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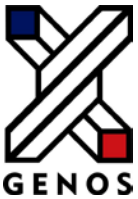
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Lectures

Thursday, October, 25th, 2018

Impaired glutamate-glutamine cycle in Huntington's disease

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The glutamate-glutamine cycle is essential for maintenance of glutamate homeostasis in the brain circumventing excitoxic conditions. This fundamental neuronal–astrocytic interaction ensures timely clearance and handling of glutamate which is essential for normal cerebral function. Not surprisingly, the glutamate-glutamine cycle seems to be affected in many pathological conditions. Essential components of the glutamate-glutamine cycle in the astrocytes are high affinity glutamate transport and glutamine synthetase.

We have recently revealed using the Huntington's disease (HD) model R6/2 mice a compromised astrocytic metabolism and regulation of glutamate-GABA-glutamine cycling resulting in impaired release of glutamine including consequences for GABA synthesis. These data were obtained from acutely isolated brain slices incubated in media containing relevant ^{13}C labelled substrates and subsequently tissue extracts were analyzed by gas chromatography-mass spectrometry. In support of our functional metabolic studies we used state-of-the-art mass spectrometry to establish a spatial brain proteome. From this, we observed altered expression of proteins in pathways related to energy metabolism, synapse function, and neurotransmitter homeostasis. Increased attention has been focused on the role of astrocytes in HD, and our data supports that therapeutic strategies to improve astrocytic glutamine homeostasis may help ameliorate symptoms in HD.

Modulation of astrocytic metabolism and neuronal signaling at the single cell level

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GABAergic plasticity is emerging as a key mechanism of shaping the neuronal networks activity. While essential role of glial cells, particularly of astrocytes, in the excitatory plasticity is well established, their involvement in GABAergic plasticity awaits systematic investigations. To address this issue, we used two models: neuronal cell culture (NC) and astrocyte-neuronal co-culture (ANCC), where we chemically induced long-term potentiation at inhibitory synapses (iLTP). iLTP could be induced both in preparations but in ANCC its extent was larger. Importantly, this functional iLTP manifestation was accompanied by an increase in gephyrin puncta size. Furthermore, blocking astrocyte Krebs cycle with fluoroacetate (FA) in ANCC prevented enhancement of both mIPSC amplitude and gephyrin puncta size but this effect was not observed in NC, indicating a key role in neuron-astrocyte cross-talk. Blockade of monocarboxylate transport with α-Cyano-4-hydroxycinnamic acid (4CIN) abolished iLTP both in NC and ANCC and in the latter model prevented also enlargement of gephyrin puncta. Similarly, blockade of glycogen phosphorylase with BAYU6751 prevented enlargement of gephyrin puncta upon iLTP induction. These data provide the first evidence that GABAergic plasticity is strongly regulated by astrocytes and the underlying mechanisms involve key metabolic enzymes. In attempt to describe the molecular mechanism of iLTP we checked the involvement of proteases in this phenomenon. We found that iLTP was insensitive to blockade of MMP-9 but it was abolished by both specific blocker of MMP-3 as well as by genetic deletion of this protease. We conclude that GABAergic plasticity in the form of iLTP depends on interactions with astrocytes as well as on proteolysis of extracellular matrix component and/or of signaling molecules by MMP-3.

NMDA receptors – the new partners of STIM proteins in rat primary cortical neurons

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Stromal interaction molecule 1 and 2 (STIM1 and STIM2) are Ca^{2+} sensors in endoplasmic reticulum (ER) and the key components of store-operated calcium entry (SOCE), which is responsible for refilling the ER with Ca^{2+} after release into cytosol. The cooperation of STIMs with the calcium channel ORAI1 is crucial for the functioning of SOCE in non-excitable cells. It has been shown that STIMs participate in SOCE also in neurons. However, STIMs seem to play different functions. STIM1 is a major activator of SOCE, while STIM2 regulates the resting Ca^{2+} level in the ER and constitutive Ca^{2+} influx into the cell. Recent research has shown that in neurons STIM proteins can affect also activation of voltage-gated channels or ionotropic AMPA receptors (our work). We expected that, in addition to them, there are other receptors activated or inhibited by STIMs, such as NMDA receptors (NMDARs).

