeostatic stimuli that results in a side-linked dominance. This evolutionary process of lateralization would provide a much more effective use of brain resources. Based on the functional lateralization that we presented here it seems to be rightful to re-name the hypothalamic sides to hypothalamic hemispheres.

This work was supported by grants OTKA104982 to T. Bartha and OTKA81745 and OTKA115613 to A. Zsarnovszky (National Research, Development and Innovation Fund, Hungary); and 11475-4/2016/FEKUT grant of the Hungarian Ministry of Human Resources.

BIOCOMPATIBILITY OF THE SU-8 IN THE CENTRAL NERVOUS SYSTEM

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Multichannel microelectrodes - implanted in the brain for recording, stimulating or drug delivery purposes - are important both in basic science and in clinics. The majority of the neural probes are silicon based. The advantage of the SU-8 material compared with the silicon is that it is more flexible, allowing a smoother coupling with the soft brain tissue.

Despite the widening use of SU-8 nowadays in the production of neural sensors, a detailed systematic quantitative study concerning its biocompatibility in the central nervous system was not performed yet.

In this project, we examined the biocompatibility of the SU-8 photoresist polymer by studying the neuron density near the device surface and by assessing the gliosis surrounding the device. Altogether 62 probes were implanted in the left and right hemispheres of 31 rats for 2 months. After 2 months, the animals were perfused, 60 µm thick horizontal sections were cut from the brains and neurons or glial cells were labeled with NeuN- or GFAP-immunostaining, respectively. Photomicrographs were taken of every section - containing the implant track - at a 10-fold magnification. The neuronal loss near the implant was examined quantitatively by a home-written cell-counting routine developed in Matlab. Neuronal densities were calculated in 20 µm wide regions up to 400 µm on the 4 sides of the tracks. The density values of each sector were normalized to the average values of the 200 to 400 µm regions. The severity of the gliosis around the probe tracks was investigated in qualitative analyses at light and electron microscopic levels.

31 hemispheres were included in the analysis. Track sides with a layer-change within 400 µm or with a defined injury were excluded from the study. The number of the analyzed photos was in average 5-to-6 per hemisphere, altogether 189 sections were evaluated. The density of neurons significantly decreased in the first 20 µm. The average normalized densities were 0.24±0.28 in the 0-20 µm distance and 0.74±0.39 in the 20-40 µm distance. From 40 µm the density of neurons was control-like.

Huge variation could be detected in the severity of gliosis even among sections of the same hemisphere. We examined the brain tissue around the track at the electron microscopic level to check whether the increased staining intensity near the track outline is a result of the increased amount of glial processes. We could detect more GFAP-positive glial processes near the track (40 µm) than in a larger distance (180 µm).

We can conclude, that the preservation of neurons near the device was very good in those cases where there were no big injuries or bleedings. At a 40 µm distance, the tissue was control-like. Our results indicate that SU-8 material enables a better neuronal survival in the close vicinity of the implant than the different types of silicone based probes which cause a significant neuronal loss typically between 50 and 100 microns from the implant surface.

POTASSIUM-DEPENDENT REGULATION OF MICROGLIAL ACTIVITY

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Ion regulation in the extracellular millieu of the brain is maintained by transporters and ion channels, with a key role for Na^+-K^+ ATPase activity. The malfunction of these molecules may lead to excitotoxicity and neuronal cell death. The extracellular accumulation of potassium is related to numerous neuropathological alterations such as spreading depolarization, ischemic stroke or epilepsy. Microglia, the main immune cells in the brain are known to play an important role in the regulation of neuronal activity, however their responses to altered extracellular potassium levels have not yet been examined in detail. The role of microglial potassium transporters in neuroinflammation or in the regulation of neuronal activity is also poorly understood.
To study the relationship between microglial activation, cytokine production and extracellular potassium levels, we performed *in vitro* experiments. Using cytometric bead array (CBA) we have measured the concentrations of 11 inflammatory cytokines and chemokines in primary microglial cell cultures, and compared these results to data obtained from astroglial and neuronal cultures. Our results showed that the cytokine production of microglia and astroglia in response to a proinflammatoy stimulus (lipopolysascharide, LPS) is modulated by extracellular potassium levels. In subsequent investigations we focused on the role of the Na^+-K^+-$2Cl^-$ (NKCC1) cotransporter in the regulation of microglial cytokine production. The selective inhibition of NKCC1 is known to reduce epileptic activity and ischemia-related brain edema, which is usually attributed to neuronal NKCC1 activity. Our results show that microglia cells express functional NKCC1 transporters, since NKCC1-specific inhibitor, bumetanide, significantly reduced the LPS-induced chemokine (CXCL1, MCP-1 and RANTES) levels in primary microglial cell cultures.
Further investigations are needed to explain the mechanisms behind potassium-dependent regulation of microglial activity as well as the cell-specific impact of NKCC1 inhibitors, both *in vitro* and *in vivo*. A better understanding of the role of microglial potassium transporters and ion channels may provide new drug targets in CNS diseases.

EXTRA-HYPOTHALAMIC CRF OVER-EXPRESSION DURING ADOLESCENCE RESULTS IN LASTING ANXIETY AND TRAUMA SUSCEPTIBILITY IN A SEX-DEPENDENT MANNER

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Accumulating evidence suggests that puberty is a developmental period exhibiting marked plasticity and additional sensitivity for environmental stimuli, including stressful events. In line with this, onset and prevalence of anxiety disorders have a peak during this period. Additionally, women are twice as likely to be affected in the development of anxiety and stress-related disorders compared to men. Despite solid epidemiological evidence of the above, mechanisms are still not understood. Corticotropin releasing factor (CRF) is a key regulator of the stress response exhibiting sex-dependent maturation during the peri-adolescent periods. Moreover, elevated CRF levels and specific polymorhisms have been associated with early-life trauma and higher risk to develop posttraumatic stess disorder (PTSD), respectively. The aim of the present study was to test if enhanced extra-hypothalamic CRF signaling during adolescence results in lasting alterations of anxiety-like traits and reactivity for traumatic stressors. To test this, we induced transient forebrain-specific CRF over-expression in male and female double mutant mice (*Camk2a-rtta2* x *tetO-Crf*) during adolescence (postnatal days PND23-44; $CRFOE_{ado}$) and assessed anxiety/avoidance and startle reactivity in adulthood (PND110-) at baseline and following a single traumatic event (predator exposure). $CRFOE_{ado}$ significantly increased anxiety in females, but not in males, indicated by increased avoidance of aversive areas in the open field, light-dark box, and predator-related cues (i.e. cat odor). Traumatic stress further increased anxiety levels in females in an additive manner. Interestingly, startle reactivity and prepulse inhibition was unaltered by $CRFOE_{ado}$, which is in sharp contrast with pre-adolescent CRFOE effects (i.e. hyperreactivity). These findings suggest that CRF hyper-signaling may mediate the long-term anxiogenic effects of adolescent stress in a sex-dependent manner, females exhibiting higher sensitivity for CRF. Moreover, these effects are highly dependent on timing as shown by contrasting phenotypic outcomes following pre-adolescent vs. adolescent CRF manipulations.

CEREBROVASCULAR DYSFUNCTION IN APOB-100 TRANSGENIC MICE

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Apolipoprotein B-100 (ApoB-100) is the major protein component of the low density and very-low-density lipoproteins that are responsible for cholesterol and triglyceride transport from the liver to the peripheral tissues. The ApoB-100 overexpressing mouse strain is a frequently used model of atherosclerosis, as the lipoprotein profile of these mice closely reflects the human plasma lipid profile and they are more susceptible to the cholesterol-enriched diet induced myocardial dysfunction. Previously, we have generated transgenic mice overexpressing the human ApoB-100 protein. ApoB-100 transgenic mice have significantly elevated serum triglyceride level, which induces oxidative stress in endothelial cells of transgenic animals. Electrophysiological recordings on transgenic hippocampal slices revealed disturbed hippocampal synaptic functions. Increased apoptosis of neuronal cells and widespread neurodegeneration were found in the brain of ApoB-100 mice, eventually leading to cognitive dysfunctions.
In the present study we demonstrate morphological and functional alterations of the neurovascular unit in ApoB-100 mice. Permeability of the blood-brain barrier (BBB) was tested by iv. injection of Evans Blue and Na-fluorescein. Neither transcellular, nor paracellular permeabilities were changed in the cortex of the transgenic mice however, obvious changes were found in the hippocampal region, where the paracellular permeability was significantly increased compared to wild types. Gene expression was monitored using real-time PCR in microcapillary fraction of transgenic and wild type brains. The level of occludin, Zoc-1, and caveolin-1 mRNA was reduced to half, while the expression level of claudin decreased slightly. A very prominent decrease was found in the expression of the homeobox gene, Meox-2, and the transporter molecule Mfsd2a. On the other hand, the level of Lox-1, the receptor that facilitates the uptake of the oxidized LDL, has increased significantly. Using transmission electronmicroscopy swollen astrocytic processes around microcapillaries were detected in the transgenic group, indicating edema of glial endfeet. Nearly half of the endothelial cell contacts displayed a discontinuous, disrupted electrondense structure in the transgenic brains, in contrast to the intact electrondense junctions of the wild types. GFAP and vimentin immunostainings also showed marked alterations. In wild type animals the GFAP staining was associated with astroglial cell bodies and brain capillaries as well, while in transgenics the GFAP staining was restricted to cellular pattern. Vimentin immunoreactivity showed a spotted staining pattern along the brain microvessels in the cortex of wild type animals, while this pattern was absent in the transgenic brains. In summary, our results demonstrate, that chronically increased serum triglyceride level leads to impaired BBB function and endothelial dysfunction in ApoB-100 transgenic mice.

DETERMINATION OF THE ESSENTIAL NUMBER OF MOTONEURONS REQUIRED TO PRODUCE FUNCTIONALLY USEFUL HINDLIMB LOCOMOTION

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Avulsion injury of one or more spinal ventral roots induces a critical loss of motoneurons followed by irreversible locomotor function impairment ranging from inadequate limb movement to complete paralysis of the limb. Recent surgical techniques facilitate improvement of limb function; however, the question still remains: how many motoneurons are exactly needed to survive and grow new axons to achieve sufficient muscle reinnervation. The aim of this study was to determine the minimum motoneuron numbers required to reinnervate the denervated skeletal muscles of the limb and produce a functionally satisfactory locomotor pattern. Since none of the commercially available methods and equipment appeared to be able to provide a quantifiable and in-depth analysis of the motor pattern of the whole hind limb, we have developed a sensitive movement recording and analysis system in order to determine the threshold of satisfactory functional reinnervation. Therefore, we combined video-based footprint and hind limb motion analysis to achieve a new and reliable assessment. Sprague-Dawley rats underwent a lumbar 4-5 (L4-5) ventral root avulsion and thereafter their L4 ventral root was reimplanted. The animals received various doses of riluzole treatment (0.4, 0.8, 1.2 and 1,6 mg/kg every second day for two weeks postoperatively) in order to rescue incremental numbers of the damaged motoneurons. Control animals received no treatment. For kinematic analysis we used a custom-made plexiglass runway with a tilted mirror fixed under the floor plate. The rats were trained preoperatively to walk from one end of the runway towards a shelter located at the other end. The locomotor pattern of the rats was recorded with high resolution (1080x720 pixels) and high speed (100 frames/s) digital cameras from both lateral and caudal aspects, simultaneously. We were able to assess nine lateral and two rear-view parameters of the hind limb movement pattern by measuring specific joint angles, footprints and gait parameters in single video frames. Four months after the operation we carried out retrograde tracing with Fast Blue in order to label and count the reinnervating motoneurons. The numbers of the reinnervating motoneurons and the functional improvement was correlated. Our results confirmed that the video-based locomotor analysis system provides detailed and useful information on the pattern and time course of the reinnervation process. Accordingly, a strong relationship between functional restoration of the movement pattern and morphological reinnervation has been detected.

MOLECULAR ASPECTS OF AGE-RELATED COGNITIVE DECLINE: THE ROLE OF CAMKII ACTIVITY IN STRUCTURAL PLASTICITY OF DENDRITIC SPINES

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Most of elderly people suffer for cognitive decline. The molecular processes underlying age-related impairments are still poorly understood but animal models provide the opportunity to study the neurobiological basis of cognitive disability in aging individuals and therefore may indicate the new pathways of successful therapies.
In our study we investigated the molecular and morphological correlates of spatial memory impairment in aged mice. The behavioral experiments conducted in the IntelliCages showed that old mice (18-20-month old) are able to learn the location of the corner with sucrose reward. However, their behavior is perseverative as they tend to prefer this corner even when sucrose is replaced by tap water. In the same paradigm young mice (3-6-month old), easily extinguish preference for the reward corner.
The cognitive rigidity is accompanied by the changes in the expression of synaptic proteins and morphology of dendritic spines in area CA1 of the dorsal hippocampus, an important brain structure involved in spatial memory formation. Old mice, as compared to young animals, show decreased levels of synaptic protein PSD-95 (postsynaptic density protein 95) and activated form of major synaptic enzyme- alphaCaMKII (Ca2+/calmodulin-dependent protein kinase II). Both confocal microscopy analysis of dendritic spines filled with GFP (green fluorescent protein) and Serial Block-face Scanning Electron Microscopy (SBEM) confirm that decreased levels of synaptic proteins is associated with shrinkage of dendritic spines and post-synaptic densities. Furthermore, we observed learning-induced differences in PSD-95 levels and morphology of dendritic spines, between old and young mice. While in young animals training reduced the levels of PSD-95 protein and resulted in shrinkage of postsynaptic densities (PSDs), in old mice PSDs grew. Moreover, old mice completely miss big dendritic spines (>0.2 μm3) with complex PSD, and unlike young mice do not generate during learning neither multiinervated spines (with 2 or more buttons) nor spines with spine apparatus.
Therefore, we hypothesized that age-dependent cognitive rigidity may results from: (1) decreased levels of synaptic proteins or (2) altered training-related remodeling of PSDs. To test these hypothesis we used AAV vectors coding shRNA against PSD-95 to decrease the levels of this protein, mutated form of PSD-95 (S73A) which prevents its CaMKII-driven degradation and CaMKII autophosphorylation deficient mutant mice (T286A). We found that local CA1 silencing of PSD-95 with shRNA impairs memory formation, but it does not result in perseverative behavior. However, overexpression of degradation-resistant PSD-95 and T286A mutation result in increased persistence during spatial memory extinction, as in old mice.
Thus our data indicate that impaired CaMKII-dependent degradation of PSD-95 underlies cognitive decline observed in aged animals.

