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ORAL PRESENTATIONS

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Phospholipid Scrambling as A Signal for Synaptic Pruning by Microglia in Developing Brain

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Background and Aim: The development of a complex nervous system is accompanied by a generation of superfluous neuronal connections that are removed when neural circuits mature. Structural and functional refinement of synaptic network is tightly related to the presence of brain immune cells microglia that actively contact and engulf unnecessary synapses. Aberrant or impaired microglial function leads to abnormal synaptic densities and dysfunctional connectivity that causes morphological, functional and behavioral deficits. Microglial phagocytic function has been implicated to have a central role in synaptic pruning; however, neuronal "eat-me" signal that discriminates weak and strong synapses remains to be identified.

Materials and Methods: Using organotypic hippocampal slice cultures and *in vivo* mouse models we investigated the role of phosphatidylserine (PtdSer) as a neuronal surface signal that labels synapses for elimination thus ensuring proper brain development and circuit maturation. Morphological findings were complemented by electrophysiological study of wild type brains and those with aberrant PtdSer scrambling.

Results: We found that PtdSer labels a subset of synapses and has an important role in neuron-microglia contacts. Synaptic development in maturing brains was affected in animals that lacked PtdSer scramblases, as assessed by morphological and electrophysiological analysis. The maturation of synaptic network followed different timelines in wild type male and female brains and these differences were mirrored in microglia function.

Conclusions: PtdSer is the first „eat-me" signal that has been established in developmental synaptic pruning of unnecessary synapses by microglia. Identification of the tag that labels synapses destined for elimination enables us to investigate how synapses are discriminated for maintenance or selection in developing brain.

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Tau Protein and Neurodegeneration

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Alzheimer's disease (AD) is one of the most common neurodegenerative disorders, incidences of which increase with age. It is expected that in aging populations of Western countries AD may become one of the main health and socio-economic problems during next decades. Molecular mechanisms of pathogenesis of AD are not well understood, therefore therapeutic treatments for this disease are symptomatic and not effective. Morphological features of AD include accumulation of neuritic plaques and neurofibrillary tangles followed by cortical atrophy with loss of substantial numbers of neurons in affected areas of brains. The main pathogenic molecules in plaques and neurofibrillarly tangles are thought to be extracellular aggregated beta amyloid peptides and covalently modified intraneuronal tau protein. However, phosphorylated tau protein is also found in cerebrospinal fluids of AD patients. Moreover, recent evidence suggests that tau can be also actively secreted from affected neurons. Whether such extracellular forms of tau protein can exert neurotoxic effects in the brain is not clear yet. In this talk we will discuss our recent results on the effects of various isoforms and aggregated species of extracellular tau on viability of isolated primary brain cells, neuroinflammatory responses in mixed neuronal-glial cell cultures. We show that extracellular tau may cause progressive loss of viable neurons and that this involves activation of certain protein kinases and sphingomyelinases. Possible mechanisms of extracellular tau-induced neurotoxicity will be discussed.

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Challenges and Advanced Strategies in Treatment of Neurodegenerative Disorders

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Neurodegeneration is a process leading to loss of structure or function of neurons, and resulting in their death. Process is progressive and causing mental or physical disability. The most common neurodegenerative disease is Alzheimer disease (AD). Treatment investigating this disease is challenging because pathogenesis of the disease is not well understood till date. Despite of millions affected people there is no pathognomonic treatment for this condition. Many drugs and therapies were investigated and most of them failed for not always understood reasons. First challenge for clinicians and researchers is accurate diagnosis which is even more important in preclinical stages. Only well selected groups can be suitable candidates for specific treatment. There is no comprehension which mechanism should be target first, and this is another challenge. The reason why multitargeted therapies and disease modifying drugs are investigating is understanding that AD could be caused by chain reactions. Early onset of AD has clear genetic impact and this group is under observation. All drugs are divided into groups according to mechanism which they are targeting to: anti-Beta-Amyloid Agents (vaccines, APP-processing Enzyme Inhibitors, Antiaggregation of Beta-Amyloid,), agents affecting Tau aggregation, agent affecting CNS receptors, inhibitors of enzymes involved in neuronal signaling, antioxidants and compounds with undisclosed targets. For many years no new compounds were established as a new drug for daily AD treatments. Heterogeneous patient groups, and not well understood pathogenesis of the disease leads to the failure. Development of disease criteria based by the biomarkers, accurate diagnosis in early stages can be promising and helpful.

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Localisation of Neurons Innervating the Porcine Testis and their Plasticity in Changing Hormonal Conditions

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Background and Aim: Retrograde tracing experiments dealing with the origin of nerve fibers that supply the mammalian testis are very scarce. Nevertheless, there is some limited evidence that the innervation of this organ originates from pelvic ganglia (PG-s), caudal mesenteric ganglion (CaMG), sympathetic chain ganglia (SCG-s) and dorsal root ganglia (DRG-s). Similarly, immunohistochemical properties of neurons supplying the testis are not well described, and their response to altered hormonal conditions is unknown. Given these considerations, we decided to investigate these issues in detail.

Materials and Methods: The present study was carried out on sexually mature boars. All the animals were injected with Fast Blue into the right testis and then divided into four groups [(group 1 – control (G1), group 2 – hemicastreatad (G2), group 3 – castrated (G3), and group 4 – castrated and injected with testosterone (G4) boars)]. After a survival period of 3 weeks, G1 animals were transcardially perfused. In pigs of G2, the right testis was removed, whereas in G3 and G4 animals both testes were removed. The pigs of G4 were injected with testosterone. After two weeks, all the animals were transcardially perfused and then the ganglia were collected. The ganglia were cut into 12 μm-thick cryostat sections. The sections were stained using antisera against TH or DβH, VACHT or CHAT, NPY, VIP and GAL. Testosterone plasma levels were evaluated with ELISA test.

Results: The traced perikarya were found in the anterior pelvic ganglion (APG), CaMG, testicular ganglion (TG), aortico-renal ganglion (ARG) and RG. FB+ perikarya were also found in lumbar (L) and sacral (S) parts of SCG. The presence of the labeled neurons was restricted to the L3–L6 and S1–S3 ganglia and also in L1-L3 and S1-S3 DRG-s.

Immunohistochemical staining revealed that FB+ neurons located in pelvic paravertebral and prevertebral ganglia of the intact boars which stained for TH or DβH and/or NPY comprised approximately 60-70% of all FB+ nerve cell bodies. Up to 10% of all FB-containing cells were VIP-positive but only about 5% contained VACHT or CHAT. Among all FB+ perikarya, only less than 1% contained immunoreactivity to GAL. Up to 30% FB+ neurons were TH- or/and DβH- and VACHT- or/and CHAT-immunonegative, so they can be referred as to nonadrenergic/noncholinergic (NANC) neurons.

In experimental animals of all the groups profound changes regarded TH+ (enormous decrease in the amount) whereas the number of GAL+, VIP+ VACHT and NANC neurons increased significantly.

Conclusions: Our experiments have shown beyond doubt the significant influence of hormonal status on the plasticity of neurons supplying the testis.

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One-Sided Axotomy-Induced Changes in the Distribution of Neurons in the Porcine Male Intramural Ganglia of the Urinary Bladder Trigone

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Background and Aim: Function of the lower urinary tract to store and periodically release urine is dependent on neural circuits located in the brain, spinal cord and peripheral ganglia. Intramural ganglia of the urinary bladder trigone (IG-UBT) are one of important sources of nerve fibres supplying the lower urinary tract in mammals including the pig.

Since our knowledge on the distribution and projections of IG-UBT neurons in the male pig is very limited, we have used retrograde tracing combined with one-sided axotomy to elucidate these questions.

Materials and Methods: The study was performed on 10 juvenile male pigs of the Large White Polish breed. All the pigs were deeply anaesthetized with sodium pentobarbital. A midline laparotomy was performed to administer the fluorescent retrograde tracer Fast Blue into the wall of the UBT. After three weeks, in the same animals right-sided axotomy of nerve fibres projecting from the anterior pelvic ganglion (APG) to UBT was carried out. One week later all the pigs were euthanized and transcardially perfused with 4% buffered paraformaldehyde to fix the tissues. Then IG-UBT were collected and properly prepared for morphological research. The tissues collected were cut with a cryostat on 12 μm thick serial sections.

Results: The IG-UBT neurons formed characteristic clusters (consisting from a few to tens neuronal cells) found under visceral peritoneum or in the outer muscular layer. After axotomy, a dramatic reduction in the number of IG-UBT neurons, amounting up to 75% as compared to the values found in the control pigs, was determined.

Conclusions: This study has revealed a relatively large population of intramural UBT neurons, which probably contribute to the complexity of the urinary bladder neural pathways.

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Importance of Calcitonin Gene-Related Peptide in Relaxation of Gut Musculature and Diverticular Disease Pathogenesis

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Background and Aim: Calcitonin gene-related peptide (CGRP) is best known for its involvement in pain transmission. However, in previous studies it was shown that another major target of CGRP innervation are the intrinsic plexuses. In animal models CGRP was demonstrated to be able to induce peristaltic reflexes, increase peristaltic threshold, relax smooth muscle cells, induce phasic contractile activity and excite myenteric neurons. Regardless, CGRP's importance in mediating gastrointestinal motor activity and its role in diverticular disease (DD) was not previously investigated. Thus, we set out to investigate whether CGRP play any role in altered colonic motility observed in DD patients.

Materials and Methods: Specimens obtained from patients undergoing surgery for colorectal carcinoma served as control (n=10) and as asymptomatic DD (ADD) (n=10); sigmoid resection after recurrent attacks of diverticulitis as symptomatic DD (SDD) (n=10). Colon sections were processed for CGRP, its receptors CRLR (calcitonin receptor-like receptor) and RAM1 (receptor activity-modifying protein 1) and NOS (nitric oxide synthase) immunohistochemistry and analyzed by means of quantitative fluorescence microscopy. Isometric smooth muscle activity was recorded *in vitro* using organ bath technique.

Results: CGRP was found primarily innervating nitrergic neurons. CGRP expression decreased up to 40%, however CRLR – increased up to 25% in SDD in all divisions of enteric nervous system. ADD patients showed intermediate values for CGRP and CRLR. Second protein necessary for CGRP signaling – RAMP1 – expression was found to be unaltered. DD smooth muscle displayed 40% ($p<0.05$) reduced relaxation responses. CGRP induced relaxation was significantly improved and only 14-17% weaker compared to control samples.

Conclusions: CGRP signaling pathway is subjected to alteration in DD. Knowing that CGRP is involved both in smooth muscle contractility and intestinal motility it is reasonable to speculate that upregulation CRLR may have develop as a compensatory mechanism for declining levels of CGRP.

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Synaptic Theory of Working Memory and its Capacity

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Working memory refers to the ability to maintain and manipulate several items of information quasi-simultaneously, as e.g. when digits in a telephone number or words in a short sentence are perceived and then recalled. Capacity of working memory is estimated to be around 4 items for most of the people. Here I present a theory of working memory in which information items are represented by population spikes (brief epochs of synchronous activity) in selected populations of neurons in cortical networks. Population spikes are observed experimentally with electrophysiological recordings but their precise functional role remains unclear. I summarize our recent work on theoretical mechanisms of populations spikes emergence in cortical neural networks. I also present an analytical estimate of how many items can be maintained in working memory, i.e. what is its capacity, and show that for realistic parameters it fits well with experimental observations.

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Neuroprosthetic Systems Based on Microstimulators

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To build interfaces between the electronic domain and the human nervous system is one of the most demanding challenges of nowadays engineering. Fascinating developments have already been performed such as visual cortical implants for the blind and cochlear implants for the deaf. Yet implantation of most electrical stimulation systems requires complex surgeries which hamper their use for the development of so-called electroceuticals. More importantly, previously developed systems based on central stimulation units are not adequate for applications in which many sites must be individually stimulated over large and mobile body parts, thus hindering neuroprosthetic solutions for patients suffering paralysis due to spinal cord injury or other neurological disorders. It has been determined that a solution to these challenges could consist in developing addressable single-channel wireless microstimulators which could be implanted with simple procedures such as injection. However, past attempts in this direction were not successful because the developed implants were stiff and too large. Further miniaturization was prevented because of the use of inductive coupling and batteries as energy sources. Here I will present preliminary results from the eAXON project which is funded by the European Research Council. The eAXON project is aimed at exploring an innovative method for performing electrical stimulation in which the implanted microstimulators will operate as rectifiers of bursts of innocuous high frequency current supplied through skin electrodes shaped as garments. This approach will allow the development of injectable stimulators with a diameter of less than 1 mm. Most of the implants' volume will consists of materials whose density and flexibility match those of neighboring living tissues for minimizing invasiveness. In fact, implants based on the proposed method will look like short pieces of flexible thread.