The aim of this study was to determine if STIM proteins interact with NMDAR and influence the Ca^{2+} influx through this receptor. In cultured rat cortical neurons we recorded single-cell Ca^{2+} levels using Fura-2AM. To determine the effects of STIMs on NMDA-induced Ca^{2+} entry we overexpressed or knocked-down STIM1 or STIM2 using shRNA and lentiviruses. The overexpression of STIMs lowers the Ca^{2+} influx via NMDAR. This process is also inhibited in neurons isolated from transgenic mice that overexpress STIM1. In turn, lowering expression of STIMs results in an increase in NMDA-induced Ca^{2+} influx. By co-immunoprecipitation assay we found a physical association of endogenous STIMs with NMDARs and by immunofluorescence staining we observed co-localization of these proteins. The interaction between endogenous proteins was confirmed by *in situ* Proximity Ligation Assay.

In conclusion, our data suggest participation of STIM proteins in neuronal signaling by the interaction with NMDAR.

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Identification of new mechanism of action of angiotensin II type 1 receptor blockers, inhibitors of cyclooxygenase-2 and fibrates – a novel modulators of glutamatergic neurotransmission?

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Angiotensin II type 1 receptor blockers (ARBs), inhibitors of cyclooxygenase-2 (COX-2) and fibrates possess well-known neuroprotective properties. Kynurenic acid (KYNA), an endogenous glutamate receptors antagonist, is produced in the brain from L-kynurenine by kynurenine aminotransferases (KAT), mainly by KAT II isoform.

The aim of our study was to analyze the effect of ARBs, COX-2 inhibitors and fibrate representative gemfibrozil on KYNA synthesis and KAT II activity in rat brain cortex *in vitro*.

The influence of ARBs (irbesartan, losartan, telmisartan), COX-2 inhibitors (celecoxib, niflumic acid, parecoxib) and gemfibrozil on KYNA formation and KAT II activity was examined. Slices and homogenates of rat brain cortex were incubated for 2 hours in the presence of L-kynurenine and examined drug. KYNA was separated by HPLC and quantified fluorometrically. Moreover, the molecular docking of drugs to KAT II and the analysis of KAT II coding genes expression in human and rat cerebral cortex were conducted.

All examined ARBs, COX-2 inhibitors and gemfibrozil decreased with different potency KYNA production and KAT II activity in rat brain cortex *in vitro.* Molecular docking showed that analyzed drugs can bind to an active site of KAT II.

Our study indicates that ARBs, COX-2 inhibitors and gemfibrozil decrease KYNA production in rat brain cortex *in vitro.* Inhibitory effect of examined drugs on KAT II activity and KYNA synthesis suggest that they may have beneficial effect in memory or psychotic disorders treatment.

This study was supported by National Science Centre (NCN) grant PRELUDIUM 4, No UMO-2012/07/N/NZ4/02088 and the grant from Medical University of Lublin No DS 448/17.

Astrocytic system N glutamine transporter in hepatic encephalopathy

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Hepatic encephalopathy (HE) is neurological syndrome associated with hyperammonemia, in which ammonia is a key pathogenic factor. One of the immediate neurotoxic effects of increased ammonia entry to the brain is an excessive accumulation of glutamine, product of ammonia detoxification in astrocytes. The increase of glutamine in astrocytes is held responsible for brain edema often observed in the acute forms

of HE. The N-system transporter SN1 specifically mediates glutamine release from astrocytes. Therefore, an inefficient SN1-dependent efflux may cause increased glutamine retention in astrocytes, further ensuing astrocytic swelling and cytotoxic component of brain edema, a clinical complication in HE.

Astrocytic swelling in cerebral cortex of mice with azoxymethane (AOM)-induced acute HE was visualized by electron microscopy (EM), and brain edema by apparent diffusion coefficient (ADC) measurement with NMR. SN1 expression in the cortex of AOM mouse was collated with *ex vivo* analysis of system N-mediated [^{3}H]glutamine transport in cerebral cortical slices from AOM mouse. Extracellular and total glutamine content was determined by HPLC and by ^{1}H spectrometry, respectively. The mechanistic relation between cerebral edema and SN1 loss was analyzed in a cerebral cortical region of mouse in which SN1 was knocked down (by ~50%) using *vivo morpholino* technique (SN1 VM).