DISCHARGE PROFILES OF JUXTACELLULARLY LABELLED NUCLEUS INCERTUS NEURONS RECORDED IN ASSOCIATION WITH HIPPOCAMPAL FIELD POTENTIAL IN URETHANE ANAESTHETISED RAT

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Theta oscillations occurring in the mammalian brain are characterized by the frequency of 3-12 Hz. This rhythm is likely to be observed in local field potential in the cortex and hippocampus, with the highest amplitude in the stratum lacunosum-moleculare within the CA1 field. Hippocampal network activity underlying theta oscillations plays an important role in numerous brain controlled functions, such as navigation in space, memory formation or generation of different behavioral states. Recent studies has shown that one of the key elements of the ascending reticular activating system, involved in the induction of hippocampal theta rhythmicity, is nucleus incertus (NI) located in the dorsal tegmental pons. NI is a bilateral structure formed of GABAergic projection neurons, adjacent to the brainstem midline right below the fourth ventricle. A subpopulation of NI neurons containing neuropeptide relaxin-3 gives rise to the relaxinergic system involved in stress response and behavioural activation of the animal. However, our knowledge of nucleus incertus and its involvement in mechanisms of theta rhythm generation remain poorly understood. The aim of our study is to characterize NI neurons on the base of their electrophysiological and also biochemical properties in reference to hippocampal oscillations.
All experiments were conducted on Sprague-Dawley rats under deep urethane anaesthesia which is characterized by sleep-like alternations of the brain state. The juxtacellular technique was used to record electrical activity and label single neurons in the NI. At the same time field potential from the hippocampus was recorded using 32 channel multielectrode arrays. After the electrophysiological experiment recorded and labelled cell was histochemically visualized and its neurochemical content was immunocytochemically determined.
Our results show that electrical activity of nucleus incertus neurons is brain state dependent. In majority of cases (ca. 90%) recorded NI neurons increased firing rate during hippocampal theta oscillations comparing to the slow wave activity (SWA). Only the minor population (ca. 10%) of NI neurons showed opposite relation and could be additionally distinguished by firing bursts at delta frequency during SWA. Action potential firing of majority of NI neurons was theta-locked regardless of their pattern of the activity (irregular, regular or bursting) however our preliminary results did not reveal dominant theta phase preference at the level of the whole population of NI neurons.
Results of this research indicate that neurons of the nucleus incertus show activity patterns that are more complex than has been previously described. Combining electrophysiological characterisation and biochemical identification of these neurons may help us to better understand the mechanisms underlying brain stem derived induction of hippocampal theta oscillations.

Funding: NSC, Poland UMO-2014/15/B/NZ4/04896.

IMMEDIATE AND DELAYED EFFECTS OF NEONATAL INFLAMMATORY PROCESS ON MMP9 AND TIMP1 MRNA EXPRESSION IN THE RAT BRAIN: RESCUE BY REPEATED TRAINING

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Perinatal brain pathologies are known to impair the development of CNS functioning and are involved in the etiology of chronic cognitive dysfunction. These conditions are associated with high production of pro-inflammatory cytokines by the cells of the immune and nervous systems. It is well established that neurons express receptors for pro-inflammatory cytokines, which provides evidence for the functioning of cytokines as neuromodulators. The exact molecular and cellular mechanisms of cytokines in the impairment of brain development have not yet been fully elucidated.
We studied the expression of neuroplasticity-regulating genes matrix metalloproteinase-9 (Mmp9) and tissue inhibitor of metalloproteinases-1 (Timp1) in the medial prefrontal cortex and hippocampus. Wistar rat pups were treated with lipopolysaccharide (LPS; 25 μg/kg i.p., P15, P18, P21), an inducer of pro-inflammatory cytokine synthesis.
Adolescent and adult LPS-treated animals demonstrated increased anxiety-like and decreased exploratory behavior in the open field arena. Impaired learning in the active avoidance task and Morris water maze was also observed. Gene expression of Mmp9 and Timp1 was differentially altered in the cortex and hippocampus of pups vs. adult untrained rats and remained unchanged in rats trained in either learning task, revealing that prolonged pro-inflammatory challenge during early postnatal development negatively affects the plasticity factors involved in memory acquisition in adulthood. These results suggest that an increase in cognitive stimulation might be an effective approach to reduce the negative effects of neonatal immune challenges on brain functioning.

Supported by RFBR 16-34-00316.

THE EFFECT OF ATYPICAL ANTIPSYCHOTIC DRUGS ON THE NEUROTROPHIC FACTORS GENE EXPRESSION IN THE MPTP MODEL OF PARKINSON'S DISEASE

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Neurotrophic factors (NTFs) play a crucial role in the treatment of Parkinson's disease (PD). Atypical antipsychotics (AAP) use in the therapy of PD for elimination of psychosis caused by dopamine agonists. Most commonly used AAP are clozapine and quetiapine. It is known that these drugs can increase expression of the glial cell-line derived neurotrophic factor (GDNF) in glial cells culture. Also there are some controversial data on the effect of AAP on the brain derived neurotrophic factor (BDNF) level. Effect of AAP on the BDNF and GDNF genes expression in context of PD previously had not been studied. Another NTF, CDNF (cerebral dopamine neurotrophic factor), had demonstrated significant therapeutic potential in PD treatment in different animal models. Whether AAP modulate expression of CDNF is also still unknown. So, the aim of our study was investigation of the effects of chronic treatment with AAP clozapine and quetiapine on the BDNF, GDNF and CDNF genes expression in the mouse brain in the pharmacological model of PD. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was used for PD modeling in mice. We have used 5 groups of animals: saline only, MPTP-only, MPTP+saline, MPTP+clozapine, MPTP+quetiapine. MPTP was administered in cumulative dose 68 mg/kg. Clozapine and quetiapine in dose 1 mg/kg were administered in 48 hours after last MPTP injection. Motor behavior of animals in the open-field and rota-rod tests was investigated on 14-th and 15-th days of treatment with AAP respectively. On 17-th day animals were euthanized, substantia nigra (SN), striatum (St) and hippocampus (Hc) were extracted. Both MPTP and AAP failed to produce any significant changes in motor behavior in the rota-rod test as well as in the open-field test. In cumulative MPTP dose less than 80 mg/kg pronounced changes in behavior may be absent due to development of presymptomatic stage of PD. In anyway MPTP treatment caused 50% depletion in tyrosine hydroxylase protein level in the ST ($p<0.001$). MPTP caused significant decrease in the BDNF mRNA level in the Hc ($p<0.001$). Vice versa MPTP alone increased the BDNF mRNA level in the SN ($p<0.05$). MPTP also decreased the GDNF mRNA level in the St ($p<0.001$). At that time, significant increase in GDNF mRNA level ($p<0.05$) in the MPTP+clozapine group in the SN was found. MPTP caused dramatically decrease in CDNF mRNA level in the SN ($p<0.001$). Simultaneously, the CDNF mRNA level was increased after MPTP treatment in the ST ($p<0.001$). In contrast, both clozapine and quetiapine decreased it to normal level ($p<0.01$). Obviously, AAP have a very limited effect on the NTFs genes expression and are unlikely to have a pronounced neuroprotective effect in the PD treatment.

The work supported by the Russian Scientific Foundation (#17-15-01021).

QSAR USED IN EVALUATION OF AGOMELATINE AND MENTHA SPICATA OILS, INTERACTING WITH SERT, D2 AND 5-HT1A RECEPTORS

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Depression and schizophrenia are severe disorders that affect many people [1]. There are several antidepressants and neuroleptics with severe side effects, which may occur at the start of treatment or at changes of dosage. Agomelatine (Valdoxan) is a new generation of melatonergic antidepressant, available for prescription in the EU since 2009 [2]. However there is extremely limited information regarding it's mechanism of interactions on the membrane receptors. Agomelatine is MT1 and MT2 agonist while simultaneously inhibiting serotonin receptors: 5-HT2C [2] and 5-HT2B [4]. Mentha spicata essential oils have 57 compounds and carvone is the major constituent. Although the percentage wildly varies in different geographic areas the other relevant compounds in Mentha oil are limonene, 1,8 cineole, sabinene [5]. In this study we used computational chemistry and bioinformatics methods to predict the possible effects of agomelatine and essential oils from *m. spicata* by interacting with serotonin transporter SERT, dopaminergic D2 and serotonin 5-HT1A receptors. In order to observe the potential effect of agomelatine and *m. spicata* oils in comparison with others widely used psychiatric drugs, we have selected a list of antidepressants and antipsychotics currently interacting with serotonin transporter SERT, dopaminergic D2 and serotonin 5-HT1A and biological activity expressed as pKi was evaluated.

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THE EFFECTS OF INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) TREATMENT ON THE MATERNAL HYPOTHALAMIC PROTEOME

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Adaptation to motherhood is associated with behavioral, neuroendocrine, emotional and metabolic changes. The hypothalamus is a main regulator of the appearance and maintenance of maternal behavior and lactation in the postpartum period. In previous studies, we identified IGF-1 as a common regulator of maternally altered hypothalamic proteins, and an increased maternal IGFBP-3 mRNA level in a preoptic microarray study confirmed by RT-PCR. We also showed that intracerebroventricular injection of IGF-1 inhibited IGFBP-3 which led to increased pup retrieval time suggesting reduced maternal motivation.
To reveal the underlying molecular mechanisms, we performed proteomic analysis on hypothalamic samples between IGF-1 treated and untreated maternal rats on postpartum day 14th. 2D-DIGE minimal stain technique combined with LC MS/MS was used to determine protein level alterations. We identified 31 significantly changed proteins, including 17 increased and 15 decreased proteins. Dyhidropyrimidinsae-related protein 2 (Dpysl2), L-lactate dehydrogenase B chain (Ldhb) and Protein disulfide isomerase A3 (Pdia3) are the proteins with the highest fold changes. Aspartate aminotransferase (Got1), Septin-5 (Sept5) and Actin 2 (Actg1) are the three most decreased proteins. The altered proteins belong to functional clusters including cytoskeleton organization, glycolysis, amino acid biosynthesis, chaperone and neuronal development. We also established molecular connections between the proteins and IGF-1 and performed a common regulator analysis with bioinformatic tools on the Pathway Studio Platform.

Grant support: KTIA-NAP_B -13-2-2014-0004 Program and KTIA_NAP_13-2-2015-0003 program.

MATERNAL SYNAPTIC PROTEOME ALTERATIONS IN THE HYPOTHALAMUS

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The period of motherhood is accompanied with several behavioral, neuroendocrine, emotional and metabolic adaptations in the brain. Although it is established that various hypothalamic networks participate in the maternal adaptations of the rodent brain, our knowledge on the molecular background of these alterations remains seriously limited. In the present study, we first determined that the functional alterations of the maternal brain can be detected at the level of the synaptic proteome in the hypothalamus. To establish synaptic proteome changes associated with motherhood, we isolated synaptosome fractions from the hypothalamus of mother rats and non-maternal control females at the 11th postpartum day. Proteomic analysis by two-dimensional differential gel electrophoresis combined with mass spectrometric protein identification established 26 significant proteins, 7 increasing and 19 decreasing protein levels in the dams. The altered proteins are mainly involved in energy homeostasis, protein folding, and metabolic processes suggesting the involvement of these cellular processes in maternal adaptations. The decrease in a significantly altered protein, complement component 1q subcomponent-binding protein (C1qbp) was validated with Western blotting. Furthermore, immunohistochemistry showed its presence in hypothalamic fibers and terminals in agreement with its presence in synaptosomes. We also found the expression of C1qbp in different hypothalamic nuclei including the preoptic area and the paraventricular hypothalamic nucleus at the protein and at the mRNA level using immunohistochemistry and in situ hybridization histochemistry, respectively. Bioinformatical network analysis revealed that cytokines, growth factors, and protein kinases are common regulators, which indicates a complex regulation of the proteome change in mothers. The results suggest that maternal responsiveness is associated with synaptic proteins level changes in the hypothalamus, and that growth factors and cytokines may govern these alterations. The conclusions of the present work contribute to establishing the molecular alterations that determine different maternal adaptations in the brain. Since maternal changes are models of neuronal plasticity in all social interactions, the reported results can affect a wide field of molecular and behavioral neuroscience.

Grant support: OTKA K116538 and KTIA-NAP_B -13-2-2014-0004 Program.

TRANSGENIC EXPRESSION OF THE HUMAN TRUNCATED TAU INDUCES NEUROFIBRILLARY PATHOLOGY IN A MOUSE MODEL OF SPORADIC ALZHEIMER DISEASE

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Alzheimer's disease is currently the most common neurodegenerative disorder that accounts for 60 to 70 percent of all known cases of dementia. Several preclinical animal models, have been developed in order to test pathophysiological mechanisms of the disease and to predict effects of pharmacological interventions. However, no mouse animal model exists, that develops multiple aspects of neurofibrillary pathology responding directly to sporadic form of Alzheimer`s disease. Therefore, there was a need to generate a novel transgenic mouse model. Our new transgenic mouse line R3m/4 expressing human non-mutated truncated tau protein with amino acid positions from 151 to 391 (3R tau 151-391) reliably recapitulates crucial histopathological features of human sporadic Alzheimer`s disease, detected as hyperphosphorylation of truncated form of tau, its somatodendritic accumulation, formation of neurofibrillary tangles in different regions of the brain with predominant pathology located in the brain stem. We have also detected accumulation of sarkosyl-insoluble complexes consisted of endogenous and truncated pathological tau proteins monitored biochemically. Importantly, the most profound histopathological and biochemical features identified predominantly in brain stem area were accompanied by significant sensorimotor impairment and reduced life span. This novel transgenic mouse model for human sporadic form of AD can serve as a valuable tool for analyzing of therapeutic efficacies of various treatments in preclinical stage.

Keywords: Alzheimer´s disease; tauopathies; transgenic mouse; truncated tau protein; neurofibrillary degeneration

ROBUST AND EFFICIENT CODING WITH GRID CELLS

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The neuronal code arising from the coordinated activity of grid cells in the rodent entorhinal cortex can uniquely represent space across a large range of distances, but the precise conditions for optimal coding capacity are known only for environments with finite size. Here we consider a coding scheme that is suitable for unbounded environments, and present a novel, number theoretic approach to derive the grid parameters that maximise the coding range in the presence of noise. We derive an analytical upper bound on the coding range and provide examples for sets of grid periods that achieve this bound and hence are optimal for encoding in unbounded environments. We show that in the absence of neuronal noise, the capacity of the system is extremely sensitive to the choice of the grid periods irrespective of the number of grid modules. However, when the accuracy of the representation is limited by the variability of the neuronal firing activity, the capacity quickly becomes more robust against the choice of grid periods as the number of modules increases. Importantly, we found that the capacity of the system is near optimal even for random period choices already for a realistic number of grid modules. Our study demonstrates that robust and efficient coding can be achieved without parameter tuning in the case of grid cell representation and provides a solid theoretical explanation for the large diversity of the grid scales observed in experimental studies. Moreover, we suggest that having multiple grid modules in the entorhinal cortex is not only required for the exponentially large coding capacity, but is also a prerequisite for the robustness of the system.