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The Different Faces of Healthy Ageing Through the Brain Imaging Perspective

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Background and Aim: Understanding the modulation of action, perception and cognition across the lifespan requires in-depth investigation of the processes governing brain tissue changes.

Materials and Methods: We use quantitative magnetic resonance imaging (qMRI) biomarkers indicative of tissue myelination and iron levels in combination with diffusion-weighted microstructural imaging and tractography to study age related white matter tissue changes in a large cohort of more than 1100 healthy participants.

Results: Using a "connectome" approach we discover five unique white matter modules with similar lifespan tissue changes that overlap spatially with distinct brain function systems and provide quantitative support for two retrogenesis hypotheses of aging.

Conclusions: We interpret our findings in the context of modular structural connectivity networks, which advance throughout the lifespan according to a hierarchical architecture of maturation and degradation, driving age related changes to cognition and behavior.

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POSTER PRESENTATIONS

P 1. The Role of Phosphatidylserine in Synapse-Microglia Interaction

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Background and Aim: One of the key features of the postnatal nervous system development is neuronal structure refinement and excess synapse removal via synaptic pruning. Synaptic pruning is crucial for healthy brain and proper cognitive development as pruning impairment might lead to various neurological disorders. One of the most promising signaling molecules in regulation of this process, is phospholipid phosphatidylserine (PtdSer), a well-known "eat- me" signal that is exposed on extracellular layer of cell membrane and thus attracts microglia, the resident brain macrophages that removes target structures by phagocytosis. Milk fat globule-EGF factor 8 protein (MFG-E8) is a PtdSer-specific opsonin that coats PtdSer-exposing structures and mediates the engulfment of targets.

Importantly, recently it has been shown that microglia also plays a major role in synaptic pruning. The aim of this study was to investigate microglia interactions with pre- and postsynaptic structures in CA1 region of developing hippocampus and the role of PtdSer in guiding microglia to target synapses.

Materials and Methods: In this study we used fixed brain slices from Thy1::GFP;Cx3cr1::tdTomato mice and stained them immunohistochemically for MFG-E8. By using laser scanning confocal microscopy we looked for the colocolization of MFG-E8, microglia and neuronal signals and evaluated the effect of the presence of MFG-E8 for the interactions of dendritic spines and axonal boutons with microglia.

Results and Conclusions: Microglia showed tendency to preferentially contact presynaptic boutons rather than postsynaptic spines. Synaptic structures labeled with MFG-E8 were contacted by microglia 2-3 times more often than those without MFG-E8.

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P 2. MFG-E8 and Active Caspase-3 in Synaptic Pruning by Microglia

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Background and Aim: Microglia is brain resident macrophages that contribute to synaptic pruning during brain development. The mechanism of synapse elimination might be mediated by microglial engulfment of phospholipid phosphatidylserine (PtdSer) on neuronal synapses. This process needs to be strictly regulated, since both underpruning and overpruning might cause several neurodevelopmental diseases. Since microglia-synapse interaction is mediated by PtdSer exposure and milk fat globule-EGF factor 8 (MFG-E8) (see Bruzas et al.) we investigated the role and the mechanism of PtdSer scrambling in developing wild type and XK-related protein 8 (Xkr8) scramblase deficient hippocampi. Xkr8 promotes PtdSer exposure on the extracellular layer of plasma membrane after it is cleaved by active caspase-3 (act-casp-3).

Materials and Methods: We labeled MFG-E8 and act-casp-3 in wild type and Xkr8 knock out mice at the peak of developmental synaptic pruning at postnatal day 15 (P15). Confocal images of immunohistochemically labeled brain slices were subjected to 3D image analysis to define individual signals of MFG-E8, act-casp-3 and the dendrites of hippocampal CA1 pyramidal neurons.

Results: We found that Xkr8 knock out females had reduced PtdSer exposure and diminished caspase-3 activation in CA1 region of hippocampus at P15. This effect was not observed in males.

Conclusions: Xkr8 may promote act-casp-3-dependent PtdSer exposure on the surface of neurons thus presenting an "eat-me" signal for microglia-mediated synaptic pruning. Impaired PtdSer scrambling affects postsynaptic compartment only in females, suggesting that developmental profile of synaptic pruning differs in juvenile male and female mice.

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P 3. Advanced 3D Image Analysis and Machine Learning to Investigate Microglia in Developing Brain

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Microglial cells are the macrophages of CNS and are associated with immune surveillance of the brain. Microglia is activated during inflammation to maintain CNS homeostasis and to defend the brain against pathogens. In the healthy brain, these cells play an important role in the remodeling of neuronal circuits and contribute to synaptic pruning in developing brain.

Microglial cells have a highly flexible morphology. Individual cells can cycle reversibly from an amoeboid to a ramified form and functional activity of microglial cells correlates with their morphological changes. This transition can be very rapid (especially in neuroinflammatory processes) or can be absent for years in healthy mature brain. The majority of studies focus on microglia features in the context of diseases and neuroinflammatory processes, but little is known about microglia role and morphological properties in developing brain. To understand microglia morphological and functional features during normal development of hippocampus, we used fixed brain slices and immunohistochemical labeling of microglial cells at different postnatal age (P8, P15, P28, P40) of wild type mice.

Comprehensive morphological analysis by 3D reconstruction and Sholl analysis we evaluated changes of microglial cells during development. To avoid commonly used highly subjective classification of microglial cells by visual investigation we used artificial intelligence (machine learning) for automatic classification of microglial cells in developing brain.

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P 4. Small $A\beta_{1-42}$ Oligomer-Induced Neurotoxicity in Primary Brain Cell Cultures is Mediated By Overproduction of Mitochondrial Superoxide

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Background and Aim: The accumulation of abnormal amyloid beta (Aβ) is involved in neurodegenerative diseases including Alzheimer's disease (AD). Recent research demonstrates importance of oxidative processes in AD. Mitochondria may have a strong contribution to reactive oxygen species (mtROS) production, however, the role of mtROS in Aβ-induced neuronal death is not entirely understood. In our study, we investigated whether various $A\beta_{1-42}$ aggregates, small (<5 nm) $A\beta_{1-42}$ oligomers, large (>5 nm) $A\beta_{1-42}$ oligomers and insoluble $A\beta_{1-42}$ fibrils are capable to induce mtROS generation in neuronal/glial cultures.

Materials and Methods: We used primary rat neuronal/glial cell cultures to analyze neurotoxic effects of $A\beta_{1-42}$. Mitochondrial superoxide (mtO_2^-) was detected using MitoSOX Red, mitochondrial membrane potential- with MitoTracker CMTM-Ros Orange dye, extracellular glutamate was measured with Amplex Red/Glutamic Acid Assay Kit.

Results: Results showed that only small $A\beta_{1-42}$ oligomers induced mtO_2^- production in neurons and microglia. Large $A\beta_{1-42}$ oligomers and $A\beta_{1-42}$ fibrils did not cause mtO_2^- production. In parallel, small $A\beta_{1-42}$ oligomers caused early mitochondrial depolarization and increase of extracellular glutamate concentration. MitoTEMPO, a scavenger of mtO_2^-, significantly decreased superoxide level in both, neurons and microglia after 1 h incubation with small $A\beta_{1-42}$ oligomers. In addition, MitoTEMPO restored mitochondrial membrane potential and glutamate level to normal level. Importantly, small $A\beta_{1-42}$ oligomer-induced neuronal death and loss was significantly decreased by MitoTEMPO after 24 h.

Conclusions: Our data show that the early event in small $A\beta_{1-42}$ oligomer-induced toxicity is production of mitochondrial superoxide in neurons and microglia, which leads to mitochondrial depolarization, release of glutamate, and subsequent neuronal death. Therefore, pharmacological inhibition of mitochondrial ROS can protect neurons against small $A\beta_{1-42}$ oligomer-induced damage in AD.

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P 5. Effect of Itaconic Acid on Neuronal Cells

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Background and Aim: Itaconic acid is an organic, unsaturated dicarboxylic acid that plays an important role in immunity. Recently, biosynthesis of itaconic acid was found in mammalian immune cells and has been linked to inflammation. This acid acts as an endogenously formed antimicrobial compound that contributes, together with several other inflammatory metabolites and cytokines, to an efficient immune response. However, effect of itaconic acid on brain cells is not investigated yet.

The aim of our study was to investigate the effect of itaconic acid on viability of cerebellum granule cells (CGC) under normoxic and hypoxic conditions.

Results: We found that itaconic acid at high physiological concentrations (8-10 mM) was directly toxic to CGC neurons and induced apoptotic cell death over 24 h incubation at normoxic conditions. Microglial cells were not affected by itaconic acid. The neurotoxic effect of itaconic acid increased under hypoxic conditions: substantial neuronal apoptosis was induced at lower, 1mM concentrations. During longer incubation time itaconic acid caused disappearance of neurons from CGC cultures: total number of neurons decreased significantly after 72 h incubation at normoxic and hypoxic conditions, but do not after 24 h.

Conclusions: Our data suggest that itaconic acid can be neurotoxic at physiological concentrations particularly under hypoxic conditions.

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P 6. Neutral Sphingomyelinase Mediates Extracellular Tau Protein Caused Cytotoxicity in Primary Neuronal-Glial Cultures

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Background and Aim: The importance of ceramide in Alzheimer's disease (AD) pathogenesis has been supported by evidence describing alternations of ceramide levels and gene related to ceramide metabolism expression upregulation in AD brains. However, the link between disrupted ceramide production, neurotoxicity and pathological exogenous proteins (Aβ or tau) still need further detailed examination. Supposedly, that pathological proteins could affect the activity of enzymes leading to the dysregulation of ceramide synthesis in brain cells. The aim of our study was to investigate the toxic effect of extracellular tau on brain cell culture and whether the inhibitor of neutral sphingomyelinase (nSMase) may suppress neurotoxicity of exogenous tau (2N4R isoform).

Materials and Methods: Cultures of rat cerebellar granule cells (CGC) were treated with various type of monomeric or aggregated recombinant human 2N4R tau plus/minus 13 μM GW4869 for 48 hours. Phagocytic activity of microglial cells in pure primary cultures was assessed using 2μm carboxylate-modified microspheres. Neuronal and microglial cells viability/densities and microglial phagocytosis were evaluated by fluorescence microscopy.

Results: Our results show that tau2N4R exhibits conformational state-independent neurotoxicity in CGC cultures. Tau2N4R causes significant loss of neurons (~70%), without any increase in apoptosis or necrosis, and microglial proliferation (~200%) after 2 days of treatment. Pre-incubation with GW4869, selective inhibitor of nSMase, restores neuronal and microglial cell densities to the control level. Further experiments have shown that GW4869 completely suppresses tau2N4R enhanced phagocytic activity of microglial cells in pure microglial cultures.

Conclusions: Our data suggest that nSMase is required for tau2N4R induced neuronal loss and microglial proliferation in CGC cultures. Moreover, nSMase contributes to the regulation of the microglial phagocytosis under exposure to tau2N4R.

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P 7. Inhibition of Complex I Protects Rat Brain Against Ischemic Damage

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Background and Aim: Ischemic stroke remains one of the leading causes of death and long-term disability in developed countries. Oxygen and glucose deprivation-induced brain cell death is closely linked to mitochondrial dysfunction due to failure of energy metabolism and opening of mitochondrial permeability transition pore (mPTP). There is growing evidence that respiratory chain complex I underlies the pathology of some neurological and heart disorders related to the increased oxidative damage. Altogether, inhibitors of complex I could be effective against ischemia-induced brain damage. So, the aim of this study is to investigate the effect of complex I inhibitors on mitochondria permeability transition and cell death during brain ischemia.