Decreased extracellular glutamine level, in conjunction with unaltered total glutamine content appeared to manifest inefficient glutamine efflux from astrocytes and its diminished conversion to glutamate in SN1 VM mouse. Swollen astrocytes were documented by EM, and cerebral cortical edema by biophysical and biochemical markers: i) decreased ADC, ii) increased extracellular taurine and iii) phosphocholine.

Collectively, the study documents SN1 deficiency-related impairment of glutamine transport from astrocytes as a contributing factor to brain edema in acute HE.

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microRNAs as regulators of hyper-excitability and neuro-inflammation in epilepsy

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Co-regulation of microRNAs and their target mRNAs by the same transcription factor as emerged as common mechanism regulating gene expression. Recent research by our group has shown that microRNA-22 (miR-22) regulates the expression of the ATP-gated ionotropic P2X7 receptor (P2X7R) and controls neuro-inflammation in certain brain regions after *status epilepticus* in mice. Since, P2X7R expression was recently shown to be induced by the transcription factor *specificity protein* 1 (SP1) in neurons. We explored whether SP1 regulates the expression of miR-22 and its target gene P2X7R after *status epilepticus*. Using pharmacological approaches, we show SP1 drives *P2rx7* mRNA and transcription of miR-22 after seizures *in vivo*. This regulatory module depends on the brain region studied and was not evident in sites of seizure-induce excitotoxicity which is calcium dependent. To confirm a role of calcium in this pathway, we manipulated calcium levels in hippocampal neurons *in vitro*, increasing levels of calcium blocked transcription of miR-22, and increased levels of P2X7R. Furthermore, removing calcium from cells also reduced miR-22. The present study shows a novel feed-forward loop where the expression of SP1, P2X7R and miR-22 is co-regulated according to calcium levels and neuronal activity.

Inter- and intra-tumoral microRNA heterogeneity

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Despite the importance of molecular subtype classification of glioblastoma multiforme (GBM), the extent of extracellular vesicle (EV)-driven molecular and phenotypic re-programming remains poorly understood. Using intracranial xenografts of patient-derived GBM stem-like cells (GSCs), we identified subpopulations with distinct transcriptomes, displaying either proliferative/nodular or migratory/invasive modes that were associated with mesenchymal-like or proneural-like subtype, respectively. To reveal complex subpopulation dynamics within the heterogeneous intra-tumoral ecosystem, we characterized protein and microRNA expression and secretion in these phenotypically diverse subpopulations of GSC. Bioinformatic analysis followed by functional EV/microRNA transfer between GSC *in vitro* and *in vivo* was used to analyze their molecular and phenotype subtype characteristics.

The highly heterogeneous profile of microRNAs expression in GBMs was distinguishable into two unsupervised classes that partially overlapped with previously determined molecular subtypes, with both subclasses of GSCs displayed differential cellular and EV microRNA profiles. The analysis of microRNA/target networks provided evidence that EV/microRNAs are modifiers of both the molecular landscape and phenotype, acting via cell type-dependent targeting. Importantly, EV proteome retained the subtype specificity and EV protein signatures were associated with worse outcome. The transfer of EVs between subpopulations of GSCs led to increased tumorigenicity *in vitro* and *in vivo* but did not cause a phenotypic switch,

facilitating the formation of inter-dependent tumor organization.

Our findings demonstrated the existence of previously underappreciated heterogeneity among cancer EVs that contribute to the diverse complexity of the brain tumor ecosystem, indicating that clinical outcome is influenced by the proportion of tumor cells of varying subtypes which by the exchange of EVs can modify molecular landscape and phenotype, acting in tumor anatomic sites-dependent fashion.

Circulating levels of BDNF and microRNAs are associated with progression of idiopathic Parkinson's disease

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MicroRNAs (miRNAs) are a class of small, non-coding RNAs that regulate gene expression at the post-transcriptional level. It has been provided the evidence that these molecules are widely expressed in the central nervous system and may play a functional role in the neurodegenerative diseases, typically by binding to the 3' UTRs of mRNAs what causes neuronal repression or degradation. Accumulating data indicate that there is a regulatory negative feedback loop between brain-derived neurotrophic factor (BDNF) and miRNAs. However, it is not known if circulating levels of above mentioned variables reflect the severity of Parkinson's disease (PD). Therefore, in the present study we investigated if the circulation level of several miRNAs and BDNF were correlated with progression of PD.