THE CORTICAL PROFILE OF SLEEP SPINDLES IN HUMANS

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It has been hypothesized that different thalamocortical networks with different cortical laminar terminuses contribute to sleep spindles of different frequencies and topographical distributions. In order to evaluate this hypothesis, we assessed the laminar profile of sleep spindles in humans using laminar microelectrode data from 5 epileptic patients undergoing presurgical electrophysiological monitoring with available postoperative histological reconstruction of the electrode track. Sleep spindles were detected in subdurally implanted corticographic grids, allowing for the separation of slow and fast spindles as well as local and widespread (global) spindles. Spindle-frequency local field potential fluctuations on the microelectrode during ECoG spindles were most prominent in the superficial layers. Current source density (CSD) was maximal in layer I-II and IV, consistent with the anatomy of the hypothesized „core" and „matrix" thalamocortical networks contributing to spindle generation. However, CSD was prominent at both depths in both local and global spindles, and CSD magnitude was strongly correlated between superficial layers and layer IV, although less so in local spindles. Fast spindle CSD was greater CSD in layer IV and slow spindle CSD was greater in superficial layers. Both single-unit and multi-unit activity was significantly associated with sleep spindle phases. Our results show that although the frequency and topographical distribution of sleep spindles affects their cortical profiles, these differences are subtle, suggesting similar generating networks for most sleep spindles.

TREATMENT OF PATIENT WITH MODERATE NEUROCOGNITIVE DISORDER

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Patient, 67 years old was treated in our hospital because of agitation, insomnia and because of delusions of reference. The patient described that she thought that members of family were following her and wanted to harm her. She had difficulties with concentration and memory. Brain CT showed diffuse cortical atrophy. The laboratory analysis showed mild elevation of glucose. Considering the clinical presentation and results of diagnostic procedure the treatment with donepezil was initiated and dosage was gradually elevated. Also, treatment with fluphenazine was initiated in dosage of 1 mg in the evening. The first few days of treatment the patient was also treated with diazepam in daily dosage of 5 mg and zolpidem in dosage of 5 mg in the evening. With this therapy the patient was calm, sleep was improved, and delusion of reference were decreased in intensity.

ROLE OF MMP-9 IN SCHIZOPHRENIA-LIKE BEHAVIORS IN RODENTS

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Schizophrenia is recognized by 3 symptoms, classified as positive, negative and cognitive. Positive symptoms may be modelled in experimental animal models by hyperlocomotion, whereas in negative symptoms lack of interest in rewards and problems in social behavior can be demonstrated. Finally, poor working memory may correlate with cognitive symptoms of schizophrenia. Herein, we employed mouse models of schizophrenia for positive, cognitive and negative symptoms and investigated the role of diminished MMP-9 in pathogenesis of schizophrenia in these animals. Mice with genetically lowered MMP-9 levels in heterozygotes (+/-,MMP-9 HET) were employed, along their wild type (WT, +/+) littermates. Since early-life stress is regarded as a factor promoting schizophrenia, we subjected the mice, in some experiments, to daily (for 21 days) encounter with an aggressive conspecific. The results indicate that alterations in the level of active MMP-9 in the brain result in increased sensitivity to locomotor hyperactivity induced by MK-801. On the other hand chronic stress, potentiates negative symptoms of schizophrenia in MMP-9 Het mice such as depressive behaviors and social behaviors impairment. Cognitive symptoms such as poor working memory can be seen in MMP-9 HET control mice. These results support the notion that MMP-9 alterations in brain may play a role in schizophrenia.

NEURONS OF THE SUPERFICIAL SPINAL DORSAL HORN THAT SHOW PHOSPHORYLATED HISTONE 3 AT SERINE 10 UPON TISSUE INJURY- ASSOCIATED PAIN

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Transcriptional changes in superficial spinal dorsal horn (SSDH) neurons are essential in the development and maintenance of prolonged pain. Phosphorylation of serine 10 (S10) in histone 3 (H3) was recently proven to occur specifically in a group of rat SSDH neurons following the activation of nociceptive primary sensory neurons by burn injury, capsaicin application or sustained electrical activation of nociceptive primary sensory nerve fibres. It was also proposed that p-S10H3 is a novel marker for nociceptive processing in SSDH neurons with high relevance to transcriptional changes and the development of prolonged pain.
In the present study we aim to clarify if these transcriptional changes apply to projection neurons, the major output elements of the SSDH circuitry, or if they are restricted to local interneurons. We combined retrograde labelling of SSDH projection neurons from the lateral parabrachial nucleus and from the periaqueductal grey matter with immunocytochemistry to reveal p-S10H3 in lamina I projection neurons at the cervical and lumbar levels. Our results will shed light on to what extent transcriptional changes effect SSDH circuitry upon tissue-injury associated pain.

DETERMINING WHITE MATTER DYSFUNCTION IN ALZHEIMER'S DISEASE BY GENERATION OF A NOVEL HUMAN BRAIN *IN VITRO* MODEL

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Alzheimer's disease (AD) is pathophysiologically characterized by the accumulation of extracellular amyloid-beta plaques and intracellular tau tangles. Recent evidence suggests a close correlation of changes in the brain's blood vessel conditions and dementia including Alzheimer's disease. The cerebral white matter is highly vulnerable to ischemic events and high power imaging studies revealed that small white matter lesions may be a third important feature of AD. Cerebral white matter lesions correlate strongly with cognitive decline and begin to accumulate before first AD symptoms occur and may therefore be important in understanding the disease progression. Understanding the role of hypoxia and hypoglycemia in the cerebral white matter requires focal control of oxygen and glucose levels within the subcortical white matter, which is vastly challenging in the rodent brain. In order to investigate the role of white matter infarcts in AD in a clinically relevant system, we needed to generate a series of new tools: (1) to recreate human cerebral grey and white matter of clinically diagnosed AD patients *in vitro*; (2) to induce *focal* hypoxia and hypoglycemia in the white matter; and (3) a super-resolution method to detect the spreading of AD associated risk factors βAMYLOID and TAU between cells.
We generated human pluripotent stem cells (iPSCs) from clinically diagnosed AD patients and their relatives. We setup derivation methods to differentiate the human iPSCs into cortical neurons, astrocytes, oligodendrocytes and microglia/macrophages. These cell types can be then assembled, co-cultured, in microfluidic devices, and Campenot chambers, and in such to establish a cortical 'grey' and 'white' matter. In order to induce focal hypoxia in the white matter compartment we created a small oxygen scavenging electrode, introduced to the axonal compartment of the Campenot chambers, mimicking focal white matter hypoxia as occurs with ageing. To trace βAMYLOID species in and around the cortical neurons and different chambers of the microfluidic device, we set up a super-resolution imaging method to detect different βAMYLOID species and the exact location in cells and how they spread between cells in the cultures.
This method allows now to directly address whether there is an underlying susceptibility of neural cells derived from AD patients to focal white matter ischemia and whether there are alterations in βAMYLOID species, between patient lines and control, both before and after focal white matter ischemia.

EFFECTS OF DIFFERENT CONTEXTUAL AND CONDITIONAL CUES AND POST-CONDITIONING COGNITIVE TRAINING ON FEAR EXPRESSION AND EXTINCTION IN RATS

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Fear conditioning is a well-known memory test, where the unconditioned stimulus can be linked to the context or to auditory or visual cues. We investigated the effects of 1) different shocking environments, 2) conditional cues and 3) engagement in cognitive tasks between retention sessions on fear expression and extinction in rats.
The study was conducted on male Wistar rats. Fear conditioning was carried out in a shocking chamber placed into a sound attenuating cube. The shocking environment could be changed by attaching, differently patterned plastic sheets to the walls. Freezing behavior was observed with a camera mounted inside the chamber. Daily test sessions were 5 minutes long. In the acquisition session, six 1 s 0.6 mA foot shocks were delivered, separated by 30 40 s random length intervals. The 10 s conditional cues were delivered before the shocks and their last second overlapped with shocking. In retention sessions shocks were not delivered.
In the first experiment, rats were tested in 6 situations. In the "light" setting the chamber was constantly illuminated, in the "light + sound" setup audible sound cues were presented. In the "dark" setting the chamber was not illuminated and in the "dark+sound", "dark+light", "dark+sound+light" paradigms an auditory, a visual cue and a combination of the two were presented, respectively. The control did not receive shocks. The experiment started with an acquisition session followed by 5 retention sessions, and another shocking session for reinstatement.
With the exception of the "light" paradigm which induced a very low level of freezing, groups did not differ significantly (first retention day, "light": 50.3 s, other groups: 115.5-196.8 s). Freezing was highest on the first retention day and fear response gradually extincted during the consecutive retentions, and increased again on the reinstatement session.
In the next experiment, the acquisition session was followed by two retention sessions at 24 hours and day 28. A 2 × 2 design was applied with the following treatments: 1.) the chamber walls were the same or different in the retention sessions than in acquisition, 2.) handling and cognitive tasks were conducted between the retention sessions, while the other group remained undisturbed.
Changing the walls had no significant effect on fear response (first retention day, different: 133.2 s, same: 184.0 s), whereas engagement between the retention days decreased freezing (undisturbed: 77.3 s, engaged: 7.3 s, $p<0.05$).
Our experiments show that the intensity of the acquired fear response was minimally influenced by the properties of the shocking environment and the applied cues. Engagement in positively rewarded tasks between retention sessions reduced fear response. However, further experiments are needed to determine whether it was due to attenuating fear memory or reduction of anxiety or both.

Study was funded by: Hungarian National Brain Research Program (NAP), contract # KTIA_NAP_13-2014-0015.

FUMONISIN B1 ALTERS THE SENSITIVITY OF NEURONAL NETWORKS AFTER ACUTE TREATMENT

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Fumonisin B1 (FB1) is a mycotoxin produced by microscopic moulds (mostly Fusarium species) which infect our major crops. The toxin inhibits the development of these plants but has also harmful effects on the animals or humans consuming the infected crops.
The main mechanism of action of FB1 is the alteration of sphingolipid biosynthesis which leads to impaired membrane characteristics and thus altered neuronal functions. It has been known that the toxin has inhibitory effects on neuronal network activities in case of chronic consumption, but according to new calcium imaging data, it has also acute excitatory effects on neural cell cultures. After these studies, we decided to examine with electrophysiological methods whether FB1 alters neuronal network activities ex vivo or in vivo after acute treatment.
In order to carry out ex vivo experiments, we treated rat brain slices with artificial cerebrospinal fluid (ACSF) containing the toxin in three different concentrations (10 μM, 50 μM and 100 μM) for 30 minutes. After the treatment we investigated electrically evoked field potentials in the hippocampus and spontaneous activity in the neocortex.
In the hippocampus, during the stimulation tests right after the treatment, the toxin did not have significant effects on the neuronal network activities in the two lower concentrations, while in case of the highest concentration, we observed an increase in EPSP slope and population spikes amplitude. However, in case of LTP induction, the highest dose had an initial excitatory effect which was reversed later due to the toxin-evoked depletion of neuronal networks, presumably.
In the cortex, during stimulation tests, similar results were seen as in the hippocampus since low and medium concentrations had lesser effect on the neurons, while the highest concentration increased the evoked potentials significantly. In convulsant (Mg^{2+}-free) medium, the increase of the toxin concentration was found to be clearly excitatory on spontaneous epileptiform activity. FB1 treatment causes longer-lasting bursts which appear with shortened latency and higher amplitude.
For the verification of ex vivo results in an in vivo model, effects of systemic administration of FB1 (7.5 mg/kg, i.p.) on evoked field potentials recorded from the somatosensory cortex after electrical stimulation of the tibial nerve are tested in urethane-anesthetized rats. These experiments are running currently.
Altogether we can say that the toxin enhanced the basic excitability of neuronal networks in brain slices but inhibited basic processes that underlie learning and memory formation seen through the decrement of LTP due to the exhaustion of neurons. Pathological, epileptic events were also stimulated, parallel with the increasing excitability of neurons.

Supported by National Research, Development and Innovation Fund, grant number: 16-1-2016-0016.

CONTEXT-DEPENDENT EFFECTS OF EARLY LIFE ADVERSITIES ON BEHAVIORAL AND NEURAL PLASTICITY OF JUVENILE ZEBRAFISH (DANIO RERIO)

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Early life environment substantially influences the physiological and behavioral phenotype due to neural plasticity peaks in sensitive developmental periods. However, it is still not well understood how stressful early experiences support the development of resilient or vulnerable neuronal states. Here we present a novel zebrafish paradigm to study early-life neural and behavioral plasticity during the first month of development. We characterized an extensive behavioral metamorphosis, including the maturation of anxiety-like and social behavior, coinciding with a highly sensitive and critical developmental time-window. We applied acute and chronic emotional stressors during this sensitive period: 2 weeks of social isolation exerted robust long-term behavioral effects. Isolated animals showed lowered novelty-induced but enhanced social anxiety, while both effects were prevented by the acute administration of the anxiolytic 5-HT1a partial agonist buspirone, implying the role of serotonin signaling in this phenomenon. To unravel the impact of serotonin and investigate possible correlations in multiple levels of neural plasticity and behavioral changes, we aim to apply immunohistochemical measurements in brain sites involved in the modulation of emotionally motivated behavior. According to our data, we hypothesize that facing stressful stimuli during early development has a context-dependent adaptive value, as individuals are able to prepare to and cope with specific environmental challenges more efficiently, while fail in response to others.