Materials and Methods: Pharmacological agents were infused to the rat tail vein for 20 min. After cervical dislocation removed brains were exposed to deep hypoxia (93% N_2, 5% CO_2, 2% O_2). Brain mitochondria were isolated by differential centrifugation. Calcium retention capacity (CRC) as a measure of opening of mPTP was assessed fluorimetrically using Calcium Green 5N dye. The activity of complex I was investigated spectrophotometrically by NADH oxidation rate. Infarct area was measured using 2,3,5-triphenyltetrazolium chloride (TTC) staining in brain slices.

Results: Our results show that ischemia increases sensitivity of brain cortex and cerebellum mitochondria to Ca^{2+}-induced mPTP opening by half and enlarges infarct area up to 38 % and 60 % respectively. Rotenone, typical I complex inhibitor, re-establishes resistance to Ca^{2+}-induced mPTP opening of both, cortex and cerebellum mitochondria to the control level. Moreover, rotenone perfusion reduces the infarction area to 7% and 14% of total cortex and cerebellum area. Another observation of our study was that anthocyanins (cianidin-3-O-gliucoside and pelargonidin-3-O-gliucoside), naturally occurring flavonoids, can act as complex I inhibitors. And infusion of anthocyanin before ischemia significantly reduces brain damages.

Conclusions: In summary, the current study suggests that inhibition of complex I has neuroprotective effect against ischemia induced mPTP opening and cell death.

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P 8. Neuroprotective Properties of Anthocyanidin Glycosides Against H_2O_2-induced Glial Cell Death

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Oxidative stress, which is characterized as an imbalance between the production of ROS and the activity of antioxidant defense system, may contribute to H_2O_2-induced CNS injury. Therefore, the blockage of ROS generation might offer a significant protection primarily of glial cells, and consequently neurons. The aim of our study was to investigate the neuroprotective effects of several anthocyanins in combination with their antioxidant/pro-oxidant activity against H_2O_2-induced oxidative stress in C6 glial cells *in vitro* (G.Ereminas et al., 2017).

We showed that all tested anthocyanins, namely delphinidin (Dp), cyanidin (Cy), malvidin (Mv), peonidin and pelargonidin (Pg) 3-*O*-glucosides, and Dp and Cy 3-*O*-rutinosides exhibited pro-oxidant behavior at low (5 μM) concentration. Whereas at 150 μM only Dp 3-*O*-glycosides and Mv3G retained such a property. Thus, the effectiveness of various anthocyanins to increase the level of H_2O_2 in the medium decreased in the order: [Dp3G=Dp3R] > Mv3G > [Cy3G=Cy3R] ≈ Pn3G ≈ Pg3G.

Based on these findings and according to the set of functional groups on the B-ring of tested anthocyanins Cy3G, Mv3G and Pg3G were selected as candidates for the protection of glial cells against H_2O_2-induced oxidative stress. Thus, it was revealed that Cy3G (5-20 μM) and Mv3G (10-20 μM) but not Pg3G protected glial cells against H_2O_2-induced necrotic cell death. Moreover, all these anthocyanins sustained the glutathione antioxidant defense system. In addition, we believe we are the first to show that Cy3G, as the most prominent antioxidant among tested anthocyanins, sustained the resting respiration rate close to the control value but did not increase the efficiency of oxidative phosphorylation in H_2O_2-affected glial cell mitochondria. Thus, we think that our findings may inspire the development of anthocyanin-based pharmaceuticals that can be used in medical brain treatment.

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P 9. Plasticity of the Porcine *Longissimus Dorsi* Muscle-Projecting Sensory Neurons 4 Days After Bupivacaine Injection in Animals Pretreated With Simvastatine or Deksamethazone

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Background and Aim: Bupivacaine (BUP) is popular local anesthetic used in the veterinary and human medicine and has a documented negative influence on the sensory neurons. Combined retrograde tracing utilizing Fast Blue (FB) and double immunocytochemistry were applied to describe the influence of the bupivacaine on the primary afferents innervating *longissimus dorsi* muscle (LDM) in pigs pre-treated with dexamethasone (DEX) or simvastatin (SIM).

Material and Methods: Young female pigs were divided into 4 groups (n=3). All animals were injected with FB into LDM. After 3 days animals of group D started to be treated with DEX, animals of group S with SIM. After next 14 days all pigs, excluding animals of control group (K) were subjected to BUP injection. BUP was injected into the LDM in this same place where FB was injected 17 days before. The animals were sacrificed after 4 days and the spinal ganglia were collected.

Results: Mean number of FB+ perikarya in animals of K group was 52±7.6 per animal. No immunoreactivity against Casp3 was observed.13.33% of labeled cells contained Gal-IR, while 20% was PACAP-IR.

4 days after BUP injection mean numbers of traced somata were significantly smaller: B:3.5±1.7, S:6.2±3.8, D:3.5±2.5.The number of immunoreactive perikarya for Casp3 increased rapidly and reached in B:24%, in S:35% in D:31%.

Gal-IR was expressed by 20% of perikarya in B group, 13% in S group while in D group this population was 27%.

The number of PACAP+ neurons increased in all groups: B:38%, S:19%, D:24%.

Conclusions: The significant decrease in the number of FB labeled cells in animals of experimental groups can be explained in two ways: it is possible that BUP causes damage to sensory nerves or inhibits the FB absorption.

In animals treated with SIM and DEX the percentage of neurons expressed the Casp3 immunoreactivity was lower which can suggest strong neuroprotective action of these drugs.

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P 10. Nerve Remodeling and Inflammation in Diverticular Disease

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Background and Aim: The enteric nervous system intrinsically coordinates digestion and integrates information from extrinsic autonomic innervation. Current research suggests that disrupted neural control of colonic motility plays a pathogenetic role in diverticular disease (DD). However, there are few studies that examine the fine structure of the enteric nervous system. Our aim was to study the ultrastructure of the enteric nervous system of the colon.

Materials and Methods: Six patients with asymptomatic DD (ADD), 10 patients with symptomatic DD (SDD), and 11 healthy patients were used for the study. SDD sigmoid colon samples were collected from patients who underwent elective surgery. Specimens of healthy (control) and ADD-affected sigmoid colon were collected from patients operated on for non-obstructing colon tumours. Samples of the myenteric and submucosal plexuses with the surrounding tissue were observed with a transmission electron microscope.

Results: Neurons in patients with SDD exhibited lamellar bodies and lipofuscin-like inclusions. Axons appeared fragmented, more swollen axons were found in the myenteric plexus of SDD samples (controls 6.9%, ADD 10.4%, SDD 13.3%, $p=0.046$). The mean area of healthy axon profiles was lower in asymptomatic patients (controls 0.69 μm^2, ADD 0.49 μm^2, SDD 0.71 μm^2, $F=10.32$, $p<0.0001$). The percentage of swollen axons (controls 4.3%, ADD 8.4%, SDD 9.8%, $p=0.01$) as well as the size of the axons (controls 0.66 μm^2, ADD 0.68 μm2, SDD 0.54 μm^2, $F=3.50$, $p=0.03$) in the submucosal plexus changed as well. Finally, mast cells were often found near nerves in the inner submucosal plexus.

Conclusions: Diseased patients show ultrastructural alterations of the enteric nerve system (ENS). The morphometric data give evidence that the disease is associated with changes related to inflammation and remodeling. Further findings might prove important in understanding the disease. This study was supported by the grant from the Research Council of Lithuania (SEN-15023).

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P 11. Interspecific Differences of Neurons Distribution Within the Sinoatrial Node Region

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Background and Aim: Intrinsic cardiac ganglia are involved in the control of the heart rate, what provides the heart with a measure of protection against the possibility of „overdrive" implied by the centrally driven system. Considering that importance of small population of neurons residing within sinoatrial node (SAN) and presumably designed for its innervation is extremely big. Rabbit, pig and sheep are widely used in cardio physiological experiments however their SAN innervation is still not fully examined. The aim of the study was to compare number and the density of neural cells in rabbit, pig and sheep SAN.

Materials and Methods: Whole mount preparations of SAN from 4 juvenile rabbits, 5 piglets' and 4 sheep's (newborn or first day dead) hearts immunohistochemically labeled for HCN4 (as conductive myocytes marker) and PGP9.5 (as general neural marker) were used for this study.

Results: Rabbit's SAN area was 18.0±2.4 mm^2, piglet's 52.2±5.2 mm^2 and sheep's 70.8±5.4mm^2. Single neurons and small ganglia were observed in all species. On average rabbits' SAN contained 12.4±1.2 ganglia that consisted of 3.6±0.3 and solitary neurons. Approximate number of neurons within rabbits' SAN was 57.2±10. The density of neurons within rabbits SAN is 3.8 neurons for 1 mm^2. Piglets' SAN contained 97.5±10.1 ganglia, from randomly chosen (168) was revealed that average ganglia consisted of 21.3±2.4 neurons and generally piglets' SAN contained more than 2000 neurons. And that were 41 neural cells in 1 mm^2 pigs SAN. Sheep SAN contained 422±18 ganglia. Ganglia size were evaluated in randomly chosen ganglia (n=423). Average ganglia were composed of 21±2 neurons, and generally sheep's SAN contained more than 18100 neurons. And it was 255 neurons in 1 mm^2.

Conclusions: The number of neurons is not dependent on the size of the SAN. Neurons number and distribution density within SAN is species dependent – 4 neurons in rabbit vs. 41 in pig vs. 255 neurons in sheep's 1 mm^2.

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P 12. Immunohistochemical Characterization of the Intrinsic Cardiac Innervation in the Mouse Ventricles

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Background and Aim: According to previously performed studies, cardiac ventricular innervation is highly heterogeneous between species. Considering major differences between mammalian heart and respiratory rates, intrinsic cardiac innervation may play a key role in the physiological regulation of cardiac parameters. In this study, the mouse, a model of a small and highly metabolically active animal, was chosen to investigate immunohistochemical features of ventricular innervation.

Materials and Methods: Three and five adult mice were used for transverse sections of cardiac ventricles and whole-mount ventricular preparations, respectively. Immunohistochemical staining for protein gene product 9,5 (PGP 9,5), tyrosine hydroxylase (TH), choline acetyltransferase (ChAT), substantia P (SP) and calcitonin gene-related peptide (CGRP) was performed. Positively stained neural structures were explored by calculating the area, nerve fibres (NFs) composition and innervation density.

Results: The mouse cardiac ventricles were innervated by the thin epicardial plexus and dense myocardial meshwork mainly composed of adrenergic TH (+) NFs. Both general and adrenergic innervation was 30% denser in the right ventricle compared to the left one, while the septum had the least innervation. Moreover, TH(+) small intensively fluorescent (SIF) cells were observed at the basal level of ventricles. Commonly, these cells were clustered into groups and situated within the epicardial nerves or separately on the ventricular walls. PGP 9,5(+) nerve cells were not found. Sensory SP(+) and CGRP(+) NFs were sparse in the epicardial nerves and myocardial meshwork, though more abundant nearby the blood vessels. Cholinergic NFs were not found.

Conclusions: Considering these findings and the mouse cardiac parameters, it is presumable that enhanced adrenergic innervation in the mouse ventricles may be related to the high heart and respiratory rates.

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P 13. Heartbeat Evoked Potentials (HEP) Capture Brain Activity that Affects Subsequent Heart Period

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Background and Aim: Beat-to-beat heart rate variability is known to be associated with neural activity not only in reflexes regulatory areas (e.g. medulla oblongata), but also in higher brain areas (e. g. ventral anterior cingulate cortex); however, this particular knowledge about influence of higher brain areas were obtained using fMRI or PET methods that have poor temporal resolution. Meanwhile, heartbeat-evoked potentials (HEP) allow to capture temporal dynamics of brain activity that is related with heart cycle. The aim of this study is to investigate temporal dynamics of neural activity that contributes to length of subsequent heart period.

Materials and Methods: Sixty healthy participants (67% males, aged 19-31 years) were asked to sit still and fixate gaze on the computer screen, while EEG and EKG were recorded. EKG R peaks were identified and used for splitting EEG data into epochs. All epochs were classified into two groups, depending on difference between adjacent R–R intervals (RRI), i.e. whether epoch-related RRI was longer (shorter) than subsequent RRI. HEPs were obtained by averaging all EEG epochs of particular group. HEPs associated with prolongation vs. shortening of RRI were compared by performing permutation statistical analysis.