EFFECTS OF PITUITARY ADENYLATE-CYCLASE ACTIVATING POLYPEPTIDE AND RADIATION ON BREAST CANCER CELL LINES

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Breast cancer is one of the most common cancers, it affects every 8 women. It is necessary to find additional treatment options. Hypophysis activating polypeptide has pro- and antiapoptotic effects, previous studies showed the peptide and its receptors presence in breast cancer biopsy samples.
Methods: In our examination we applied breast cancer adenocarcinoma cell lines, MCF-7 and MDA MB231. In our project we examined the effect of PACAP1-38 and PACAP6-38 (PAC1 receptor antagonist) in different concentrations. Nearly half of breast cancer patient receive radiotherapy, in second part of our study we examined how can PACAP1-38 and 6- 38 treatment influence the effectiveness of radiation. We used 5 different concentration value of PACAP 1-38 and 6-38. We measured MTT test to examine cell viability. We applied radiation dosages which usual in clinical and experimental conditions: 0,5Gy; 1Gy; 2 Gy; 4 Gy. The effects of radiation were tested on colony formation assays. The effects of PACAP1-38 and 6-38 and radiation were examined with R&D „Human Apoptosis Array kit" and „Cell Stress Array kit"
Results: We detected significant cell viability decrease after PACAP1-38 and 6-38 treatments with MTT assays. The effectiveness of radiation was enhanced with the two forms of the peptide. We measured significant cell number decrease with colony formation assays. We detected fluctuation in the expression fluctuation numerous cytokines, chemokines and bioactive factors which effects the cell physiology.
Discussion: Our future aim is to find additional therapeutic options in the treatment of breast cancer. We plan to examine the effect of PACAP and other factors which can influence the cell viability correspondingly PACAP.

Supported by: EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, GINOP-2.3.2-15-2016-00050 ''PEPSYS, MTA-TKI, KTIA_13_NAP-A-III/5, TÁMOP 4.2.4.A/2-11-1-2012-0001 „Nemzeti Kiválóság Program", MTA TKI, TÁMOP 4.2.2.A-11/1/KONV-2012-0024, ÚNKP-16-2-I. New national excellence program of the Ministry of Human Capacities, the National Research, Development and Innovation Fund K119759. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

HYPOCRETIN (OREXIN) SIGNALING IS CRITICAL IN SUSTAINING THETA/GAMMA-RICH WAKING BEHAVIORS THAT DRIVE SLEEP NEED

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The two hypocretin (orexin) neuropeptides (hereafter referred to as 'Hcrt') have potent neuromodulatory activity in a variety of neural circuits important for motivated and survival behaviors. They were shown to be critical for the stability of behavioral states, although the mechanisms underlying this, and the symptoms that emerge in their absence in narcolepsy, are not well understood. We showed that while *Hcrt*-KO mice respond to 6-h sleep-deprivation (SD) with a slow-wave-sleep (SWS) electrocorticographic (ECoG) δ oscillation rebound as powerful as *WT* controls, spontaneous waking fails to induce a subsequent SWS δ power reflecting prior waking duration. This correlates with impaired θ (6.0-9.5 Hz) and fast-γ (55-80 Hz) activity in spontaneous wakefulness. We algorithmically identified a theta-dominated-waking substate (TDW) underlying motivated behaviors, and typically preceding cataplexy in *Hcrt-KO* mice. *KO* mice fully implement TDW when waking is enforced, but spontaneous TDW expression is greatly reduced, due mainly to reduced TDW bout duration. A reformulation of the classic sleep homeostasis model, where homeostatic pressure rises exclusively in TDW, rather than in all waking, predicts δ power dynamics both in *KO* and *WT* mice, baseline and recovery SWS. The low homeostatic weight of *KO* mice' spontaneous waking correlates with decreased cortical expression of neuronal activity-related genes (*Bdnf*, *Egr1*/*Zif268* and *Per2*). Baseline TDW stability therefore relies on Hcrt to sustain θ/fast-γ network activity and associated neuronal plasticity, while other arousal circuits sustain TDW during SD. We propose that TDW identifies a discrete brain activity mode which is regulated by context-dependent neuromodulators, and acts as major driver of sleep homeostasis. Hcrt loss causes impaired TDW maintenance in baseline waking, and blunted δ power in SWS, reproducing respectively, narcolepsy excessive daytime sleepiness, and poor sleep quality.
To tackle the circuits mediating these effects, we generated conditional KO (cKO) alleles of *Hcrtr1* and *2* receptor genes and selectively inactivated the receptors in Noradrenergic (NA) and Dopaminergic cells. These mice show specific defects in adapting brain electrocortical activity to changing behavioral contexts. The electrocorticogram (ECoG) of NA cell-specific *Hcrtr1* cKO (*Hcrtr1*$^{Dbh\text{-}CKO}$) mice was examined in distinct behavioral paradigms. While the baseline waking ECoG was almost normal, exposure to challenging contexts led to more profound spectral changes. Our data evidence the role of Hcrt-to-NA signaling in building an appropriate θ/fast-γ response in stress-associated environments, a slowing of the ECoG in its absence, but also behaviors in which Hcrt-to-NA signaling may serve to curb hyperarousal. Furthermore, we show that altered waking causes alterations in ensuing SWS quality, with a selective deficit in the slow-δ oscillatory component following wakefulness with a deficit in θ/fast-γ activity.

SYSTEMIC INFLAMMATION ALTERS NEUROINFLAMMATION AND OUTCOME AFTER CEREBRAL ISCHEMIA IN STROKE PATIENTS AND EXPERIMENTAL ANIMALS

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Objectives: Systemic inflammation contributes to unfavourable outcome in patients with cerebrovascular disease, but the mechanisms involved are poorly understood. The central effects of systemic inflammation on neuroinflammatory changes after stroke in patients has not been previously investigated and how it compares to the changes seen in experimental animals are also unclear.
Methods: To investigate the potential cerebral effects of peripheral inflammation, patients presenting with ischemic stroke and elevated systemic inflammatory burden (increased total white blood cell count, elevated erythrocyte sedimentation rate, C-reactive proteinlevels and/or evidence of infection) were compared with stroke patients without an evidence of elevated systemic inflammatory burden at admission. Immunohistochemistry to detect neuronal injury and markers of inflammation was performed on post mortem, paraffin embedded brain tissues. Data from clinical studies were compared with those from mice with or without systemic inflammation preceding experimental stroke.
Results: In stroke patients, evidence of systemic inflammation at admission was associated with increased recruitment of granulocytes and microglia / macrophages in the area of the infarct and the peri-infarct zone. Blood leukocyte levels at admission, but not at later time points predicted the extent of inflammatory cell recruitment in the brain parenchyma after stroke, with stronger association with microglia / macrophage numbers as compared to granulocytes.Interestingly, systemic inflammatory burden positively correlated with survival in stroke patients.In mice, systemic inflammation preceding experimental stroke resulted in worse neurological outcome, which was associated with increased microglial activation, granulocyte recruitment andlarger BBB injury. However, elevated systemic inflammatory burden is associated with more prolonged neuroinflammatory responses in the human brain compared to that seen in experimental animals.
Conclusions: To our knowledge, this is the first study to systematically evaluate inflammatory changes in the human brain induced by systemic inflammation and stroke. Our data suggest systemic inflammatory actions could contribute to neuroinflammation in patients after stroke, which could be therapeutically targeted.

ANALYSIS OF SPINAL NEURONAL NETWORKS CONTROLLING FORWARD AND BACKWARD LOCOMOTION

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Locomotor behavior is vitally important for the animals as a basic form of subject's progression. Higher vertebrates including humans are capable not only of forward locomotion but also of walking in different directions relative to the body axis (backward, sideward, etc.). While the neural mechanisms responsible for the control of forward (FW) locomotion were studied in considerable detail, the mechanisms controlling steps in other directions are mostly unknown. The purpose of the present work was to investigate the distribution of spinal neuronal network controlling forward (FW) and backward (BW) locomotion. We performed electrophysiological recording of cats (n=15) decerebrated at precollicular-postmammillar level that were FW and BW walking after epidural stimulation (ES) of various lumbarsacral segments. Then, the c-Fos immunostaining was used for defining the activating interneuronal population during FW vs BW locomotion in several cats (n=8). We found that the neuronal network affecting control of FW locomotion was distributed broadly in the spinal cord and could be triggered by ES of all tested spinal segments from L3 to S2. Whereas the neurons participated to BW locomotion were activated only in confined position from caudal L5 to caudal L7 with the peak in L6 spinal segment. The pattern of c-Fos immunopositive cells distribution at frontal sections was in general similar in BW and FW stepping cats: we observed three gray matter regions contained distinct groupings of c-Fos+ neurons. The first region is located in the lateral dorsal horn of segments L3-S2 (laminae I-VI), and can be related to the sensory processing in this area. The second region is located at laminae VI, VII, and X boundary, in segments L4-S1. It was shown earlier that the neuronal populations of this region are involved in the regulation of posture and locomotor movements. The third region is located in ventromedial area of segments L2-L5 overlapping with the lamina VIII that contains commissural neurons and also associated with central pattern generating elements responsible for ipsilateral flexor-extensor and left-right alternation. Some differences were observed between cats stepping FW and BW: in BW cats more c-Fos+ neurons were located within the second region (at laminae VI, VII, and X boundary) in segments L6-L7. Data obtained by combination of neurophysiological and immunohistochemical approaches confirm that interneurons involved in the control FW and BW stepping are distributed non-equally within the spinal cord gray matter.

The study was supported by the Russian Foundation for Basic Research (RFBR grant #16-04-01791 and RFBR grant #7-04-01822), and by Russian Science Foundation (RSF grant #14-15-00788).

NEUROTOXIC EFFECTS OF CATIONIC PAMAM DENDRIMERS AND ITS REVERSION BY SURFACE FUNCTIONALIZATION

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Drug nanocarrier systems has been tested as a potential tool to overcome the different problems of neuropharmacology. One of the most promising polymers studied are polyamidoamine (PAMAM) dendrimers, which are hyperbranched macromolecules organized from a core that gives way to expansive growing layers, terminating in a surface of primary amines positively charged at physiological pH. A key aspect to study for their application in effective therapies is their biocompatibility. In this sense, it has been described that the great number of surface positive charges induce cytotoxic effects in different cell types.
In accordance with this, we focused in the study of the possible alterations induced by these compounds in physiological neuronal activity and the possibility to revert their toxic effects by surface chemical modifications. To this aim, in this report we studied the effects in hippocampal neurons of fourth generation PAMAM dendrimers with a complete positive charged surface (G4) compared with dendrimers modified in a 25% of their terminal groups with folate (PFO25) and polyethylene glycol (PPEG25), two common molecules used to functionalize dendrimers.
The results show that G4 dendrimers induce high cytotoxicity in cell viability assay, which is attenuated by both chemical modifications, being PPEG25 the most biocompatible of them. By the other hand, patch clamp studies were performed loading the register pipette with different dendrimers as in the perforated patch clamp techniques and registering capacitive currents during 30 minutes. G4 dendrimer treatment shows a significant increase in membrane charge transferred of capacitive currents after 10 to 15 minutes of registration, which demonstrate an increase of membrane permeability and suggest a loss of its integrity. For the case of PFO25 and PPEG25 it was not observed significant changes in capacitive currents. Thereafter, the effect of dendrimers in Ca2+ transient increments was studied and results show a significant increase of intracellular Ca2+ with a complete disruption of normal pattern of transients for G4 treatments, whereas for both PFO25 and PPEG25 it was possible to observe a normal pattern of transients but with an increase of its frequency. Finally, it was studied the influence of these effects in synaptic activity by patch clamp registers. Results show a significant increase of frequency of for G4, but not for PFO25 and PPEG25 treatments. No significant differences were observed in amplitude for any treatment.
In conclusion, these results demonstrate that cationic G4 dendrimer induces excitotoxicity in neurons which would be generated by the disruption of membrane integrity and subsequent increase of its permeability leading to the increase of intracellular Ca2+. In addition, it has been demonstrated that these toxic effects are reverted by the modification of 25% of PAMAM surface with folate and polyethylene glycol.

AUTOPHAGY AND APOPTOSIS MODULATION AS MECHANISM OF DETERIORATING EFFECT OF PROTON PUMP INHIBITORS ON EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Background: Multiple sclerosis (MS) is chronic demyelinating disease of CNS. In MS, proton pumps inhibitors (PPI) are used to prevent gastrointestinal mucosa damage during corticosteroid therapy in the disease relapse. It is known that PPI modulates autophagy, the process of intracellular digestion, which can have immunomodulatory effects. However, effects of PPI in neuroinflammation were not investigated so far.
Objective: The objective of this study was to investigate effects of PPI on experimental autoimmune encephalomyelitis (EAE), animal model of neuroinflammation, and its effects on autophagy and apoptosis induction.
Methods: Dark Agouti rats, 8 weeks old, were immunized with spinal cord homogenate and complete Freund's adjuvant. Rats were divided into four groups: 2 control (untreated) groups and 2 experimental groups treated with PPI, Pantoprazole (Controloc), intraperitonealy (20mg/kg of body weight). The first experimental group was treated with controloc from the day of immunization until 7th day after immunization (d.a.i), for analysis of Controlocs' effects in the inductive phase of EAE. This rats were sacrificed 7th d.a.i, together with one group of untreated immunized rats and healty, non-immunized rats. Popliteal lymphatic nodes were taken from these rats and their proteins were isolated, quantified and prepared for further analysis. The second experimental group was treated with controloc from the day of immunization until 21st d.a.i. and rats were further observed until 40th d.a.i. for clinical score of EAE investigation. Treated rats were compared with another untreated immunized group of animals. Immunoblot analysis of popliteal lymph node homogenate was used to investigate potential immunomodulatory effect of PPI on autophagy and apoptosis induction, and mechanisms involved in this process. Primary antibodies for pAMPK, tAMPK, Actin, p62, NBR-1, pmTOR, tmTOR, ULK-1, PARP, Casp3, GADD, XBP-1(Cell signaling) were used.
Results: Our results revealed that controloc significantly deteriorate clinical signs of EAE. Comparing to healty, non-immunized animals, EAE rats had increased levels of p62 and NBR-1, cargo receptors that are selectively degraded in autophagy. Additionally, phosphorilation of autophagy activating kinase, AMPK was decreased, while phosphorilation of autophagy inhibitors, mTOR and ULK-1 was increased in EAE rats. Autophagy inhibition was in correlation with PARP and Caspase activation, apoptosis markers, and increased expression of GADD and XBP-1, endoplasmic reticulum stres markers. PPI treatment increased AMPK phosphorilation, and decreased mTOR and ULK-1 activation, p62 and NBR1 accumulation, PARP and Caspase activation and GADD and XBP-1 expression.
Conclusion: PPI have negative effect on clinical score of EAE most probably. through autophagy activation which is in corelation with apoptosis and ER stress inhibition in the inductive phase of the disease.

Keywords**:** EAE, autophagy, apoptosis, ER stress

HIPPOCAMPAL NOS-1 INHIBITION INTERFERS WITH COCAINE SENSITIZATION EXPRESSION AND SYNAPTIC TRANSMISION

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Repeated administration of psychostimulants such as cocaine (COC) causes progressive increases in locomotor activity, called “behavioural sensitization” that underlies drug-seeking behaviour and relapse. The hippocampus (HP) is a brain region implicated in associative learning processes that occur during addiction. A major form of synaptic plasticity in HP is long-term potentiation (LTP) characterized by an enduring increase in the efficacy of glutamatergic synaptic transmission. Nitric Oxide (NO) is a neurotransmitter that participates in HP synaptic plasticity and in behavioural effects of COC. Results from our group showed a key role of the NOS-1/NO/sGC/cGMP pathway in the development of COC sensitization and in the associated enhanced HP synaptic plasticity, because inhibition of NOS-1 during COC administration prevented sensitization. The aim of this work is to evaluate if NOS-1 inhibition after development of COC sensitization was able to reverse its expression, and to characterize the HP participation in this phenomenon. To this purpose, male Wistar rats were administrated with COC (i.p. 15 mg/kg/day) or saline (SAL) for 5 days, one group was sacrificed 60 min. after last administration; a second group received the NOS-1 inhibitor 7-nitroindazole (7-NI, 50 mg/kg/day) or vehicle (VEH) for the following 5 days; and a third group received a single intra-HP 7-NI (16.31 µg/µl) or VEH infusion. The last two groups received a COC or SAL challenge 24 hs after the last 7-NI or VEH administration. To evaluate sensitization expression, locomotor activity was measured on days 1, 5 and in the challenge day. To measure HP synaptic transmission, the threshold to generate LTP was assessed by multi-unitary extracellular recordings on day 5 and in the challenge day. In addition we quantified NOS-1 protein levels by western blot, and NOS-1 and the cAMP response element-binding (CREB) gene expression by RT-PCR. Our results show that COC sensitized animals expressed increased NOS-1 protein and gene expression, CREB levels, and enhanced HP synaptic transmission on day 5. Furthermore, systemic or intra-HP NOS-1 inhibition reversed sensitization expression and the associated enhanced HP synaptic plasticity. We conclude that HP has a fundamental role in COC sensitization expression, emphasizing the NO participation in this phenomenon, and the interference of NO signaling pathways could be considered as pharmacological target to treat drug addiction and/or relapse.

THE ROLE OF SLEEP SPINDLES IN OVERNIGHT VERBAL MEMORY RETENTION IN TEMPORAL LOBE EPILEPSY PATIENTS

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Declarative memory performance and hippocampal functioning are highly associated in the healthy population. Learning induced memory consolidation causes increased coupling between medial temporal lobe (MTL), thalamus and frontal areas during sleep, resulting in an increase of sleep spindles and SWS (active system consolidation, Diekelmann et al., 2010). Our aim was to investigate the modulatory effect of learning on sleep spindles in temporal lobe epilepsy (TLE) patients and to see whether the association between declarative memory consolidation and sleep parameters are altered or intact in epilepsy.
We administered a modified version of the Rey Auditory Verbal Learning Task (RAVLT) to TLE patients (n=22) undergoing presurgical epilepsy evaluation. Baseline sleep EEG was measured prior to learning nights. Learning was measured on three consecutive evenings. Delayed recall was measured 30 minutes after learning and memory retention was measured in the morning, after waking. Sleep stages were detected manually, sleep spindles were detected with an automated threshold-cutting method based on individually adjusted slow and fast sleep spindle frequency values.
In summary, most patients show a relatively intact learning curve on the majority of the experimental evenings, and normal retention rates on the following mornings. Intra-individual variance of sleep spindle parameters show a rather stable pattern throughout consecutive learning nights. Inter-individual differences in the laterality of the epilepsy, medications during the VEEG monitoring, and the comparison between retention rates, sleep spindle density, duration and amplitude are still under analysis.

ANTAGONISM PROVIDES BALANCE - INVESTIGATION OF EXCITATORY AND INHIBITORY MODULATION OF DOPAMINERGIC NEURONS' ACTIVITY

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Dopamine (DA) synthesizing neurons within ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) of the mammalian brain form the core of reward and motivation system. Tonic release of DA into target structures supports animals' basal motivation and motor functions, while phasic increase of released DA signals reward and induces synaptic plasticity. Different modes of DA release arise from changes in dominant firing pattern of dopaminergic neurons. Altered electrical activity of DA neurons is involved in the pathogenesis of drug addiction, depression and several other psychiatric diseases. Level and pattern of DA neuron activity derives from balance between excitatory (mainly glutamatergic and cholinergic) and inhibitory (GABAergic) inputs. Glutamatergic and cholinergic agonists increase firing rate of DA neurons and augment release of dopamine in the forebrain. Although NMDA receptor is believed to be crucial for induction of DA neuron bursting activity, existence of NMDA-independent mechanisms underlying this activity pattern can't be excluded.
Our study was aimed to determine how disruption of GABAA receptor will affect electrical activity of DA neurons and what is the role of cholinergic modulation in development of burst firing of DA neurons. We have used two genetically modified strains of mice: one with selective, inducible knock-out of gamma-2 subunit of GABAA receptor (Gabrg2) and one with inducible deletion of NR1 subunit of NMDA receptor. In this way we could control time and target of deletion of selected receptor subunit. Extracellular *in vivo* recordings of DA neurons activity were conducted on urethane anesthetized animals and combined with iontophoretic drug application. Responses of VTA and SNc dopaminergic neurons to administration of bicuculline, muscimol (GABAA antagonist and agonist, respectively) and carbachol (non-selective cholinergic receptor's agonist) were tested. We have observed differences between mutant and control animals - both in baseline activity and in responses bicuculine and muscimol. DA neurons lacking Gabrg2 had increased spontaneous firing rate and displayed reduced responsiveness to GABAA receptor compounds. During experiments with carbachol administration we found that majority of recorded neurons in both experimental groups responded with increase of firing rate to iontophoretically applied drug. Surprisingly, subpopulation of neurons, both in controls and in mutant animals developed robust, prolonged bursts under the influence of carbachol.
Our results show that despite deletion of gamma-2 subunit DA neurons can still form functional GABAA receptors, however such alternation modulates activity of dopaminergic neurons. Our results from NR1 subunit knock-out animals show that activation of cholinergic receptors can be sufficient to switch DA neurons to bursting mode of firing in NMDA receptor independent manner.

Funding: NSC, Poland, PRELUDIUM 2015/19/N/NZ4/00960.

CORRELATION BETWEEN ELECTRICAL PERCEPTUAL AND PAIN THRESHOLDS (EPT/EPP) AND THERMAL COMPONENTS OF QUANTITATIVE SENSORY TESTING (QST)

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Electrical perceptual threshold (EPT) and electrical pain threshold (EPP) testing measure cutaneous sensibility and pain, respectively. However, the pathways mediating these electrically evoked responses are poorly understood. Research to date has compared and found weak correlations between EPT and mechanical detection thresholds (von Frey filaments) suggesting preferential activation of large diameter afferents and the dorsal column tract. However, a detailed comparison between EPT and EPP to a comprehensive battery of quantitative sensory testing (QST) measures has yet to be undertaken.
In the present study, we investigated the intra-rater reliability and relationship between EPT and EPP and each component of QST (cold and warm detection thresholds, cold and heat pain thresholds, mechanical detection threshold, vibration detection threshold and pressure pain threshold) in 17 healthy volunteers across select lower limb dermatomes (L3, L4, L5 and S1). Differences in thresholds across dermatomes were analysed using one-way ANOVA with Holm-Sidak multiple comparison post-hoc tests and expressed as mean +/- SEM. Relationships between EPT/EPP and QST were determined using Pearson's correlation coefficient. Intra-rater reliability was determined using intra-class correlation coefficient (ICC).
Similar decreases in proximal to distal dermatomal sensitivity in EPT (S1 > L3- P= <0.001; S1 > L4- P= 0.015; L5 > L3- P= 0.016) and warm perception thresholds (S1 > L3- P= <0.001; S1 > L4- P= <0.001; L5 > L3- P= <0.001; L5 > L4- P= 0.021; L4 > L3- P= <0.001) were observed. Furthermore, significant correlations between warm perception and EPT in individual dermatomes as well as when data from all dermatomes were combined (L4- R=0.56 and P=0.023; all dermatomes- R=0.53 and P<0.001) were found. Similarly, EPP had a significant correlation with cold pain threshold across dermatomes (R= -0.36 and P=0.035). EPT and warm detection threshold testing was found to have similar intra-rater reliability scores (EPT ICC: 0.734; warm ICC: 0.737).
These data demonstrate that EPT and EPP show strong correlations with thermal sensory pathways. Furthermore, EPT and EPP may induce activity in small diameter, thermo-responsive afferents. Based on these findings, further research is required to determine whether EPT and EPP may be used to monitor spinothalamic tract integrity in spinal cord injured subjects. EPT may also provide a novel, reliable and semi-automated method of assessing small fibre function in patients with lumbar radiculopathy.

INTERICTAL FUNCTIONAL CONNECTIVITY IN PATIENTS WITH UNILATERAL MESIAL TEMPORAL LOBE EPILEPSY

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In this study, we aimed to find electrophysiological evidence that high frequency interictal functional connectivity of the mesial temporal lobe in unilateral epilepsy (MTLE) can lateralize the affected hemisphere. In order to acquire precise electrophysiological signal from the mesial temporal lobe, a semi-invasive technique (foramen ovale; FO) was used that can capture brain mechanisms invisible to the scalp EEG. Our analysis was restricted to gamma frequency band, since higher frequency oscillations are often related to epileptic activity. Twelve unilateral (7 left and 5 right) epileptic patients were involved in this study. Five separate, one minute long, interictal EEG recordings were analyzed for each patient. Phase synchronization (phase lag index; PLI) was applied to measure functional connectivity in gamma (30-45 Hz) frequency band. PLI within the FO contacts (ipsi- and contralateral) and between the FO contacts and scalp electrodes was determined. We have found a prominent increase (11 out of 12 patients) in the local synchrony of the FO contacts in the affected side compared to the healthy side. Furthermore, the right FO contacts showed an increased synchronization with the ipsilateral temporal scalp electrode in patients with right MTLE compared to left MTLE patients. Our results are in line with previous scalp EEG and intracranial EEG researches indicating that increased interictal synchronization especially in higher frequency bands is a robust indicator of the epileptic zone.

PASSAGE THROUGH THE OCULAR BARRIERS AND BENEFICIAL EFFECTS IN RETINAL ISCHEMIA OF TOPICAL APPLICATION OF PACAP1-38 IN RODENTS

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The neuropeptide pituitary adenylate cyclase activating polypeptide (PACAP) has two active forms, PACAP1-27 and PACAP1-38. Among the well-established actions are PACAP's neurotrophic and neuroprotective effects, which have also been proven in models of different retinopathies. The route of delivery is usually intravitreal in studies proving PACAP's retinoprotective effects. Recently, we have shown that PACAP1-27 delivered as eye drops in benzalkonium-chloride was able to cross the ocular barriers and exert retinoprotection in ischemia. Since PACAP1-38 is the dominant form of the naturally occurring PACAP, our aim was to investigate whether the longer form is also able to cross the barriers and exert protective effects in permanent bilateral common carotid artery occlusion (BCCAO), a model of retinal hypoperfusion. Our results show that radioactive PACAP1-38 eye drops could effectively pass through the ocular barriers to reach the retina. Routine histological analysis and immunohistochemical evaluation of the Müller glial cells revealed that PACAP1-38 exerted retinoprotective effects. PACAP1-38 attenuated the damage caused by hypoperfusion, apparent in almost all retinal layers, and it decreased the glial cell overactivation. Overall, our results confirm that PACAP1-38 given in the form of eye drops is a novel protective therapeutic approach to treat retinal diseases.

Supported by GINOP-2.3.2-15- 2016-00050 "PEPSYS", the National Research, Development and Innovation Fund K119759, MTA-TKI, PTE AOK KA Research Grant, Bolyai Scholarship, National Brain Research Program (KTIA_13_NAP-A-III/5), TAMOP 4.2.4.A/2-11-1-2012-0001 „National Excellence Program", The UNKP-16-3-IV, UNKP-16-4-III, UNKP-16-4-IV, New National Excellence Program of the Ministry of Human Capacites, and Centre for Neuroscience, University of Pecs, EFOP-3.6.1.-16-2016-00004, Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs. The role of neuro-inflammation in neurodegeneration: from molecules to clinics, EFOP-3.6.2-16-2017-00008

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

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Several studies have demonstrated that the molecular and structural composition of the extracellular matrix (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 versican reactions showed diffuse neuropil staining at very early embryonic stage (E13.5), 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, aggrecan and HAPLN1 were restricted to the neuropil of some brainstem nuclei, in contrast to HA, neurocan and TN-R which were found throughout the brainstem. Our results show that at early stages of development only a diffuse staining of ECM molecules is present in the neuropil of 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 detected spatiotemporal differences in the distribution of different ECM molecules both in the neuropil and perineuronal net in various brainstem areas. It is expected that the pattern of ECM expression appears to be related to the functional maturation of brainstem neural circuits, which is also evident in other developmental processes such as neurogenesis, synaptogenesis or synaptic plasticity.

Support: OTKA K 115471, MTA TKI 355

ANALYSIS OF TEMPORAL FREQUENCY IN LOCAL FIELD POTENTIAL OF RAT VISUAL CORTEX

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Numerous analytical methods have been applied to investigate the neuronal processing of information about temporal frequency content of visual stimuli. The most encountered methods are 1) direct measurement of response amplitude, e.g. an amplitude of averaged visual evoked potential, and 2) assessment of response magnitude after transformation of the signal from time to frequency domain.
In the current study we have attempted to find out which of these two approaches yield the best results for different frequencies of visual stimuli. Local field potentials were recorded during visual electrophysiology experiments performed on anesthetized rats in response to LED light flashing at various temporal frequencies in a range from 0.5 to 15 Hz. Visual responses were collected from all layers of the primary visual cortex.
We conclude that it is not optimal to use the same paradigm to analyze the whole spectrum of temporal frequencies. We found that for frequencies lower than 2 Hz it is difficult to draw conclusions based on power spectrum alone, and the estimation of the visual evoked potential amplitude by direct measurement should be also performed. On the other hand, for higher frequencies (> 2 Hz) the assessment of evoked potential in time domain was highly inaccurate, therefore we suggest to transform signal to frequency domain and draw conclusion based on the peak at stimulation frequency, instead. Further, our results point on the advantages of using the Welch method instead of the periodogram for the analysis of signals in the frequency domain.