Results: HEP amplitudes at middle of diastole were more positive at centroparietal scalp sites before prolongation of RRI (i.e. before decelerating of heart rate) compared to amplitudes before shortening of RRI (i.e. before accelerating of heart rate).

Conclusions: HEP allow to capture activity in higher brain areas, that contributes to prolongation of subsequent heart period.

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P 14. Validation of Parkinson's Disease Sleep Scale-2 in Lithuanian Patients

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Background and Aim: Sleep disturbances and nocturnal disabilities play an important part of non-motor symptoms in Parkinson's disease (PD). Parkinson's Disease Sleep Scale -2 (PDSS-2) assess a wide spectrum of disease specific sleep problems and is easy to administer as a patient self-rating scale. The aim of this study is to translate and culturally adapt PDSS-2 for Lithuanian PD population.

Materials and Methods: This study was carried out at the Department of Neurology, Hospital of Lithuanian University of Health Sciences.

The study consisted of two parts: evaluation of both PDSS-2 Lithuanian translations by PD patients and interviews regarding any issues on Lithuanian version of PDSS-2.

Patient inclusion criteria were PD diagnosis, age above 18yo. Exclusion criteria were non-native Lithuanian speaker and patients diagnosed with dementia.

PDSS-2 was translated into Lithuanian by two independent professional translators.

After first part of the study our group drew a conclusion to validate and back-translate patients' preferred Lithuanian translation of PDSS-2.

Multidisciplinary team included two professional forward translators (from English into Lithuanian), one backward translator (Lithuanian into English), primary investigator and study supervisor.

Results: 41 patients with PD completed the PDSS-2, and their mean score was 21 out of 60. The score was equal or greater than the cut-off value of 15, indicating poor sleep, in 27 patients (65.5%).

35 patients of PD answered both forward translations. B translation was preferred over A translation (25.7% vs. 20%; $p>0.05$. 54.3% of respondents preferred both Lithuanian translations equally or refrained from answering). Patients prioritized 'drebulys' (Lithuanian translation of word 'tremor') over 'tremoras' (45.7% vs. 40%, 14.3% of patients refrained from answering). B translation was chosen for further PDSS-2 validation for Lithuanian PD population. After completed back-translation of PDSS-2 and discussing Lithuanian translation of PDSS-2 with 6 PD patients (mean age 62yo, mean duration of interview 4:40min) some linguistic changes were applied to Lithuanian version of PDSS-2.

Conclusions: After the completion of linguistic validation steps the PDSS-2 is considered ready for clinical validation in Lithuanian PD population.

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P 15. Descriptive Epidemiological Analysis of Primary Pediatric Malignant CNS Tumors in Lithuania in 2007–2016

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Background and Aim: Primary central nervous system tumors in children are one of most common diagnosed pediatric tumors and are related with high mortality. The aim of this study was to evaluate the epidemiological characteristics of primary pediatric CNS tumors in Lithuania.

Materials and Methods: 106 pediatric patients (0–18years) were diagnosed with primary CNS tumor in Lithuania in 2007–2016. Data for the study was gathered from National Cancer Institute and Kaunas Clinics, Hospital of Lithuanian University of Health Sciences databases. Tumors were classified according to histopathological type following WHO 2016 classification of tumors of central nervous system.

Patients were grouped according to the WHO standard age groups and 0–4 year age group was divided into two subgroups (0–2, 3–4 years).

Results: The most common primary pediatric tumor was medulloblastoma (17%). Male predominance was noticed. Average age at diagnosis was 9.1years (±5.58). Recurrence of the tumor occurred for 11.32% patients. Recurrence of the tumor was related with early mortality. 34.9% of pediatric patients diagnosed with primary CNS tumors died during analyzed period (62.2% male).

Conclusions: The incidence of primary pediatric tumors in Lithuania has tendencies to increase. General epidemiological characteristics were found similar to reported in other European countries or USA.

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P 16. Brain Dopaminergic System: Genetic Variations, Personality Traits and Alcohol Addiction Risk

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Background and Aim: Brain dopamine system is involved in response to reward and reward associated cue, predicted reward error estimation, learning and motivation. Genetic polymorphism of dopaminergic system in brains determines personality traits such as proneness to anxiety, hopelessness, impulsivity, openness to experience, which might increase vulnerability to various addictions. This study applies genotyping to determine genetic polymorphism for DRD2/ANKK1, COMT and SLC6A3 genes that are involved in dopaminergic activity. Carriers of COMT (rs4680) 472G>A variant have slower enzymatic rate of COMT leading to increased dopamine in frontal cortex which suppresses impulsive response to cue associated with reward; DRD2/ANKK1 (rs1800497) 2137G>A variant is associated with lower D2 receptor amount and lower affinity; SLC6A3 (rs27072) 328C>T variant results in increased activity of DAT1 transporter and decreased amount of extra-synaptic dopamine in ventral striatum. Attention to gene polymorphism and addiction related personality traits is the novelty of our research.

Materials and Methods: Seventy four LSMU employees and patients from LSMU Neurosurgery Department were genotyped to determine MAF of chosen genetic variants. Risk of alcohol use disorder was assessed by Alcohol Use Disorders Identification Test (AUDIT). Personality traits will be assessed by specific scales: Barrat Impulsiveness Scale-11 (BIS-11) for impulsivity and impulsivity dimensions, Substance Use Risk Profile Scale (SURPS) for proneness to anxiety, hopelessness, impulsivity, and novelty seeking.

Results: Frequency of alleles were as follows: COMT rs4680 A: MAF=0.48; DRD2/ANKK1 rs1800497 A MAF=0.22; SLC6A3 rs27072 T MAF=0.30.

Milestones of the Project: To genotype and perform gene association and gene-gene interaction of DRD2/ANKK1, COMT and SLC6A3 with personality traits and alcohol addiction in large population-based cohort.

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P 17. Psychiatry or Neurology: A Case Study

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Background and Aim: A 40-year old patient was experiencing severe anxiety for 6 months. She was not able to relax and did not see the meaning to live or exist. A case study reveals an obsessive-compulsive disorder with mixed anxiety disorder complicated by epileptic seizures. The aim of this study is to present a complex case in order to understand if the epileptic fireplace has caused obsessive-compulsive and anxiety symptoms, or is it a comorbidity of these diseases.

Materials and Methods: Psychiatric history was collected, medication treatment was prescribed, computed tomography (CT) scan was performed, electroencephalogram (EEG) was recorded.

Results: Patient was diagnosed with obsessive - compulsive disorder, with mixed anxiety disorder. Medication treatment: Mirtazapine 30 mg; Escitalopram 10 mg; Alprazolam 0.5 mg. Medication treatment lasted 4 months. Patient's condition stabilized. After a while patient started doing much worse and started to have panic attacks. Patient was diagnosed with panic attacks and was admitted to a hospital psychiatric unit. CT scan showed no pathology. EEG showed an intense tendency to synchronized parosomic activity, exacted on the left temporal derivatives of the "sharp" wave group with reflection in frontal leads on both sides. Patient was diagnosed with panic disorder and was recommended to observe the dynamics due to changes of EEG. Medication intervention in hospital was continued. Patient's state improved. Medication doses were reduced. After one month of treatment patient arrived feeling better, however, psychiatrist suspected maniac state. For that reason the medications were reduced. Patient's condition stabilized. Minimal doses were prescribed. Patient was recommended to psychoanalyst. She attended three times (once a week) and disappeared. From that time on she did not answer psychiatrist or therapist phone calls.

Conclusions: It is important to find out if the symptoms have been caused by hyperactive waves in the frontal lobe. It would be useful to apply anticonvulsant medication alone and to monitor the patient's condition. Also it is important to find out if the symptoms were caused by neurological or psychiatric disorder.

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P 18. Menstrual Cycle Influence on Functional Hyperemia in Prefrontal Cortex

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Hormonal fluctuations during the menstrual cycle may affect various cognitive abilities (E.G.Souza et al., 2012). Despite a significant amount of literature published in recent years, this subject still lacks a consensus (D.I.Miller & D.F.Halpern, 2014). Whether these hormonal changes affect functional hyperemia in prefrontal cortex is a question that hasn't yet been resolved. This work is aimed to compare prefrontal cortex hemodynamic measures of two female groups during the different phases of menstrual cycle.

A total of 30 healthy 22±2.5 year-old females were examined: 17 females were in their luteal phase and 13 females in the follicular phase. The phase of the menstrual cycle and hormone levels were assessed theoretically using subjects report and calculated following canonical menstrual cycle. Functional hyperemia of the prefrontal cortex was measured using optical spectroscopy fNIR400 device (Biopac). For mental stimulation, we used the Wisconsin Card Sorting Test. Repeated measures ANOVA results show that there is no significant difference in functional hyperemia of prefrontal cortex between groups of females in the luteal phase and follicular phase. No significant difference was spotted for the analogous optodes of the two groups.

This may suggest that the menstrual cycle does not influence functional hyperemia of the prefrontal cortex, though more refined approach may be needed.

Neuroscience today tends to rely on male subjects, due to widespread belief that circulating sex hormones in females may "complicate" the results. However, it is evident that sex-related differences depend mostly on the task. More importantly, with example of our study, and regarding other researchers (Souza et al., 2012), we show that menstrual cycling does not necessarily influence cognition within females, within the frame of menstrual cycles.

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P 19. Oral Contraceptive Users Are Less Reactive to Visual Emotional Stimuli Than Naturally Cycling Women: An Event Related Potential Study

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Background and Aim: First introduced more than 50 years ago, oral contraceptives are used by more than 100 million women all over the globe. Sex hormones are closely linked to women's emotional well-being which is crucial for their social behavior, psychological health and decision making. There is scarce information available about the impact of synthetic components of oral contraceptives on women's cognitive abilities, brain structure and functions. The late positive potential (LPP) is a reliable electrophysiological index of emotional perception in humans. Higher LPP amplitude reflects stronger allocation of attentional resources to emotional than neutral stimuli. The amplitude is the largest for the stimuli most directly related to biological imperatives.

Materials and Methods: The current study examined differences in response to visual emotional stimuli from the International Affective Picture System between oral contraceptives users (OC, n = 39) and naturally cycling women (NC, n=39) using event-related potentials (ERPs) method.

Results: Study revealed the following:

- NC demonstrates significantly higher global brain activity than OC in the time interval from 565 to 1000 ms after the onset of the emotional stimulus.
- The largest and significant topographic differences between NC and OC groups in time interval from 160 to 1000 ms were found in central (C2, C4) and centroparietal (CP2, CPz) scalp areas.
- NC women demonstrate higher LPP amplitude (350 – 700 ms) in central, centroparietal areas when reacting to all emotional visual stimuli (high – low arousal, negative – positive valence) compared to OC women.
- In both groups (NC and OC) LPP amplitude is the largest for the most arousing stimuli (erotic and highly unpleasant) and the lowest for neutral ones.

Conclusions: To conclude, this study highlights NC women tendency to draw more attention to visual emotional stimuli compared to OC users. All women demonstrate attention bias towards pleasant pictures when compared to unpleasant stimuli with the same level of arousal (high or moderate).

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P 20. Do Females Pay More Attention to Changes of Own Body Signals?

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Background and Aim: The ability to detect signals that derive from one's body is called interoception. It covers both proprioceptive and visceroceptive signal perception. Interoception is an important function that helps in understanding the own body as 'self'. Impaired awareness of detecting changes of internal physical and physiological conditions is observed in psychiatric disorders, such as depression and anxiety. Interestingly, the prevalence and symptomatic manifestation of the disorders is more pronounced in females than in males. These observations raise questions whether females differ from males in their ability to be more focused and sensitive to their own body signals. Thus, our aim of the experiment was to evaluate gender-related effect on proprioceptive information processing.

Materials and Methods: Ten males and 10 females (20-27 years of age) enrolled in the experiment of electroencephalographic proprioceptive evoked potentials (PEP). A 64- channel EEG was recorded during the proprioceptive stimulation that consisted of a brisk change of weight of a handheld load. The stimulation was administered to left and right hands in the regular order.

Results: The results revealed that females had more positive responses than males between 48 ms and 232 ms after the stimulation. Also, stronger response activity in females than in males during this time interval was prominent independently from hand stimulated. In addition, as predicted, during 48-200 ms time interval hand-related contralateral brain activity patterns were detected, although laterality and gender did not interact with the results.