Supported by the Polish National Science Center grant Symfonia 1 (2013/08/W/NZ4/00691)

P21 RESTRICTS PROLIFERATION OF NEURAL PROGENITORS IN MOUSE HIPPOCAMPUS AFTER DNA DAMAGE

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Background: Irradiation results in inhibition of hippocampal neurogenesis, an injury process implicated in neurocognitive impairment. How DNA damage following irradiation leads to impaired neurogenesis remains unclear. Disruption of neuronal development in mouse hippocampus after irradiation is regulated by p53. In the absence of p53, there is enhanced depletion of neural stem cells and profound inhibition of neurogenesis in mouse hippocampus after irradiation. The cyclin-dependent kinase inhibitor 1, or p21, is known to negatively regulate neural stem cell proliferation. As a major downstream effector of p53, it mediates p53-dependent cell cycle arrest in response to DNA damage. Here we asked whether p21 played a role in the disruption of neuronal development after irradiation.
Materials and Methods: Ten-week-old male mice, wild type (+/+) or knockout (−/−) of the *p21* gene were given graded single doses of cranial irradiation. Two bromodeoxyuridine (BrdU) injection schedules (BrdU given daily for 7 days at 4 weeks and mice killed at 9 weeks after irradiation; or a single BrdU dose at 4 weeks after irradiation and mice killed at 2 hours, 2 days, 1 or 5 weeks after BrdU) were used for cell fate mapping. Neural progenitors and neurons in dentate gyrus were identified using standard phenotypic markers by immunohistochemistry. Cell numbers were estimated by non-biased stereology. The effects of *p21* genotype and irradiation on cell numbers were analyzed by 2-way ANOVA.
Results: In *p21*+/+ mice, irradiation induced acute apoptosis of proliferating type 2 neural progenitors and DCX-positive (+) neuroblasts in the subgranular zone of the dentate gyrus. This apoptotic response was independent of p21 and the number of apoptotic cells remained unchanged in *p21*−/− mice compared to +/+ mice after irradiation. In non-irradiated mice, p21 knockout was associated with an increase in the total number of proliferating (BrdU+) cells, and proliferating type 2 (BrdU+/Tbr2+) cells. Consistent with inhibition of hippocampal neurogenesis, a reduction in neuroblasts and newborn neurons (BrdU+/NeuN+ cells) was observed 9 weeks after irradiation irrespective of *p21* genotype. Irradiation also resulted in loss of proliferating (Ki67+), newborn (BrdU+) and total type 1 (nestin+/GFAP+) cells, the putative neural stem cells, and *p21* genotype had no independent effects on these cell numbers after irradiation. Following a single BrdU dose given after 4 weeks, there was an increase in total BrdU+ cells, BrdU+ type 2 (Tbr2+) cells and BrdU+ neuroblasts (DCX+) but not BrdU+ type 1 (nestin+/GFAP+) cells at 2 hours and 2 days after BrdU in irradiated *p21*−/− mice compared to irradiated wild type mice.
Conclusion: Absence of p21 is associated with increased proliferation of type 2 neural progenitors in mouse dentate gyrus with or without irradiation. The *p21* genotype does not alter the extent of depletion of neural stem cells and inhibition of neurogenesis after irradiation.

EFFECT ON HEMATOLOGICAL AND NEUROCHEMICAL END POINTS IN PESTICIDE EXPOSED FARM WORKERS

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The high use of pesticide in agriculture has been found to be linked with adverse health effects and neurological problems in humans. In view of increasing risk of pesticide toxicity among farm workers, present study has been carried out on agriculture workers (age 16 - 65 years) of Sagar (MP) district of India to investigate the adverse effects of pesticide on hematological and Neurochemical alterations in pesticide exposed farm workers. An significant increased count of RBC (16%), WBC (11%), Lymphocytes (32%), Haematocrit (9%) and increased levels of alanine aminotransferase (15%), gama glutamyltransferase (88%), triglycerides (133%), TC/HDL ratio 78%,, total cholesterol (38%)has been reported in farm workers as compared to controls. The value of creatinine (39%), blood urea nitrogen (26%) and uric acid (44%) also found increase in these individuals. An increased in the level of lipid peroxidation, and decreased levels of reduced glutathione, superxide dismutase and catalase associated with decreased levels of acetylcholinesterase activity has also been reported in pesticide exposed farm workers as compared to controls. These preliminary findings suggested that overuse of pesticides increased the risk of adverse health effects including neurologic dysfunction among agriculture workers.

Keywords: Pesticide; Biomarkers; oxidative stress; acetylcholinesterase; farm workers

ELECTRIC AXON GUIDANCE IN EMBRYONIC RETINA: INVOLVEMENT OF INTEGRINS AND EXTRACELLULAR CALCIUM IONS

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Growing axons are directed not only by chemical signals but also by electric fields in a process known as galvanotropism. The axons of embryonic brain, spinal cord, and retina extend along the extracellular voltage gradient towards the cathode. In the embryonic central nervous system, positive direct current (DC) potentials are generated by neuroepithelial cells' sodium transport, of which disruption results in erroneous path-finding of newborn neurons' axons (Yamashita, 2013). Thus, the electric field plays a pivotal role in orienting axons. However, there is no experimental evidence for the cell surface molecule that is activated asymmetrically in electric fields. Here I show that integrins and the extracellular Ca2+ are involved in galvanotropism. Retinal strips of chick embryos were embedded in Matrigel, and cultured in the electric field of the same strength as that in vivo (15 mV/mm). Matrigel contained the same extracellular matrix proteins as in the embryonic retina, laminin and collagen, to which integrins bind. Retinal ganglion cell (RGC) axons, which express integrins, extended towards the cathode. Monoclonal anti-chicken integrin □1 antibodies, TASC and W1B10, significantly enhanced the cathodal growth. A reduction in the extracellular free Ca2+ with EGTA (1 mM) also enhanced the cathodal growth, which suggested that millimolar Ca2+ inhibits axon growth. In the presence of EGTA at 2 mM, however, the axons extended in all directions even in the electric field, suggesting that Ca2+ at physiological concentration is necessary for determining the direction of axon growth. In the presence of Mn2+ (1 mM), which non-specifically activates integrins, the axons formed local meshes even in the electric field. These results suggested that integrins mediate electric axon guidance and that the extracellular Ca2+ exerts inhibitory effects on axon growth. The amplitude of the positive DC potential is largest at the periphery of the embryonic retina, where the neuroepithelial cells most actively proliferate. The DC potential is almost null at the ventral portion of the optic cup, where the future optic disc is formed. RGCs, the first type of retinal neurons, are born at the central part of the optic cup, and their axons are directed towards the future optic disc by the voltage gradient.

EFFECT OF COENZYME Q10 ON THE MODEL OF EXPERIMENTAL DEMENTIA OF ALZHEIMER'S TYPE IN RATS

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Alzheimer's disease (AD) is one of the most common neurodegenerative disorders in elderly people. It leads to the progressive loss of mental and behavioural and cognitive impairments. Recent studies showed that antioxidants may have important roles as a neuroprotective for AD. In this study, we aimed to investigate biochemical and histopathological changes in the brain tissue, along with depression and anxiety stress test, and effects of CoQ10 prophylactic treatment on these parameters in the model of experimental dementia of Alzheimer's type in rats.
Rats were divided randomizedly four groups. CoQ10 (12 mg/kg/day; i.p.) were administered to the Lesion and CoQ10 (L-C) group for 3 weeks. On 22thday, rats were injected in bilateral intracerebroventricular streptozotocin (ICV-STZ) (3 mg/kg). The same experimental procedure were applied to other groups (only vehicle administered sham group (S), only ICV-STZ administered lesion group (L) and CoQ10 pretreatment sham group (S-C)). After two weeks, rats were tested by the open field and the forced swim tests. The brain tissues were analyzed for levels of AChE enzyme activity and total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI). Neuron damage and iNOS were showed histochemically.
As a result, we demonstrated that depression and anxiety like behaviors were increased in the L group. The model of experimental dementia of Alzheimer's type (group L-C) was decreased by the administration of CoQ10. Furthermore the AChE enzyme activity levels were measured in the cerebral cortex area. It was found significantly increased ($p<0,05$) in the L group when they compared with the S group. Gliosis, fibrillar bundles, hemorrhagic areas, hyperemia and focal edema areas were observed in the L group, whereas only gliosis and vascular proliferation were observed in the L-C group.
This study showed that CoQ10 may be used as a supplement in the Alzheimer' s disease treatment, however CoQ10 has required advanced additional studies.

Keywords: Alzheimer, Dementia, Depression, Coenzyme Q10, Stereotaxi, Oxidative Stress, Antioxidants.

EPIGENETIC ANALYSIS OF THE PROLIFERATION AND MATURATION DYNAMICS OF OLIGODENDROCYTES IN PREGNANT ANIMALS IN A MODEL OF MULTIPLE SCLEROSIS

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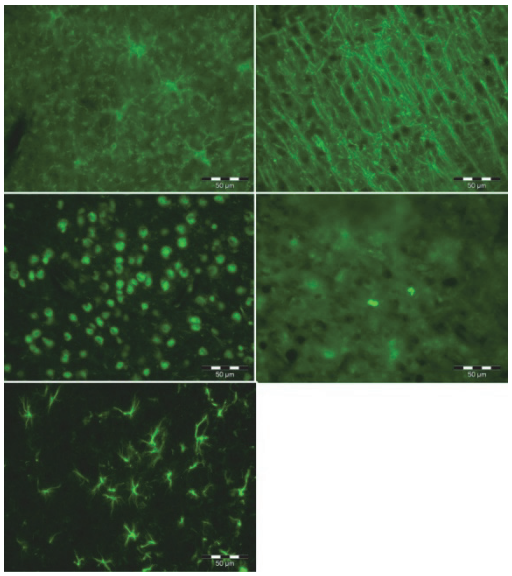
Introduction & Objectives: Multiple sclerosis is a demyelinating disease of central nervous system in which auto-reactive T-lymphocytes are produced to target the myelin antigens. It is more common in women than man and there are several studies suggesting the adverse effects of MS are suppressed during pregnancy. In the present study, we aim to compare changes in the density of oligodendrocyte precursor cells as well as epigenetic mechanisms functioning in and their proliferation dynamics in experimental autoimmune encephalomyelitis (EAE) induced nullipar, pregnant and postpartum C57 BL6 mice. Moreover we purpose to investigate the alterations in myelin formation and gliosis processes between those groups.
Materials & Methods: Animals were divided into 2 main groups as healthy and EAE-induced. Healthy groups were further subdivided into nullipar, pregnant, post-partum groups. EAE-induced groups were further subdivided into nullipar (sacrified at 17th or 26th day after EAE induction), pregnant and post-partum groups. 10 µm-thick coronal brain slices were obtained. Immunofluorescence stainings were performed for NG2 to analyse oligodendrocyte precursor cell density; for HDAC1 to analyse histone deacetylation patterns; for MBP to analyse myelin formation; for Ki67 to analyse proliferation dynamics and for GFAP to analyse gliosis processes. Results were compared for each of the experimental groups.



	NG2	MBP	HDAC1	Ki67	GFAP
Nullipar, Healty	4.67	4.83	2.00	4.00	5.00
Pregnant, Healty	2.33	2.17	5.00	3.00	2.00
p	*0.099*	*0.068*	*0.034**	*0.480*	*0.043**
Pregnant, Healty	2.00	4.00	4.00	5.00	5.00
Post Partum, Healty	5.00	3.00	3.00	2.00	2.00
p	*0.034**	*0.317*	*0.317*	*0.025**	*0.034**
Pregnant, Healty	5.00	4.33	5.00	3.00	5.00
Pregnant, EAE Induced	2.00	2.67	2.00	4.00	2.00
p	*0.043**	*0.261*	*0.043**	*0.317*	*0.043**
Nullipar, Healty	3.00	5.00	5.00	4.33	5.00
Nullipar, EAE induced (17th day)	4.00	2.00	2.00	2.67	2.00
p	*0.500*	*0.043**	*0.034**	*0.261*	*0.043**
Nullipar, Healthy	5.00	2.67	4.00	4.00	5.00
Nullipar, EAE induced (26th day)	2.00	4.33	3.00	3.00	2.00
p	*0.043**	*0.261*	*0.317*	*0.500*	*0.043**
Nullipar, EAE induced (17th day)	5.00	4.00	4.33	2.67	5.00
Pregnant, EAE Induced	2.00	3.00	2.67	4.33	2.00
p	*0.043**	*0.456*	*0.239*	*0.197*	*0.043**
Nullipar, EAE induced (17th day)	5.00	2.00	2.83	2.67	5.00
Nullipar, EAE induced (26th day)	2.00	5.00	4.17	4.33	2.00
p	*0.043**	*0.043**	*0.361*	*0.197*	*0.043**
Post Partum, Healty	2.00	5.00	5.00	2.00	2.00
Post Partum, EAE Induced	5.00	2.00	2.00	5.00	5.00
p	*0.025**	*0.025**	*0.034**	*0.034**	*0.034**
Nullipar, EAE induced (26th day)	2.00	5.00	4.17	3.00	2.00
Post Partum, EAE Induced	5.00	2.00	2.83	4.00	5.00
p	*0.034**	*0.034**	*0.361*	*0.500*	*0.043**
Pregnant, EAE Induced	2.00	4.00	2.67	2.17	2.00
Post Partum, EAE Induced	5.00	3.00	4.33	4.83	5.00
p	*0.034**	*0.317*	*0.239*	*0.068*	*0.043**

Results: Both gestation and post-partum periods as well as disease induction were shown to cause statistically significant alterations on the number of polydendrocytes. MBP immunostaining results suggested that immunostaining intensity was higher in EAE-induced nullipar animals sacrified at 17th day of induction compare to healthy nullipars but it was lesser compared to that of sacrified at 26th day of induction. While the HDAC1 staining intensity was enhanced together with pregnancy, it was quite the opposite for the EAE-induced groups of nullipar and post-partum animals. Obtained from Ki67 immunostaining results cell proliferation was shown to be increased in healthy pregnant animals while to be decreased in healthy post-partum subjects. Both gestation and EAE induction resulted in increase in GFAP positive cell numbers. However, it was found to be higher in EAE-induced post-partum group compared to EAE-induced pregnant group. Number of astrocytes were increased during EAE progression in post-partum animals while they were decreased in healthy post-partum group.
Conclusion: To sum up, it was thought that pregnancy might be the cause of alterations in the number of polydendrocytes, degree of myelin content, astrogliosis and related epigenetic processes which may be associated with the recovery in disease clinical course during pregnancy.