Conclusions: The component of PEP potential elicited at around 200 ms is considered to represent orienting response to the stimulus. Our results suggest that females pay more attention than males to changes of their body position in time and space.

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P 21. 40 Hz Steady-State Responses and Individual Sensory Preference

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Background and Aim: Fast repetitive sensory stimulation elicits a periodic steady-state response (SSR) which can be recorded with electroencephalogram (EEG). The response has the resonant frequency close to the frequency of stimulation. Nevertheless, behavioral correlates of this phenomenon are not known and there is an urgent need to look for behavioral correlates of 40 Hz SSRs due to their impairment in psychiatric disorders. We investigated the relationship of 40 Hz SSR elicited with auditory, visual and somatosensory stimulation and an individual sensory preference for learning.

Materials and Methods: Twenty-nine young healthy right-handed male subjects received visual, bilateral auditory and left hand somatosensory stimulation at 40 Hz frequency with concurrent EEG recording. The preferred sensory modality used for learning was measured through scoring obtained by the VARK questionnaire. 40 Hz SSR power was analyzed at five regions: somatosensory response at frontal and right centro-parietal, visual at frontal and centroparietal, and auditory at fronto-central electrodes.

Results: All 40 Hz stimulation elicited SSR responses. The highest response was to auditory stimulation. A negative correlation was found between auditory SSR power and VARK questionnaire auditory score (ϱ=-0.43; p=0.02).

Conclusions: The present study demonstrates possible relationship between auditory SSR and an individual sensory preference for auditory learning.

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P 22. Microsaccades and Illusory Motion

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Background and Aim: Oculomotor events such as microsaccades are thought to be highly related to the perception of visual illusions, such as the "Rotating Snakes", Fraser-Wilcox which are known to produce the strong perception of an illusory motion. Despite the popularity of the topic, not all aspect of this phenomena is thoroughly investigated.

Materials and Methods: Therefore, in this study, a total of twelve healthy subjects (5 males; 7 females) were asked to evaluate a set of visual stimulus, their eye movements were recorded using EyeLink 1000 and microsaccades were detected with a velocity-based algorithm (Engbert and Kliegl 2003). All participants had a normal or corrected-to-normal vision, and no apparent physical, neurological, and psychiatry disorder were present. We investigated whether there were any differences in microsaccadic eye movements regarding participants age and sex.

Results and Conclusions: In general, results show that younger adults (21 – 24 yr.) had slower microsaccades but higher microsaccade rate comparing to older adults (44 – 56 yr.) ($p<0.5$). Moreover, young adults are prone to see illusory motion more often than older participants (36% vs. 25% respectively). We found that women tend to have higher microsaccade rate and faster eye movements comparing to men ($p<0.5$). However, no significant correlations were found between the perception of illusory motion and microsaccadic eye movements. Nevertheless, the neural background of illusion perception could not be distinguished from oculomotor events.

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P 23. Visual Responses in the Rat Superior Colliculus Neurons Are Susceptible to Two Types of Adaptation

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Background and Aim: Repeated presentation of any stimulus usually leads to the reduced response amplitude. This phenomenon is called adaptation. In visual system adaptation helps to maintain responsivity of neurons during changing lighting conditions by reducing response amplitude and thus avoiding saturation. An ability to respond rapidly under different light conditions is especially important for superior colliculus (SC) neurons, responsible for visual attention, including the detection of visual objects potentially dangerous to the subject. Although adaptation in the primate collicular neurons has received much attention, little is known about quantitative properties of adaptation in rodent SC, an increasingly important model for vision research. Our aim was to establish temporal and spatial properties of adaptation in the rat SC neurons.

Materials and Methods: We employed single-unit extracellular recordings in urethane anaesthetized (1.4 g/kg) adult female rats. Visual stimuli were presented on a computer monitor by employing PsychoPy software.

Results: When visual stimuli were repeatedly presented in the same location, ON responses were reduced in the majority of SC single units. The recovery of response amplitude required several seconds and was much slower in the neurons possessing small diameter receptive fields (RFs, <20 degrees). However, when the adaptor stimulus was presented outside the small diameter RF, in the area of putative surround inhibition, responses to visual stimuli presented inside RF were enhanced in 7 out of 11 units. OFF responses were much more robust: they were less reduced by repetitive stimulus presentations, recovered faster than ON responses (<1 s), and adaptation effects were spatially limited to a fraction of the RF area.

Conclusions: We demonstrate that in rat SC neurons visual responses are shaped by at least two forms of adaptation of a complex spatiotemporal structure that can profoundly shape visual information processing in SC.

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P 24. GABA PAM Drugs Effect on The Oppel-Kundt Illusion Visual Processing for People With Schizophrenia Spectrum Disorders

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Background and Aim: A dysfunctional visual processing, visual stimulus integration and GABAergic dysfunction were found among patients with schizophrenia spectrum disorders (SSD). Better understanding of GABA positive allosteric modulators (PAM) effect on the Oppel-Kundt illusion manifestation for patients with SSD is needed to get insight in illusion genesis and visual dysfunction among people with SSD.

Materials and Methods: Subject and control group were patients from Hospital of Lithuanian University of Health Sciences (LUHS), Kaunas Clinics. Study protocol was approved by LUHS Bioethics Center. Exclusion criteria were any impairment causing inability to perform a test. The stimuli: white spots were presented horizontally against a black background according to the Oppel-Kundt pattern. Using computerized equipment, the subjects adjusted the unfilled part of the stimulus to be equal in length to the referential one. The number of the filling spots in the referential interval varied from 0 to 19. Each subject repeated experiments 10 times. GABA PAM prescriptions gathered from medical records. ANOVA, Bonferroni post-hoc test and T-test were used for statistical analysis.

Results: Study group: 30 persons with SSD and 25 matched individuals. During 170 of experiments patients involved in the study were on various GABA PAM. There were 130 experiments when patients were not on GABA PAM. Patients who were on GABA PAM during the experiment have made smaller errors than patients who were not on GABA PAM when presented from 1 to 18 referential interval filling spots, difference of the errors between two groups varied from 2,87 to 4,6 arc min. Patients who were on GABA PAM during the experiment have made notably larger errors than comparison group when referential line was filled with 0, 3, 9–14,19 spots.

Conclusions: The Oppel-Kundt illusion tended to manifest weaker for patients with SSD, but stronger for those who received GABA PAM.

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P 25. Study of Vertical – Horizontal Illusion With Different Stimulus Orientation

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Background and Aim: Imperfections of visual perception, particularly geometric optical illusions, allow us to explore in more details the principles of the visual image processing. Illusions' investigations extend our knowledge concerning the structure of the sensory systems and can be helpful in finding and developing new biomedical solutions. The aim of the present pilot study was psychophysical examination of the factors influencing the magnitude of vertical – horizontal illusion in experiments with stimuli made up of segments of real and imaginary lines.

Materials and Methods: In experiments, we investigated three modifications of the vertical-horizontal illusion, and the task of observers was to determine (by adjusting the test line segment to be equal in length with the referential one) the point of subjective equality. The length of the referential (vertical or horizontal) line or the empty spatial interval between the dots was fixed at 66 min of arc; the width of the lines and dots size was about 1 min of arc. The experiment consisted of 16 series for each of three different stimuli group; in each group 4 different stimulus orientations were used.

Twenty five naive observers (13 females, 12 males), with normal or corrected to normal visual acuity, took part in experiments. Observers' age ranged between 18 to 55 years. Participants gave their informed consent before taking part in the experiments, which were performed in accordance with the ethical standards of the 1964 Helsinki Declaration.

Results: It was determined in experiments that for all stimulus orientations the regularities of the illusion magnitude changes are qualitatively similar for types of stimuli, i.e., consisted of the real or imaginary line segments. It was demonstrated that for stimuli with the imaginary elements, the greatest effect was gathered for the changes in horizontal position of the vertical segment dividing the horizontal spatial interval. For stimuli with the real lines, the strongest effect was obtained for vertical positioning of the horizontal line relatively to the vertical one. A simple computational model was successfully applied to fit the experimental data obtained in the present study.

Conclusions: Vertical – horizontal illusion is more prominent for stimuli made up of real line segments. The illusion was strongest for stimulus orientation equal to 0º and weakest for orientation equal to 90º for all groups of stimuli.

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P 26. Filled-Space Illusion Caused by Continuous Distractor

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Background and Aim: In the filled-space illusion, the filled part of stimuli seems longer in juxtaposition with the empty one. The aim of present study was to try to develop a quantitative model capable to account the illusory effects induced by stimuli with continuous and discrete distractors.

Materials and Methods: In present study, we examined the strength of the filled-space illusion as a function of degree of contextual continuous filling of the referential interval of the three-dot stimulus. In the first series of experiments, the length of the line segment (placed in the center of the referential interval) varied from zero to complete filling of the interval. In the second one, the length of the segment was constant but the extension of the referential interval varied (the positions of the terminal spots of the referential interval moved aside from the line segment ends symmetrically, thus, forming the empty gaps and producing discontinuities in the filling). Subjects adjusted the length of the empty test interval to that of the reference, and the matching errors were considered as the illusion magnitude.

Results: The data obtained showed regular overestimation of the referential interval with completeness of the interval filling. The dependencies established were used to develop a new quantitative model, which was successfully applied to fit the experimental results of the present study and those obtained earlier for conventional Oppel-Kundt stimuli.

Conclusions: It was demonstrated that the model calculations appropriately correspond to all variations of the illusion magnitude changes for stimuli with continuous and discrete distractors.

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P 27. Variations of Experimental Data in the Length-Matching Task

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Background and Aim: The aim of the present study was to measure and compare variations of the Oppel-Kundt and Müller-Lyer illusion strength. The inter-subject and within-subject variations were considered.

Materials and Methods: Three spatial interval patterns were used in the experiments, because such the configuration of stimuli eliminates left-right asymmetry and produce stronger illusions. Oppel-Kundt illusory figures consisted of vertical stripes, dots or a single horizontal line. Müller-Lyer stimuli comprised wings composed of lines or dots. Twenty nine subjects participated in the experiments. The subjects' task was to adjust the length of the middle stimulus interval to be subjectively equal to the flanking reference intervals. Twenty trials were included for each data point analysis.

Results: At present study, the Oppel-Kundt and Müller-Lyer illusions were approximately equal in strength. The stimuli made up of lines produced distortions of 17.6 arc min and 16.6 arc min, respectively. The dotted stimuli induced 19.8 arc min vs. 16.2 arc min illusions. The strength of the Oppel-Kundt illusion evoked by the vertical stripes was 21.3 arc min. The two illusions did not differ according to their manifestation manner. The averaged individual standard deviation (SD) ranged from 4.1 arc min up to 4.6 arc min for the dotted versions of the Müller-Lyer and Oppel-Kundt figures, respectively. For control stimulus, SD was 2.9 arc min. The SD of the means ranged from 9.0 arc min to 10.5 arc min for the Müller-Lyer figure made up of lines and Oppel-Kundt figure with dots, respectively. For control stimulus, SD was 4.6 arc min.

Conclusions: The Oppel-Kundt and Müller-Lyer illusory figures produced stronger variations than that obtained with the control stimuli of the same length.

The perceived length variations were similar for both the Oppel-Kundt and Müller-Lyer illusions.

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P 28. Lateralization of Parietal Lobes for the Processing of Categorical and Coordinate Spatial Relations in Left and Right Handers: A fNIRS Study

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Background and Aim: The brain is hypothesized to process visuaspatial information in two ways: categorical (assigning objects to abstract classes e.g. above/below...) and coordinate (assigning objects by distances e.g. 1 m, 1 cm...). Many experiments have corroborated the hypothesis, associating left hemisphere with the categorical perception and the right hemisphere with the coordinate, emphasizing the importance of the parietal regions. Most studies focused on the brain lateralization of right handers, excluding left handers, as they usually show atypical lateralization. The goal of this study was to find evidence for categorical/coordinate task brain lateralization in both right and left handed people, using functional near-infrared spectroscopy (fNIRS).

Methods: For the experiment subscribed 28 right handers and 17 left handers. Each participant, while their parietal regions were being scanned with NIRScout fNIRS device, completed a blocked experiment, consisting of a cup and a dot. For the categorical task, they had to decide, by a button press, if the dot was inside or outside the cup, for the coordinate task, if the vertical distance between the dot and the horizontal line of the cup was shorter or longer than the horizontal line of the cup.

Results: Statistical analysis revealed Task*Handedness interaction for the right superior parietal lobe and the left supramarginal gyrus. Right handers showed activation in both regions for the coordinate task, but no activation for the categorical task. This successfully replicated the findings of previous positron emission tomography studies, demonstrating the importance of the region in precise visuospatial navigation. Left handers showed no significant activation in the parietal lobes.

Conclusions: This study proved that fNIRS can be applied on the researches of spatial relations processing. Right handers, as stated in previous studies, probably process categorical relations in the prefrontal areas. The question still remains about the regions of such processing in left handers.

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P 29. Application of fNIRS for Diagnosing Major Depressive Disorder and Evaluating Treatment Effectiveness of Transcranial Magnetic Stimulation

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Background and Aim: Major depressive disorder (MDD) is the leading cause of disability worldwide, leading to substance abuse and high suicide rates. For its diagnosis and clinical evaluation, medical doctors are widely using various depression rating scales and relying on patients' feedback. In recent years, there has been an increase in neuroimaging studies on MDD, showing reduced brain activation in the limbic system and the prefrontal cortex. Functional near-infrared spectroscopy (fNIRS) is quickly rising as a new tool for depression diagnosis, inexpensive and non-invasive, that could solve the problem of bias and possible manipulations of scale results. In our study, using fNIRS, we scanned MDD patients, who were being treated with drugs, and also drug-resistant patients, before and after transcranial magnetic stimulation (TMS) treatment.

We hypothesized to see decreased brain activation for both groups, but an increase in activity after TMS treatment.

Methods: A fNIRS device was made to measure left anterior prefrontal cortex (laPFC) activity changes, while patients (4 drug non-resistant; 5 drug-resistant) and 5 controls were performing a working memory Wisconsin Card Sorting Task. Drug-resistant patients were scanned two times: one before and one after the treatment (10 Hz left dorsolateral prefrontal cortex stimulation). The oxygenated hemoglobin concentrations were calculated using a custom MatLab script, based on the intensity changes of 735 nm and 890 nm wavelengths.

Results: Both patient groups showed poorer results in the performance of the Test in comparison to the control group, making more mistakes and taking more time to complete it. Patients showed significantly decreased laPFC activity. Drug-resistant patients had an increase in laPFC activation after TMS treatment, which was not significantly different from the control group. The fNIRS data strongly correlated with depression scale results.

Conclusions: fNIRS could be potentially used not only to aid diagnosing MDD, but also for the evaluation of treatment effectiveness. However, it is still not clear if the method could be applied for diagnosis of the earliest stages of depression.

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P 30. Role of Variants at SIRT1 Gene in Pituitary Adenoma

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Background and Aim: Pituitary adenomas (PAs) accounts for approximately 10% off primary brain tumors. It is usually located in the front two-thirds of the pituitary gland and may extend into surrounding structures resulting a lot of complications: headache, visual impairment and behavioral changes (American brain tumor association, 2017). The present study aimed to determine the association between two sirtuin 1 (SIRT1) gene polymorphisms (rs4746720 and rs3740051) and PA development and investigate how they associated with gender.

Materials and Methods: The study included 70 patients with a diagnosis of PA. The reference group involved 120 healthy subjects. The genotyping of SIRT1 rs3740051 and rs4746720 were performed using the real-time polymerase chain reaction method. The potential associations with single nucleotide polymorphisms (rs3740051, rs4746720) were evaluated for all patients.

Results: Genotyping results revealed that rs4746720 AA variant was observed with a frequency of 100 % in all study groups. Statistical analysis of SIRT1 rs3740051 did not reveal significant differences in genotype (AA, AG, GG) distribution between PA and control groups: 78.6%, 18.6%, 2.8% vs. 85.0%, 14.2%, 0.8%, respectively (p=0.301). Analysis of genotype distribution between females and males did not exhibit any significant differences either: genotype (AA, AG, GG) distribution in PA and control females (79.0%, 18.4%, 2.6% vs. 87.5%, 10.9%, 1.6%, respectively, p=0.517) and in PA and control males (75.0%, 21.9%, 3.1% vs. 82.1%, 17.9%, 0%, respectively, p=0.359).

Conclusions: The SIRT1 rs3740051 and rs4746720 polymorphisms were not associated with PA development.

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P 31. Chromatin Immunoprecipitation (ChIP) Assay Optimization and Runx3 Targets Analysis in Glioblastoma Cells

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Glioblastomas are the most aggressive malignant brain tumours with significant heterogeneity in molecular profile and patient outcome. Tumour heterogeneity is also determined by genes behaving as bifunctional modulators. Runt-related transcription factor 3 (Runx3) was described as both tumour suppressor and activator in vast of cancers as well as in gliomas. Bi-functioning of Runx3 may be explained by transcription factor ability to initiate or repress target genes expression. The aim of this study was to optimize ChIP experimental conditions and to analyze expression of selected Runx3 targets in human glioblastoma cells.

Chromatin immuneprecipitation – ChIP-qPCR assay was applied to analyze transcription factor Runx3 and its targets interaction in human glioblastoma U87 cell line. U87 cells were cross-linked using 1% formaldehyde at 70% confluence. The following steps included cell lysis, DNA ultrasonication, immunoprecipitation, immunocoplex purification with magnetic beads, DNA extraction and qPCR.

Optimization part showed that initial cell number, time of crosslinking and quenching as well as sonication and immunoprecipitation setting are critical ChIP factors. Runx3 showed high transcriptional interaction rates with AKT1, CLDN1, AKT1, ABCC1 genes, 78%, 54%, 51% and 36%, respectively, as compared to input control.

ChIP assay is commonly used for research of protein-DNA interactions that are present in living cells. The optimization of ChIP assay is one of the most complicated steps, which is the key to success of the entire research. Runx3 role in glioblastoma is undeniable, nevertheless the exact functioning is still unclear and Runx3 is often described as a "tumour modifier". ChIP data revealed the presence of Runx3-target complexes in U87 cells demonstrating Runx3 role for its targets regulation. Nevertheless, further analysis is necessary to show whether the increase or decrease in Runx3-target interaction and as well expression appears after Runx3 induction.

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P 32. The Role of Sema3c Protein *in vitro* Angiogenesis

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Background and Aim: Angiogenesis is one of the key processes in the growth and development of tumor. Class 3 semaphorins (Sema3) are characterized as Nrp/plexin-mediated axon guidance factors involved in tumor angiogenesis by interacting with the VEGF signaling pathway. Numerous studies showed that Sema3A and Sema3F function as inhibitors of tumor angiogenesis. However, the role of Sema3C in tumor angiogenesis is poorly understood: recent studies show that Sema3C is critical for the gastric cancer angiogenesis, on the other hand, this protein act as a selective inhibitor of pathological retinal angiogenesis. Therefore, in order to address the later problem, we examined Sema3C effects on microcapillary formation by human umbilical vein endothelial cells (HUVEC) *in vitro* angiogenesis assay.

Materials and Methods: Semaphorin-IRES-Venus encoding bicistronic expression vectors were constructed and used for transfection of 293FT cells. Sema3-A, F and C expression was confirmed by Western-blot and reverse transcription-PCR (RT-PCR) analyses. For microcapillary formation and cell migration assays, HUVEC cells were suspended in medium collected from transfected 293FT cells and seeded on 96-well plate coated with Geltrex for tube formation analysis and on 96-well Oris Cell Migration Assay plate. Results were analyzed after 16 and 24 h with Image J/Angiogenesis analyzer programs and statistically evaluated by the two-tailed Student's t-test.

Results: Western-blot and RT-PCR analyses showed that Sema3-A, F, and C were expressed in 293 FT cells and secreted in cell medium. Angiogenesis assay revealed that Sema3C significantly inhibited the formation of a tube-like HUVEC network, compared to control. Migration assay showed that Sema3C strongly induced HUVEC cell migration.

Conclusions: Our research indicates that Sema3C functions as an inhibitor *in vitro* angiogenesis. Further studies on Sema3C interaction with Nrp/Plexin complexes should reveal molecular aspects of Sema3C effect observed in our study.

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P 33. GFAP Expression in Varying Degrees of Glioma

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Background and Aim: Glial fibrillary acidic protein (GFAP) is an intermediate filament that provides mechanical support to astrocytes. Rs2070935 is single nucleotide polymorphism found in the promoter region of GFAP that could affect transcriptional activity. The aim of this study is to investigate GFAP expression at mRNA, protein levels in varying degrees of glioma and evaluate possible links with polymorphisms of rs2070935.

Materials and Methods: Sample size of 50 glioma tumors was investigated. GFAP expression at mRNA level was identified using quantitative reverse transcription polymerase chain reaction (qRT-PCR) with SYBR GREEN dye. Translational activity of the following gene was detected using western blot assays. Rs2070935 genotypes were identified using qPCR with TaqMan probes.

Results: This study found that grade II gliomas exhibited greater mean GFAP mRNA and protein expression than grade IV tumors ($p<0.0001$). GFAP transcriptional activity correlated negatively with increasing tumor grade ($r=0.6469$, $p<0.0001$). Comparison of GFAP transcriptional and translational activities revealed a positive correlation ($r=0.6546$, $p<0.0001$). Rs2070935 CC homozygotes featured greater amounts of total GFAP protein in grade II glioma, compared to CA heterozygotes ($p=0.0238$). Rs2070935 AA genotype is associated with poor prognosis for glioblastoma patients ($p=0.0007$).

Conclusions: A negative correlation between GFAP expression and glioma grade was observed. Rs2070935 is a potential prognostic marker for glioblastoma patients. Associations were discovered in grade II gliomas between increased GFAP protein levels and CC genotype of rs2070935.

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P 34. Investigation of Metallothionein MT1A, MT1E, MT1X, MT2, MT3 Expression, MT2 Single Nucleotide Polymorphism and MT1A Gene Promoter Methyation in Gliomas

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Background and Aim: Gliomas are primary brain tumors that originate from glial cells. Glioblastoma is the most common and aggressive type of glioma. Glioblastomas develop from lower grade gliomas such as astrocytomas grade I-III or manifest quickly without previous indications as astrocytoma grade IV. Metallothionein (MT) genes expression plays a crucial role in carcinogenesis of diverse malignancies. We proposed MT genes as prognostic markers for gliomagenesis.

Materials and Methods: MT1A, MT1E, MT1X, MT2, MT3 expression was measured by qRT-PCR using SYBR Green in 57 tumor tissue of patients with different grade glioma. Methylation status of MT1A gene promoter was determined in 53 tumor samples using MS-PCR. rs28366003 polymorphism is an A/G substitution of MT2 gene located in core promoter region between TATA box and TSS. SNP was genotyped by TaqMan assay in 143 glioma patients blood DNA. Statistical analyses were made to determine associations between gene expression, methylation status and SNP variants.

Results: MT1A, MT1E, MT1X, MT2, MT3 genes expression was elevated in high grade gliomas (grade III-IV) comparing with low grade gliomas (grade I-II). Statistically significant differences were determined for MT1A (Mann-Whitney test, $p<0.05$). High MT1A, MT1X and MT3 gene expression was associated with shorter patient survival rate ($p<0.05$). MT1A gene promoter was methylated in 67% of specimens (36/53). MT1A gene promoter methylation was not associated with gene expression. We determined that genotype frequencies of AA, AG, GG are 93.7%, 6.3%, and 0%, respectively. There are no associations between gene expression level and genotype.

Conclusions: Expression of MT genes increased in high grade gliomas and could be associated with gliomagenesis. However, further tests are needed to confirm this hypothesis.

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P 35. Possible Correlation of Metallothionein Content and Quantity of Some Trace Metals in Blood of Patients With Glioblastoma

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Background and Aim: Metallothioneins (MT) are cysteine-rich proteins, known to maintain homeostasis of trace elements, protect against heavy metal toxicity and damage caused by free radicals or pharmacological agents used in cancer therapy. The aim of the study was to find out correlation between MT content in blood and brain tumour tissue and content of trace elements zinc (Zn), copper (Cu), selenium (Se), lead (Pb) and cadmium (Cd) in blood of patients' with Glioblastoma.