NEUROBIOLOGY UNDERLYING DIVERSITY IN SOCIAL BEHAVIOR: IMPLICATIONS FOR AUTISM

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Voles provide an excellent opportunity to explore the neural and genetic mechanisms contributing to the diversity in social behaviors and mating strategies. Prairie voles are highly social, biparental and socially monogamous, while meadow voles are asocial and promiscuous. The oxytocin system facilitates several aspects of social behaviors associated with monogamy in prairie voles, and diversity in this system contributes to diversity in social behaviors. Oxytocin receptor (OXTR) signaling in the nucleus accumbens (NAcc) and prefrontal cortex (PFC) is critical for pair bond formation between mates in prairie voles. Diversity in expression patterns of the OXTR within the brain contribute to diversity in social behaviors across and within species. Prairie voles have high densities of OXTR in the NAcc compared to meadow voles. In prairie voles, oxytocin links the neural encoding of the social signature of the partner with the rewarding aspects of mating through interactions with dopamine and by coordinating communication across a neural network linking social information with reward. Genetic polymorphisms robustly predict natural variation in OXTR expression in the striatum of prairie voles, which predict pair bonding behavior and resilience to neonatal social neglect. We have also explored the capacity of prairie vole to display empathy-like behavior, specifically consoling. Prairie voles increase their partner-directed grooming toward mates that have experienced an unobserved stressor. This consoling response is abolished blocking oxytocin receptor antagonist into the anterior cingulate cortex, a region involved in human empathy. Finally, loss of a bonded partner results in the development of depressive-like "grieving" behavior. Infusion of oxytocin into the NAcc prevents social loss-induced depression. Studies using intranasal oxytocin and behavioral genetics suggest that the role of oxytocin on social attachment and social cognition is conserved from rodent to man. In humans, intranasal oxytocin enhances eye gaze into the eyes of others, the ability to infer the emotions of others from facial cues, empathy, and socially reinforced learning. Thus the oxytocin system may be a viable target for drugs to improve social functioning in autism. Melanocortin agonists, in particular, evoke endogenous oxytocin release, facilitate social bonding, and activate oxytocin-dependent neural networks via enhancing oxytocin receptor signaling, and thus represent a novel therapeutic strategy for improving social function in autism spectrum disorders.

DISTRIBUTION OF THE NEUROPEPTIDES AMYLIN, VASOTOCIN AND VIP IN THE BRAIN OF ZEBRA FINCHES (TAENOPYGIA GUTTATA) AND THEIR CHANGES WITH RESPECT TO PARENTAL BEHAVIOUR

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The neural basis of parental care is one of the least explored research area in social behavioural neuroscience. Most of the studies are conducted on rodents and limited to maternal behaviour. However, many species, including humans, share parental effort among the male and the female. Therefore it is important to use biparental species as models for investigating the neurobiology of parental care. In zebra finches, both sexes incubate the eggs and feed the hatchlings. Neuropeptides, such as nonapeptides (including vasopressin in mammals and its homologue arginin vasotocin (AVT) in birds), vasoactive intestinal polypeptide (VIP) and amylin play important roles in the regulation of various social behaviours both in birds and mammals. The role of AVT and VIP has been studied in the context of gregariousness, pair bonding and territorial interactions but not in parental behaviour in birds. Amylin has been shown to influence maternal behaviour in rodents, but its role and distribution is unknown in the avian brain. Here we describe the changes in the distribution of AVT, VIP and amylin in the zebra finch brain according to the reproductive status and sex.
Brains of male and female zebra finches were compared when paired or feeding hatchlings to control birds living in same sex groups. AVT and VIP were labelled on brain sections using immunohistochemistry, while in case of the amilyn in situ hybridization histochemistry was used in the absence of a specific antibody. The labeling signal has been quantified and statistically compared among groups with special emphasis to the regions of the brain's social behavioural network.
Both AVT and VIP were less expressed in the paired finches compared to both group living or parental birds in the bed nucleus of the stria terminalis and in the medal preoptic area (POM). AVT was also less abundant in the paired birds within the lateral septum and the paraventricular nucleus of the hypothalamus.
Amylin showed a much wider distribution across brain regions than those found in rodents where only the preoptic area contains amylin neurons. A strong label was found in the striatum and several brain regions of the social behavioural network. Regions responsible for the generation of birdsong also contained amylin positive cells. Area-X, a striatal region specific to singing males appeared as a weakly labelled region in males but not in females. There was also difference between the two sexes in the label of the POM. Nucleus accumbens and higher vocal center (a pallial area necessary for song production) showed both sexual and reproductive status dependent differences.
The decrease in the expression of AVT and VIP in regions of the social network of the paired individuals is probably due to their mild social deprivation compared to the other groups. Our data suggest an evolutionary conservative role of the observed neuropeptides in the regulation of parental behaviour in both males and females.

SECRETAGOGIN REGULATES PAIN-INDUCED STRESS

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Our ability to take action upon perilous environmental calls is an evolutionary clue for survival. An immediate response is enabled via the hypothalamus-pituitary-adrenal medulla (HPA) axis which triggers epinephrine release to adjust metabolic status to increased constitutional and neural need. Environmental challenges may need prolonged alertness, however; the ability to support reactivity is of substantial evolutionary benefit to defend against repeated or long-held attacks. Here, we show a novel mechanism in the mammalian brain which enables us to give a delayed response to stressful stimuli. We identified secretagogin, a recently explored calcium-sensor protein in norepinephrine-expressing brainstem neurons, including the locus coeruleus. In the brainstem, pain-induced stress increased tyrosine hydroxylase (TH) phosphorylation specifically at the 31 serine residue (pSer31) which was absent in secretagogin-null mice, and human subjects who deceased in acute stress showed increased secretagogin and pSer31 levels. Locus coeruleus neurons expressed ciliary neurotrophic factor (CNTF) receptor type 2. We show that formalin-induced stress increased CNTF level in the cerebrospinal fluid and application of recombinant CNTF on pontine explants triggered phosphorylation of TH which was paralleled with ERK-phosphorylation. Secretagogin silencing blocked CNTF-induced phosphorylation of TH and ERK. In the medial prefrontal cortex (mPFC), ascending norepinephrinergic brainstem afferents expressed pSer31 in their terminals and secretagogin was present in mPFC synaptosomes. Stress increased pSer31 in mPFC in wild-type, but not secretagogin-null mice mPFC. Further, secretagogin-null mice show reduced scores in pain-induced behavioral paradigms. Conclusively, we suggest that secretagogin is a critical regulator of pain-induced, norepinephrine-mediated stress in mammals.

MODULATION OF HIPPOCAMPAL THETA RHYTHM BY MUSCIMOL INFUSIONS INTO THE MAMMILLARY BODY AND ANTERIOR THALAMUS IN URETHANE-ANESTHETIZED RATS

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Two closely related structures, the mammillary body and anterior thalamic nuclei, are critical for proper processing of several aspects of memory, however their exact role has remained unclear. In both the structures, neurons firing according to hippocampal theta rhythm have been found, mainly in the medial mammillary nucleus (MM) and anteroventral thalamic nucleus (AV). We argue that the mammillary body - anterior thalamic nuclei axis not only relays theta signal, but may also modulate it. Our previous study has revealed that injection of muscimol to the MM attenuates theta rhythm activity in the hippocampus (HP) in urethane-anesthetized rats. In this study, we compered the effect of pharmacological activation of GABAergic transmission in the AV and MM on hippocampal theta rhythm.
Our main goal was to investigate whether local administration of GABAA receptor agonist (muscimol) into the AV affects theta rhythm in the HP in urethane-anesthetized rats. Secondly, we wanted to compare the effect of inactivation of GABAergic transmission in the AV and MM on hippocampal bioelectric activity.
Male Wistar rats were implanted with unilateral recording electrodes into the dorsal HP and unilateral injection cannula into the AV. Animals received microinjection of either muscimol (n = 5) or water (n = 5). 1-min tail pinch stimulations were applied at 5- and 10-min intervals to evoke theta rhythm episodes in the HP. Changes in local field potential were assessed on the basis of percent change of total EEG signal power for 1-Hz bands.
Virtually no significant changes of EEG signal power were observed within theta frequency bands after muscimol injection into AV. The infusion had minor effect only in 5-6 Hz band, increasing total power up to 240% in comparison to the pre-injection conditions (100%). Completely different effect was observed after muscimol injection into the MM - significant decrease of EEG signal power, down to 19% and 48%, in 3-4 Hz and in 7-8 Hz band, respectively. In the same time, the injections into both the structures increased total power in delta frequency bands, however in different range, up to 262% and 2415%, after intra-AV and intra-MM infusion, respectively.
We found that intra-AV muscimol injection did not affect sensory-elicited theta rhythm in the hippocampus in urethane-anesthetized rats. Our study also revealed that inactivation of GABAergic transmission in the anterior thalamic nuclei and mammillary body has completely different effect on hippocampal theta rhythm.

This research was supported by the National Science Centre (DEC-2014/12/S/NZ3/00621).

INTRINSIC OPTICAL SIGNAL IMAGING AND SIMULTANEOUS HIGH-RESOLUTION CORTICAL ELECTROPHYSIOLOGY USING A FLEXIBLE, TRANSPARENT MICROELECTRODE ARRAY

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Summary: Multimodal data integration can yield important insights into brain processes and structures in addition to spatiotemporal resolution complementarity. In our work, the high spatial resolution of intrinsic optical signal imaging (iOS) and the outstanding temporal resolution of micro-electrocorticography (µECoG) is combined in visual stimulus experiments in anaesthetized cat. Optical signals reflected from the exposed brain surface are acquired through a cranial window, while the visual cortex is covered by a transparent polymer electrode array used for EEG recording. Microsystem design, testing, surgical procedures, stimulus and acquisition protocols of this novel neuroimaging approach will be described in details.
Motivation and results: iOS records tiny changes in optical reflection of the exposed cortical surface due to local hemodynamic changes. iOS has been demonstrated to study the function of the neuronal circuitry of the visual cortex evoked by retinal stimuli. Cortical electrophysiology performed in the very same area may provide additional insight into the connectivity between functional domains. Our aim is to investigate the simultaneous use iOS and µECoG techniques by introducing a transparent polymer based subdural microelectrode array into the optical recording chamber. The 8 micron thick, 32-channel microelectrode array is composed of polyimide (PI) substrate and indium-tin-oxide (ITO) metallization. Its technology relies on MEMS processes. The robustness of its site impedance is proven in electrochemical impedance spectroscopy performed in long-term soaking tests and after the in vivo implantation. To demonstrate the feasibility of the combined optical-electrical recording, we have run several stimulus protocol to excite retinal cells of an anaesthetized cat and measured the evoked optical and electrical responses of the visual cortex (area A18) in a synchronized manner with the µECoG sheet. A customized recording chamber is fabricated to accommodate the flexible µECoG array into the optical path of the imaging without deteriorating the circulation of the pressurized ACSF (artificial cerebrospinal fluid) medium above the exposed brain region. Optical quality of iOS signals (605 nm) is also evaluated without the polymer microelectrode as a reference. During visual stimulus, local field potential on 32 channel of the ECoG was simultaneously recorded and evaluated. As far as we know, this is the first demonstration of a flexible, PI/ITO/PI based microelectrode array in combination with in vivo intrinsic signal imaging.

FOS PROTEIN IN MEDIATION OF STRESS RESPONSE INDUCED BY SINGLE SHORT-TERM MATERNAL SEPARATION IN OLFACTORY NEUROGENIC AREAS

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Adverse effect of repeated separation of rat pups from their mother on processes of neurogenesis has been previously described. However, the influence of single short-term maternal separation (MS) on neurogenesis has not been studied yet. The aim of this work was to assess the effect of this stressful event on neurogenesis of neonatal rats and to elaborate the possible role of Fos protein in mediating the stress response induced by separation. We have investigated Fos expression in cells of neurogenic areas: the subventricular zone (SVZ), rostral migratory stream (RMS) and in the olfactory structures: the olfactory bulb (OB), accessory olfactory bulb (AOB) and the anterior olfactory nucleus (AON).
Rat pups were exposed to single separation from the mother on postnatal day 7 (P7), 14 (P14) and 21 (P21) for 2 hours and immediately transcardially perfused. Fos immunopositive cells were quantified in the SVZ, RMS, OB, AOB and in the AON. To reveal whether newborn cells produce Fos protein, pups were injected by BrdU 10 days before maternal separation at P21 and double immunohistochemistal labeling for Fos and BrdU was performed.
There were no Fos+ cells neither in the SVZ of P7 and P14 control nor experimental animals. In the SVZ of P21 control rats a few Fos+ cells were observed and exposure to MS significantly increased their number. In the RMS of P7, P14 and P21 control rats were no Fos+ cells and separation from the mother didn't induce Fos expression in any experimental rat. MS induced notable changes in production of Fos protein in all examined olfactory areas. The number of Fos+ cells was increased in all OB layers. Increased number of Fos+ cells after MS was observed in AOB layers of P14 rats, where MS also induced early production of Fos protein in glomerular, nerve and granular layer. In the AON, the enhancement of Fos protein expression induced by MS was age dependent. BrdU+ cells did not colocalized with Fos+ cells in any assessed areas.
Our results suggest that some SVZ cells have complete precondition requisite for the Fos signal transduction and they are unlike RMS included in circuitry activated by MS. We can conclude that the AOB can be activated besides pheromone-like chemo-signal also by MS. Our finding also indicates that stress due to MS can induce Fos protein production in cells, which don't produce this protein under physiological conditions. The absence of colocalization of Brdu and Fos in all examined regions indicates that 10 day period after BrdU administration isn't long enough to provide the evidence about functional significance of newly born cells. Activation of important olfactory centers, the OB and AON indicates that single short-term maternal separation is a stressful signal which is mediated by olfaction.

Supported by VEGA 2/0159/17; VEGA 2/0069/15.