Materials and Methods: The research was conducted using whole blood/plasma and brain tumour tissue of patients' (n=34) with Glioblastoma (WHO IV, confirmed pathologically) undergoing a surgical intervention. Metal content was determined by inductively coupled plasma mass spectrometry, and MT content was assayed spectrophotometrically.

Results: The results of our study showed that after surgical intervention the analyzed parameters decreased ($p<0.05$) as follows: MT content in blood (0.27±0.01 vs. 0.20±0.01 nM/ml), Pb content in blood (1.79±0.85 vs. 1.46±0.85 μg/dl), and Cd content in blood (0.06±0.04 vs. 0.05±0.03 μg/dl). Although literature suggests that Zn is expected to induce expression of MT, our regression analysis demonstrated inverse dependence of blood MT concentration on Zn content ($\beta=-0.002$, $p<0.05$) after surgical intervention. Cu and blood MT were related directly ($\beta=0.001$, $p<0.05$). Survival after brain tumour removal was inversely related with age ($r=-0.349$, $p<0.05$). Men tended to have higher MT concentration in brain tumour as compared to women (75.68±55.72 *vs.* 52.53 ±17.69 μg/g, $p<0.05$).

Conclusions: Analysis of our data showed that content MT in blood of patients' with Glioblastoma correlate with concentrations of Zn and Cu. Survival also correlates with age of patient's with Glioblastoma. Content of MT in brain tumour correlates with gender.

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P 36. The Antioxidant Effects of Green Tea Extract in Mice Brain Affected By Cd, Ni and Pb

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Background and Aim: The toxic manifestations of heavy metals, such as Cd, Pb and Ni, is not clear, but it's presumed that they can cause oxidative stress and it is implicated in aging and many diseases such as neurodegenerative disorders and various cancers. So, to reduce deleterious effects of these metals, it is necessary to develop a protective formulation that is effective in multi directional manner to living cells. One of possibilities is to focus to natural products (for example, green tea extract (GTE)), because plants react to heavy metal toxicity through immobilization, chelating and compartmentalization of the metal ions.

The aim of this study was to evaluate the antioxidant effects of GTE on the content of MDA and GSH in the mice brain affected by Cd, Ni and Pb.

Materials and Methods: Experiments were done on outbred white laboratory mice using intraperitoneal injections of $CdCl_2$, $NiCl_2$, $Pb(CH_3COO)_2$ and/or GTE solutions. The exposure-time was 14 days. GSH was measured by reaction with 5,5'-Dithiobis(2-nitrobenzoic acid) to give a compound that absorbs 412 nm light wavelength. Lipid peroxides were estimated by measuring thiobarbituric-acid reactive substances and were expressed as malondyaldehide (MDA).

Results: Our results showed that in Cd and Cd+GTE, Ni and Ni+GTE, Pb and Pb+GTE treated mice groups the content of GSH in mice brain was decreased by 24% and 33%, 18 and 30%, 31 and 35%, respectively, as compared to control. Meanwhile, in GTE treated mice group the content of GSH was at the control level.

In another series of experiments we indicated that Cd, Ni and Pb increased LPO in mice brain by 18%, 19% and 12%, respectively, as compared to control. Meanwhile, in other treated mice groups: GTE, Cd+GTE, Ni+GTE and Pb+GTE the content of MDA was decreased by 18%, 31%, 19% and 15%, respectively, as compared to control mice.

Conclusions: Our studies showed that GTE protected lipids from peroxidation, but didn't protect GSH from oxidation in brain of mice affected by Cd, Ni and Pb.

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P 37. Antioxidant Enzymes Activities in Mice Brain Under Buckwheat Extracts Treatment

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Background and Aim: The unique composition of Buckwheat contributes to their various health benefits such as anti-oxidative, anti-cancer, anti-hypertension, anti-diabetic, cholesterol-lowering, and cognition-improving. Buckwheat tends to contain high amounts of certain bioactive components such as rutin, therefore, showing higher efficiency in preventing/treating various disorders. The present study was conducted to evaluate the effects of Buckwheat blossom and leaf extracts on the enzymatic activities of superoxide dismutase and catalase in mice brain.

Materials and Methods: Experiments were done on 4-6 weeks old outbreed mice. Activities of enzymes were determined after 21-day of Buckwheat extract intragastrical administration. Control animals received the same volume of saline. Because buckwheat extracts were made in ethanol, thus second control group received the same volume of ethanol as well. Enzymes activities were evaluated spectrophotometrically.

Results: Our data showed that blossom as well as leaf extracts decreased SOD activity in brain of mice by 28% and 27% respectively as compared to the control group, while in ethanol treated brain of mice SOD activity found to be 17% lower. However, activity of CAT significantly increased after both buckwheat extracts treatment as well as under ethanol effect.

Conclusions: Our studies disclosed that repeated administration with Buckwheat blossom as well as leaf extracts has an impact on enzymatic activities of both antioxidant enzymes in brain of mice. The stimulating impact of ethanol on activities of both enzymes of mice brain has also been detected.

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P 38. Protective Effect of Zinc Against Nickel Induced Adverse Effects in Brain of Mice

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Background and Aim: There are proposed a number of possible toxicity mechanisms of nickel (Ni), including disruption of trace element homeostasis and formation of ROS. The aim of this study was to assess the effects of Ni on the activity of δ-aminolevulinic acid dehydratase (δ-ALAD), the content of reduced glutathione (GSH) and malondialdehyde (MDA) in brain of mice and to establish the ability of zinc (Zn) to protect brain from adverse effects of Ni.

Materials and Methods: For the single metal exposure, mice were once i.p. injected with a solution of $NiCl_2$ (96μmol Ni/kg bw) or/and $ZnSO_4$ (24μmol Zn/kg bw). For the repeated exposure, mice received 14 injections of $NiCl_2$ (19μmol Ni/kg bw) or/and $ZnSO_4$ (24μmol Zn/kg /bw) solutions. The control mice received injections of saline solution. The activity of δ-ALAD was examined by the method of Sassa. GSH was measured by reaction with DTNB, MDA was determined after reaction with thiobarbituric acid.

Results: Single exposure to Ni suppressed activity of δ-ALAD by 21% ($p<0.05$) and increased content of brain MDA by 78% ($p<0.05$). Single Zn^{2+} injection before Ni^{2+} diminished the suppressing effect of Ni on enzyme activity and provided some protective effect against Ni induced brain lipid peroxidation (LPO). Repeated Ni exposure showed statistically significant brain δ-ALAD activation and GSH depletion (by 26%) as well as increase of LPO (by 53%). Repeated Zn pre-treatment restore activity of δ-ALAD and content of GSH to the control level, while partly protected brain from Ni induced LPO.

Conclusions: Single as well as repeated exposure of Ni has adverse effects on brain δ-ALAD activity, just as on oxidative stress markers. However Zn provided significant protective effect against Ni induced toxicity.

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P 39. Oxidative Stress Induced Protein Carbonylation and Lipid Peroxydation in Experimental Animals

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Background and Aim: Oxidative stress is an imbalance of pro-oxidant/antioxidant homeostasis which occurs in organisms because of various exogenous and endogenous factors. One of those is big amounts of metals such as aluminium or iron which we used to induce oxidative stress. The main aim was to measure the level of this process in mice liver and brain.

Materials and Methods: Oxidative stress level was measured by detecting protein carbonylation and lipid peroxydation using protein carbonyl groups and malondialdehyde (MDA) as biomarkers.

First, mice liver or brain homogenate was prepared. Protein level in homogenate which should be less than 10 mg/ml was measured using Warburg-Christian method. The homogenate was treated in the following ways: in the first group of samples $AlCl_3$ was added, in the second group – $FeCl_3$ to induce oxidative stress. Remaining samples were made as control groups: control and control incubated.

For detecting protein carbonyl groups 2,4-dinitrophenylhydrazine was added to homogenate, which leaded to formation of stable dinitrophenyl hydrazone product. This then was detected using a method of spectrophotometric quantification of the acid hydrazones at 370 nm.

For detecting MDA, the reaction with thiobarbituric acid was made and MDA concentration was evaluated using spectophotometric method at 540 nm.

Results: MDA level in mice liver samples with $AlCl_3$ was the same as in control incubated and it was 2 times higher than in control ($p<0.05$). Highest level of MDA was found in samples treated with $FeCl_3$ which was 33 times higher comparing with control ($p<0.05$). Similar results were found while comparing MDA level in mice brain samples. In those with $FeCl_3$ MDA level was 24 times higher than in control ($p<0.05$).

There were no statistically significant results comparing protein carbonyl level in mice liver and brain.

Conclusions: Results have shown that aluminium ions have no higher effect on homogenate than control incubated while iron ions have a strong effect in inducing oxidative stress and high MDA levels were found in those samples.

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P 40. Effects of Aluminium on Iron and Magnesium Concentrations and Lipid Peroxidation in Aluminium-Exposed Mice Brain

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Background and Aim: The toxicity of aluminium (Al) is mediated by free radical generation causing lipid peroxidation, and by induction of changes in the distribution of some elements. This study was aimed to evaluate the long-term effect of Al on the level of malondialdehyde (MDA), the final product of lipid peroxidation, and to determine concentrations of Al, magnesium (Mg) and iron (Fe) in mice brain and blood.

Materials and Methods: Experiments were done on 4-6 weeks old Balb C mice who received drinking water supplemented with AlCl3 (50 or 100 mg $Al^{3+/}$kg body weight) for 8 weeks. Control mice had pure drinking water. Lipid peroxides were estimated by MDA level. Al, Mg and Fe concentrations were determined by inductively coupled plasma mass spectrometry (using NexION 300 D).

Results: Exposure to high-dose Al (100 mg/kg bw) caused an increase of blood MDA concentration by 61% ($p<0.05$) as compared to control. The increase in blood MDA by 18% after treatment with low-dose Al (50 mg/kg bw) was not statistically significant. The exposure to both doses of Al did not change MDA concentration in the brain. The high-dose Al caused a two-fold increase in the blood Al concentration ($p<0.05$), as compared to control. However, the low dose did not change Al concentration in the blood. The low and high doses of Al statistically insignificantly decreased the brain Al concentration. Exposure to high-dose Al resulted in an increase of Fe concentration in the blood by 21% ($p<0.05$) and brain by 36% ($p<0.05$). The low-dose Al almost did not change Fe concentration in the blood but induced a statistically significant increase of the brain Fe concentration by 35%. Exposure to both doses of Al did not induce statistically significant changes of Mg concentrations in the brain or blood in any experimental group.

Conclusions: Long-term oral exposure to high-dose Al caused an increased lipid peroxidation and higher concentrations of Al and Fe in the mice blood as compared to control. The both low and high doses of Al resulted in increased Fe concentration in mice brain.

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P 41. Effects of Aluminium on Redox Status and Concentrations of Antioxidants Selenium and Zinc in Mice Brain

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Background and Aim: Aluminium (Al) disturbs redox status of cells and changes homeostasis of some antioxidant elements. The aim of this study was to determine the effects of Al on total thiol level (TTL) and concentrations of antioxidants selenium (Se) and zinc (Zn) in mice brain and blood.

Materials and Methods: Experiments were performed on 4-6 weeks old Balb C mice using 8-week oral treatment with drinking water supplemented with $AlCl_3$ (50 or 100 mg Al^{3+}/kg bw). Control mice had pure drinking water. TTL was measured using a kit from Rel Assay using LX-20 Pro auto-analyzer (Beckman-Coulter, Woerden, The Netherlands). Concentrations of Al, Se and Zn were measured by inductively coupled plasma mass spectrometry (using NexION 300 D).

Results: Exposure to low-dose Al (50 mg/kg bw) and high-dose Al (100 mg/kg bw) caused a decrease in brain TTL by 25% ($p<0.05$) and 23% ($p<0.05$), respectively. Administration of low-dose Al caused a statistically significant increase in plasma TTL by 22%, in regard to control. However, an increase in plasma TTL by 18% was statistically insignificant after treatment with the high dose. There was a two-fold increase of blood Al concentration in the mice group treated with high-dose Al, as compared to control ($p<0.05$). After administration of low-dose Al, blood Al concentration was the same as in control. Administration of any dose of Al caused a statistically insignificant decrease in the brain Al concentration. Se concentration in the blood was significantly higher in both low and high Al dose groups by 51% ($p<0.05$) and 46% ($p<0.05$), respectively, as compared to control. However, the both doses of Al almost did not change concentration of Se in the brain. Both doses of Al did not induce statistically significant changes in Zn concentrations in mice brain and blood in any experimental group.

Conclusions: Effects of aluminium on total thiol level in mice brain and blood were opposite: reduced in the brain and increased in the blood. The exposure to Al resulted in increased blood Al and Se concentrations but had no influence in the brain.

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P 42. Artificial Neural Networks in Traumatic Brain Injury: Predicting Outcomes after Surgical Removal of Acute Subdural Hematoma

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Background and Aim: Prognostication of acute traumatic brain injury (TBI) remains challenging. We evaluated prognostic ability of artificial neural network (ANN) for predicting outcomes after surgical removal of acute subdural hematoma.

Methods: Consecutive TBI patients who underwent surgical evacuation of acute subdural hematoma in a period from January 2009 until January 2016 were prospectively evaluated for age, gender, admission Glasgow Coma Scale (GCS) score, hematoma thickness, midline shift and surgical management. Discharge outcomes were assessed using the Glasgow Outcome Scale (GOS). ANN and Radial basis function neural network (RBFNN) were designed to predict in-hospital mortality rate and unfavorable outcome at hospital discharge. Training set consisted of 70% of the data, and the remaining 30% set was used to test the models.

Results: Six-hundred and ninety-five patients (73.4% male; median age 59 years) were studied. Decompressive craniectomy was performed in 266 (38.3%) patients. In-hospital mortality rate was 32.1%, and poor discharge outcome (death, persistent vegetative state or severe disability) rate was 60.4%. For mortality prediction, the best ANN model with 10 hidden neurons showed 75.6% accuracy in training dataset and 74% accuracy in test dataset (51.5% specificity, 84.5% sensitivity). The RBFNN with 40 neurons in a hidden layer had higher accuracy at level of 79.7% in training data set and 77.4% in test dataset (62.1% specificity, 84.5% sensitivity). The best ANN model with 10 hidden neurons predicted poor outcome with 79.9% accuracy in training dataset and 79.3% accuracy in test dataset (84.9% specificity, 70.7% sensitivity). The RBFNN performed with better accuracy of 80.9% in training dataset and 80.8% in test dataset (84.9% specificity, 74.4% sensitivity). The ANN and RBFNN outperformed accuracy of binary logistic regression.

Conclusions: ANN can be useful to support prediction of outcomes after surgical treatment of acute subdural hematoma.

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P 43. Auto-Associative Network Implementation in the Neuromorphic Chip „Spikey“

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Background and Aim: Simulations of computational models of brain activity are computationally expensive and require high resources of computational power and energy. Neuromorphic computing systems are designed to accelerate neuronal network emulations and use analogue circuits to mimic neurobiological architectures. One of such systems, created by the Electronic Vision(s) Group in Kirchhoff Institute for Physics, Heidelberg University, Germany, is the "Spikey" chip. This neuromorphic system comprises 384 neurons and 98304 synapses and emulates neuronal activity 10000-fold faster than biological real-time. The aim of the study is to estimate the simulation efficiency and learning accuracy of the auto-associative memory model, implemented on the Spikey neuromorphic system.

Materials and Methods: „Spikey“ chip is programmable with the PyNN API in Python programming language. Auto-associative neural network was programmed using PyNN API. Network consists of leaky integrate-and-fire neurons connected all-to-all by excitatory synapses and a group of all-to-all inhibitory connections controlling a spiking rate. Symmetric and asymmetric STDP learning rules were used for excitatory-excitatory synaptic connections, while excitatory-inhibitory and inhibitory-excitatory synaptic connections had constant weight values assigned at the start of the experiment. Spike bursts with the varying spike counts were used as the inputs.

Results: Pattern recall accuracy was highest with the symmetric STDP learning rule and a larger number of spikes in the spike bursts. There is a strong interference with the network activity from the hardware inherent noise and spike delays, reducing overall recall accuracy and learning capacity.

Conclusions: Although network size limitations and hardware noise make implementation and simulation of the neural network models difficult, the „Spikey“ neuromorphic system offers great possibilities for the standard computational hardware, when computational speed is a big concern.

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P 44. Optimization of the Spiking Neural Network Parameters Using Genetic Algorithm in a Computational Model of Schizophrenia

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Background and Aim: Computational models of spiking neural networks, employed to understand the dynamical mechanisms of impaired gamma frequency oscillations in schizophrenia, usually are complex nonlinear systems and have a large number of free parameters. Whole parameter space exploration and estimation of the optimal parameter sets in complex biological models is a difficult task. The aim of this study was to assess the performance of the genetic algorithm in a parameter identification problem.

Methods: Computational model of a spiking neural network is composed of 800 pyramidal neurons (PCs), 150 regular-spiking interneurons (RSIs) and 50 fast-spiking interneurons (FSIs) described by the integrate-and-fire models (K.Spencer, 2009). All cells are randomly interconnected and have recurrent connections between each other. The background activity in the cortex is represented by a Poisson noise input to the network cells (PCs, RSIs and FSIs) at 100 Hz. In addition, network receives 20 Hz and 40 Hz drive excitatory stimulation. Genetic algorithm is applied to estimate the optimal synaptic weights to PCs, RSIs, FSIs and GABA receptor-gated channel time constant.

Results: The optimal parameter set of synaptic weights to PCs, RSIs, FSIs and GABA receptor-gated channel time constant is obtained and allows reproducing synchronous network oscillations at 20 Hz and 40 Hz, observed experimentally in healthy and pathological conditions.

Conclusions: Genetic algorithm is an effective tool for large-scale nonlinear optimization problems and can be applied to estimate the optimal parameter set in a computational model of schizophrenia-affected neural network.

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P 45. Emergent Dentate Granule Cell Excitability Control Via Kv4-Cav3 Channel Nanodomain Interactions: A Computational Study

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Background and Aim: Since long neuroscientists have viewed and described intracellular Ca^{2+} dynamics in terms of amplitude and duration, deriving various phenomena based on these two variables. In recent years it has become clear that such a description is not sufficient and spatial variability between the Ca^{2+} source and Ca^{2+} effectors has to be understood and included. However, both measuring or modeling intracellular spatial interactions is a difficult task. Our aim in this study was to combine two simulators, NEURON and MCell, into a novel dentate granule cell (DGC) model with phenomena emergent due to Kv4-Cav3 channel nanodomain interactions via Ca^{2+} sensitive KChIP3 protein.

Materials and Methods: NEURON 7.4 simulator was used to model DGC electrical compartments, whereas MCell 3.4 was used to model subcellular dynamics, such as Ca^{2+} diffusion, buffering and Ca^{2+} binding to KChIP3. A novel DGC model (H.H. Jerng et al., 2005) was simplified and used in NEURON 7.4, while methods to convert currents to ionic fluxes were adapted from a computational study (D.Keller et al., 2006). Simulators were combined via Python 2.7.14.

Results: The model shows how nanodomain interactions influence cell excitability by controlling the gain of DGC spike firing. With intact Kv4-Cav3 interactions, DGC showed a lower spike gain compared to no interactions, reproducing the results observed experimentally (D.Anderson et al., 2010).

Conclusions: Our model shows how Kv4-Cav3 channel nanodomain interactions play a role in regulating DGC cellular excitability. Including nanodomain channel interactions and protein localization, especially in the dendritic spine, can help integrate and solve a myriad of existing questions in neuroscience, such as synaptic tagging, differences between short-term and long-term plasticity, specificity of neuromodulation and others.

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P 46. Detection of Myofascial Trigger Points Using Noise Components of Surface Electromyographic Signal

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Background and Aim: In this study, a new method of detection of facial half with myofascial pain syndrome (MPS) is suggested. In the beginning, hypothesis was raised that because of myofascial trigger points (MTPs), biochemical balance in muscle tissue would be locally affected, which should be possible to evaluate by comparing differences of electrical impedance of different fascial halves.

Materials and Methods: Method of MTP identification is based on 50 Hz power line noise component's amplitudes in records of surface electromyographic signals (sEMG). Because of increased electrical impedance of muscle tissue with MTPs, 50 Hz power line noise's voltage amplitude changes too. Research was done using sEMG signals of Muscullus Masseter chewing muscles. By using mathematical computing software MATLAB, software tools for automatic sEMG signal rectification, segmentation by activity periods and parametrization using statistical moments of higher order were created. There were 19 patients who participated in research (17 women, 2 men), as well as a control group of 12 people. Also, there were 6 signals, which were acquired before and after application of local anesthesia using local numbing agent lidocaine.

Results: Statistical analysis of results revealed, that difference of 50 Hz power line noise component's amplitudes between left and right facial halves in control group were smaller than in patients group.

Conclusions: Research preliminarily confirms the hypothesis of biochemical balance and electrical impedance. Analysis of this statistical parameter of sEMG signal could be used as one of the criteria for objective MPS diagnosis or automated tools.

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P 47. What Minds Require of Brains: A Phenomenological Consideration of the Evolution of the Central Nervous System Towards Abstraction

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Background and Aim: The organization of the brain and how it implements the mind has yet to be understood from a broader perspective. Psychologists Tomasello, Kahneman and Tversky, neuroscientists Damasio, Graziano, Panksepp, and others have contributed new insights. We describe the human mind as a system of cognitive frameworks, and we consider how aspects of the functional organization of the brain may have evolved to implement such a mind.

Materials and Methods: We organize a diagram of the components of the brain in terms of their functions, as given by the textbook *Brain Function and Its Origins (*G.E Schneider, 2014), and encyclopedic sources. We compare these functions with a system of cognitive frameworks by which the mind M = S1 S2 interacts with the world W and balances what we intuitively know (S1 = Kahneman's System 1) with what we consciously don't know (S2 = System 2). We assign the brain's components to six major categories: Senses (W=>M) and Movement/Action (M=>W), Homeostasis (S1) and Consciousness (S2), Learning/Memories (S2=>S1) and Emotion/Motivation (S1=>S2).

Results: We note the evolution of the central nervous system by which the world is modeled increasingly abstractly in terms of icons, then indexes, and ultimately by symbols in the neocortex. The symbolic mind divides the global workspace into perspectives. The will arises in logically balancing S1 and S2, which may typically be championed by right and left hemispheres. This balancing occurs in the basal ganglia of apes. Humans are distinguished by playing games with semantic (S1) and syntactic (S2) obligations, namely, answering every question. Flexible creation of these shared worlds expanded the cerebral cortex.

Conclusions: The requirements of a mind can be variously implemented by the brain. The brain may be thought of as relating advocates for distinct cognitive frameworks. Right and left hemispheres may champion S1 and S2.

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P 48. Affect Regulation and the Autonomic Nervous System in Psychotherapeutic Process: A Critical Review

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Background and Aim: The psychotherapeutic process and its neurobiological background recently becomes the object of contemporary neuroscience. We can mention at least three psychotherapeutic paradigms deeply integrated with nowadays neuroscience: affective neuroscience (J.Panksepp, 2004), neuropsychoanalysis (M.Solms, 2015; G.Northoff, 2009) and interpersonal neurobiology (L.Cozolino, 2002, 2006; D.Siegel, 2012). Autonomic nervous system plays an important role in affect regulation both of patient and psychotherapist while the process of psychotherapeutic interaction. The main aim of the report is to observe and summarize the main research activities and approaches towards the role of autonomic nervous system in psychotherapeutic process.

Materials and Methods: The report compares main concepts of autononomic nervous system in psychotherapeutic process and come in touch with several case studies on peculiar problematic. Also we deal with researches of attunement within interaction of two brains, especially in relation to arousal and energy shifts (A.Schore, 2003) and brain-brain interactions which promote the development of specific cerebral circuits (C.Trevarthen, 1993).

Results: In the report the activity of sympathetic and parasympathetic systems are observed in correlation with main points of psychotherapeutic relations. The actual theories related to this subject (S.Porges „vagal system") and related therapeutic concepts such as the neuropsychoanalytic vision of limbic system and several structures in the brain processing the emotion and memory (M.Solms & O.Turnbull, 2002) are also mentioned.

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