NEURONAL REGULATION OF ASTROGLIAL ENERGY METABOLISM

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Glucose is the main energy source for the mammalian brain. However neurons are the most energy-consuming cell type in the nervous system, they aren't main cells that take up glucose from blood. This role is played by astrocytes, glial cells. In response to a neuronal signal, astrocytes release lactate, a glucose-derived metabolic substrate for neurons[1]. Previous studies revealed that neurons can regulate expression of metabolic enzymes in astrocytes[2], however molecular basis of this phenomenon are still unknown. Our present research is focused on an identification of regulatory factors released from neurons and affecting astrocyte metabolism.
To address this issue we carried out experiments which demonstrate that incubation of rat hippocampal astrocytes in neuronal conditioned medium (NCM) elevates the level of mRNA, the abudance of protein level and the activity for crucial glycolytic enzymes (aldolase, pyruvate kinase) in astrocytes. For aldolase the increase in mRNA level was observed only for one brain-expressed isozyme (ALDOA), although no rise in the enzyme activity or the protein level was noted. In contrast, for pyruvate kinase the two-time increase in mRNA level and protein abudance was observed for both brain-expressed isoforms (PKM1, PKM2) and these changes translate into significant growth in enzyme activity. Simultaneously, the increase of ATP level is observed in cultured glial cells. Moreover, our data reveal that factors released from neurons to a medium are heat-unstable and differ on molecular mass (below 10 kDa and between 30-100 kDa).
Taken together, it may be presumed that neuronal modulation of astrocytic metabolism is based on regulation of the expression and the enzymatic activity of pyruvate kinase, and is mediated by secretion of some molecules with distinct molecular mass.

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VASOPRESSIN AS A SOCIAL HORMONE: LESSONS FROM THE FIRST BUT STILL UP-TO-DATE MUTANT RODENT MODEL, THE BRATTLEBORO RAT

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Vasopressin is an ancient and ubiquiter molecule with primary water regulatory action. The forerunner of the knockout animals, the spontaneous mutated Brattleboro rat was also discovered based upon its enhanced water intake and urination. However, subsequent studies attributed vasopressin to wide range of behaviors from learning and memory to social investigation. Anatomical work has shown that the vasopressinergic fibers are more dense in male rats than females and are obliterated entirely by castration. This fact might contribute to the repeatedly described memory deficit of male but not female Brattleboro rats, supporting the important role of vasopressin in male behavior. Indeed, social memory was also impaired in male, but not female Brattleboro subjects. However, interstingly enough, aggression, a male type behavior was influenced by vasopressin only in lactating females, and not males, being lower in vasopressin-deficient subjects, together with lower impulsivity. In conjuction with this kind of maternal neglect, vasopressin-deficient mothers were more careless during spontaneous, but not during induced maternal behavior. In males a site-specific action of vasopressin on aggression was observed; enhanced vasopressin signalling in medial amygdala lead to a friendly, social and lower aggressive state. All in all, although vasopressin is thought to be the male hormone of social bound, its role increases in females during lactation being an important contributor of defensive maternal behavior (spontaneous care, impulsivity and aggression).

THE NUCLEUS INCERTUS SPECIFICALLY TARGETS NEURONS RESPONSIBLE FOR THE FORMATION OF CONTEXTUAL MEMORY

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The hippocampus plays an essential role in declarative memory formation by encoding episodic memories, and establishing associations between their features. A well-known example of this hippocampal function is contextual fear conditioning (CFC), in which the subject has to associate a context (conditioned stimulus, CS) with an aversive event (unconditioned stimulus, US).
The encoding of CFC is mediated by the pyramidal cells (PC) of the hippocampal CA1 region. CA1 PCs receive the contextual information (CS) from the CA3 and the primary sensory information (US) from the entorhinal cortex, which inputs target different parts of their dendritic tree. The very delicate timing of these inputs is essential for the CA1 PCs for being able to forge an associative link between them. The GABAergic somatostatin (SOM)-positive interneurons located in the stratum oriens of the hippocampus play an essential role in the temporal regulation of these inputs. SOM cells receive glutamatergic and cholinergic excitatory inputs from the medial septum (MS). These inputs promote memory encoding and theta-generation and carry information about the state of the animal during US. The permissive role of these SOM cells is so strong that, if they are artificially inhibited directly during US presentation, mice are unable to make CFC memory traces. However, it is unknown, whether there is any natural subcortical inhibitory input targeting the hippocampal SOM cells that could prevent the formation of unnecessary contextual memories of everyday events.
Using retrograde tracing and confocal fluorescent microscopy in vesicular GABA-transporter (vGAT)-reporter mice, we found that the GABAergic cells of the pontine nucleus incertus (NI) send a strong inhibitory input to the hippocampus and MS. Cre-dependent viral anterograde tracing in vGAT-Cre mice showed that NI innervates hippocampal SOM cells very selectively, and also establishes synaptic contacts with glutamatergic and cholinergic MS cells, whereby it can decrease the activity of hippocampal SOM cells indirectly. Our correlated light and electron microscopic investigations showed that NI establishes gephyrin and GABAA receptor containing symmetrical synapses. In addition, optogenetic stimulation of NI fibers showed picrotoxin-sensitive inhibitory postsynaptic currents on hippocampal SOM interneurons and an inhibition of hippocampal sharp-waves in vitro. Post hoc reconstruction of these cells showed that these SOM positive cells are oriens-lacunosum moleculare (O-LM) cells or projection interneurons.
Our results show that inhibitory neurons in the NI of the brain stem are in ideal position for effectively vetoing the activity of hippocampal SOM cells, whereby they could regulate septohippocampal theta-generation and prevent the formation of unnecessary contextual memories. Behavioral data are presentment in a parallel poster.

MOLECULAR CHANGES IN THE CA1 REGION OF THE DORSAL HIPPOCAMPUS DURING FEAR EXTINCTION

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Context fear conditioning is a form of associative learning in which the aversive stimulus is associated with the new context. Extinction training, in which a previously trained animal is exposed to the context without presentation of the aversive stimulus, results in reduction of conditioned fear responses. It is still not fully understood what regions and molecular processes contribute to the context fear memory extinction. In the current study we investigated the contribution of the area CA1 of the dorsal hippocampus in extinction of contextual fear memory. The function of this region in memory formation is well documented, however its contribution to memory extinction is still speculative.
We found that in mice after fear memory extinction the levels of glutamatergic marker, PSD95 protein, decreased in most layers of the hippocampal CA1 field. These changes were absent in CaMKII-T286A mutant mice which have impaired fear memory extinction. Furthermore, we observed that fear memory extinction resulted in decreased density of dendritic spines containing PSD95 (PSD+) in the *stratum oriens* and increased density of dendritic spines PSD+ in the *stratum lacunosum moleculare* of CA1 principal neurons. Since decreased expression of PSD95 suggests degradation of this protein as well as inhibition of the region we applied chemogenetic manipulations to test the role of functional inhibition of CA1 in memory extinction. In this experiment we used AAV2/9 coded DREADD synapsin-hM4Di-mCherry, which inhibiting cell activity upon stimulation with CNO. C57BL/6 mice were transfected with AAV2/9 into area CA1 of the dorsal hippocampus. Inhibition of CA1 pyramidal cells with DREADD-CNO system during extinction session prevents consolidation of fear memory extinction. Overall, our data suggests that context fear memory extinction involves inhibition of the dorsal CA1 area. Moreover, controlled protein degradation is necessary for this process to occur.

ON THE MECHANISM OF PROTECTIVE AND ANTIOXIDATIVE EFFECT OF INSULIN ON BRAIN CORTICAL NEURONS UNDER CONDITIONS OF OXIDATIVE STRESS

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Numerous evidences have been obtained in recent years that insulin possesses neuroprotective effect. Both experimental studies and clinical trials of insulin suggest that it is promising for treatment of neurodegenerative and other brain damage diseases. But the mechanism of its protective effect is far from being understood. The aim of the present work was to study how insulin modulates various protein kinase activities and Bax/Bcl-2 ratio in rat brain cortical neurons under the development of oxidative stress induced by hydrogen peroxide and to try to understand the role of these insulin effects in the mechanism of its protective action. The effect of insulin on cortical neurons was shown to be the higher, the higher was its concentration in the nanomolar range (1 nM < 10 nM < 100 nM), no difference was found between the effects of 100 nM and 1 μM insulin. Insulin was shown to possess the antioxidative effect in a wide range of concentrations, from 1 nM and higher. The protective effect of insulin was not revealed in the presence of an antagonist of insulin (and IGF-1) receptors and an inhibitor of AMP-dependent protein kinase (AMPK) - BMS 754807 and CC, respectively. Inhibitors of PI3-kinase/Akt and MEK1/2 /ERK1/2 signal pathways significantly decreased the protective and antioxidative effects of insulin. The modulation of various protein kinase activities by insulin was studied by immunoblotting method. 100 nM and 1 μM insulin was found to increase Akt basal activity and its activity after the cortical neuron's exposure to hydrogen peroxide (estimated as the level of pAkt Ser473). Thus, 1 μM insulin increased the basal Akt activity more than 5 times. It significantly increased the activity of this enzyme 5, 30, 45 min and 1, 2, 4 and 6 h after neurons exposure to hydrogen peroxide as compared to the effect of this prooxidant alone which also activated Akt. The increase of ERK1/2 activity by 100 nM and 1 μM insulin was revealed at early stages (5-30 min) of exposure of cortical neurons to hydrogen peroxide. It was evaluated as the increase of pERK1/2 level [pERK1 (pThr202/pTyr204) and pERK2 (pThr185/pTyr187)]. The modulation of AMPK activity by insulin in the dynamics of development of oxidative stress in cortical neurons will be described. The Bax/Bcl-2 ratio was found to increase as a result of brain cortical neuron's exposure to hydrogen peroxide, but preincubation with insulin prevented such changes. The studies of the mechanism of action of various protectors having antioxidative effect (e.g. carnosine, insulin, flavonoids, gangliosides, vitamin E etc) are of importance for revealing such complexes of these compounds, the components of which may enhance the protective effects of each other. Such approach is of interest as clinical trials showed unfavorable effect of long administration of high doses of viitamin E to patients with various diseases.

The work was supported by grant from the Russian fund for basic research No 16-04-408.

STORM SUPER-RESOLUTION IMAGING OF DENDRITIC CB1 CANNABINOID RECEPTORS

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Endocannabinoid signaling is considered as one of the most important regulatory mechanisms to fine-tune neurotransmitter release in an activity-depenent manner. According to the widely accepted modus operandi, endocannabinoids are released from the postsynaptic neuron, travel backwards via the synaptic cleft and exert their effect on presynaptically located CB1 cannabinoid receptors. However, emerging evidence accumulates that besides the ubiquitous function as retrograde synaptic messengers, endocannabinoids have also important cell physiological functions as autocrine signals. For example, it has been demonstrated recently (Maroso et al., 2016, Neuron, 89:1059-1073) that CB1 receptors control dendritic excitability via the facilitation of h-currents mediated by the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. Remarkably, the CB1-HCN pathway is tonically active in CA1 superficial pyramidal cells in the hippocampus, whereas it is not functional in the CA1 deep pyramidal cells. In the present study, we sought to delineate the nanoarchitecture of the molecular machinery underlying the CB1-HCN signaling pathway and to establish if a specific subcellular compartment provides the platform for its operation. However, in contrast to the well established presynaptic accumulation of CB1 receptors on axon terminals, a direct and knockout-validated anatomical evidence for dendritic CB1 receptors was still missing likely owing to the much lower copy number of somatodendritic CB1 receptors. Therefore, we applied STochastic Optical Reconstruction Microscopy (STORM), which relies on the localization of single fluorophores and hence it is endowed with a superior detection sensitivity compared to conventional imaging approaches. To ensure the cell-type-specific sampling and to provide an appropriately detailed subcellular context for the STORM signal representing the localization of the CB1 receptors, we first performed whole-cell patch-clamp recordings from superior and deep pyramidal cells in acute mouse hippocampal slices and filled the neurons with biocytin during the electrophysiological recordings. Next, we carried out correlated confocal and STORM imaging, and interpreted the super-resolution data within a cell-type- and subcellular domain-specific context by the VividSTORM software. The results unequivocally show that very low levels of CB1 receptors are present in the dendritic shafts of secondary dendrites of the superficial pyramidal cell in the stratum radiatum. The STORM signal intensity in superficial pyramidal cells, but not in deep pyramidal cells, was signficantly above the noise level as validated in control cells obtained from CB1 knockout mice. These findings demonstrate that STORM imaging is capable to uncover the nanoscale distribution of previously hidden molecular targets and opens the way for future investigations on the organization of the dendritic CB1-HCN pathway in the healthy and diseased brain.

THE TRANSLATIONAL IMPLICATIONS OF A NOVEL, EXTENDED VERSION OF THE BELLMAN EQUATION- LESSONS FROM MINDFULNESS COGNITIVE BEHAVIORAL THERAPY

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The Bellman equation is a central theorem in reinforcement learning, it defines the value of a given state as the sum of the immediate reward received upon entering a state and the discounted value of future states that may be obtained starting from the current state. The value of state is determined by agent related attributes (action set, policy and γ discount factor), the agent's knowledge of the environment (described by the reward function) and environmental factors hidden to agent (given by the transition probability). Generally, model-based reinforcement learning problems use the model to conduct forward-looking simulations for the sake of making predictions and/or optimizing policy in a way that the cumulated sum of the reward is maximized in the long term. Under model-based reinforcement learning scenarios, predictions (e.g. attempts to determine the value of state) are deduced from information contained in the model. In model-free learning on the other hand, the agent obtains information about its environment by trial and error and computes estimates of the value of states or state-action pairs, in a way that estimates are cached. Accordingly, learning is governed by the utility of a stimulus or outcome with respect to its predicted cumulative future value discounted as a function of time, embodied by the reward prediction error. It is evident that both model-based and model-free accounts of reinforcement learning utilize temporal discounting.
Change of reward-related processes is well documented in several mental disorders and symptoms (e.g. anhedonia of depression, anxiety), hence conceptualization of mental disorders using the Bellman equation may carry translational aspects. Mindfulness is an emerging technique that focuses on intentional direction of attention to ongoing events and experiences in an open and receptive manner. Efficacy of mindfulness therapy has been observed in management of diverse conditions including chronic pain management, chronic stress, anxiety and depression, however its exact mechanism and precise relation with reward (reinforcement learning)- related processes is yet to be elucidated.
In the current presentation, based on the critical evaluation of reports dealing with the influence mindfulness therapy has on reward-related processes in mental disorders, we introduce a new derivate of the Bellman equation, that can account for the alteration of attentional processes. We propose that the transition probability, a function of the environment that is beyond the control of the agent may be further specified in a way that motivational salience as well as other attributes of the environments are modelled independently. Furthermore, we will describe the potential translational aspects of our new model.

This study was supported by the Hungarian Brain Research Program (KTIA_13_NAP-A-V/2).