Selected miRNAs were determined by array cart and real-time qPCR using specific primers TaqMan miRNA assay (Life Technology, Carlsbad, CA, USA) in the serum of patients with iPD and age-matched healthy subjects. The concentration of BDNF was determined by ELISA (R&D System, Minneapolis, MN, USA).

qPCR results indicated that the concentration of BDNF, miR-1, miR-7, miR-16, miR-22, miR-23b, miR-29c, miR-30a-5p and miR-301 were statistically lowered in the serum of patients with iPD compared with age matched healthy subjects. Both plasma BDNF and miRNa-s levels negatively correlated with the Hoehn and Yahr scale.

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Circulating microRNA as a biomarker of epileptogenesis and epilepsy in the rat model of temporal lobe epilepsy

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Epilepsy frequently develops as a result of brain insult, ex. brain injury or stroke. Currently there are no tools to predict which patients suffering from trauma will eventually develop epilepsy or how severe it is going to be. In recent years small non-coding RNAs are proposed as biomarkers for neurological diseases. Particularly microRNAs are interesting candidates, as several of them were described changing their levels in the brain of epileptic subjects. There is evidence suggesting that microRNAs levels are altered also in the plasma, making them attractive candidates for peripheral biomarkers of epilepsy. This study was conducted to evaluate usefulness of plasma miRNAs as biomarkers of epileptogenesis and epilepsy.

In our studies we aimed at detecting plasma miRNA differentiating (i) between animals with epilepsy form those in preclinical phase; and (ii) between animals with different epilepsy severity. We used the rat model of temporal lobe epilepsy induced by the status epilepticus evoked by the stimulation of the lateral nucleus of the amygdala. Animals were continuously video and EEG monitored for 6 months. Blood was collected at 14, 30, 60, and 90 days after stimulation from tail vain. Blood plasma was separated and processed using Affymetrix miRNA 4.1 array strip microarrays. We have found that levels of miRNA in plasma are altered during epileptogenesis and differentiate between different epilepsy phenotypes. miRNA may become a useful biomarker of epileptogenesis/epilepsy as well as severity of the disease.

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Friday, October, 26th, 2018

Role of toll-like receptors in perinatal brain injury

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Preterm birth and infectious diseases are the most common causes of neonatal and early childhood deaths worldwide. In surviving infants, infection/inflammation is associated with increased morbidity including chronic lung disease, brain injury, and permanent neurological deficits. Toll-like receptors (TLRs) are innate immune receptors that react to molecules expressed by bacteria, as well as molecular pattern molecules that are released from injured tissue in the absence of an infectious agent. Thus, TLRs are critical in both pathogen-induced and non-pathogen-induced inflammation. TLRs are expressed in the brain during development and are regulated after systemic inflammation as well as after hypoxia-ischemia. Further, we have shown that activation of TLRs can increase the vulnerability of the developing brain to hypoxic-ischemic injury. Our data show that deletion of TLR2 is neuroprotective, while stimulation of TLR2 by gram-positive bacteria increases the vulnerability to subsequent hypoxic events, thus increasing the risk of brain injury. Mechanisms of TLR-induced injury and potential therapeutic strategies will be discussed.

New neuroprotective strategies for the neonatal brain

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Perinatal brain lesions are a leading cause of death and disability in children. Such damage can be induced by multiple factors, varies in severity between individuals, affects infants of different genetic backgrounds and occurs at various stages of the physiological developmental program. Several experimental studies have engaged in the understanding of brain disease pathophysiology and in the development of strategies that may be beneficial for the neurological outcome of infants. In addition, a few clinical trials have recently tested the neuroprotective effects of magnesium sulfate, melatonin, and EPO. To further identify novel targets for neuroprotection, we will present a translational bioinformatics investigation, with integration of human and mouse molecular and neuroimaging datasets to provide a deeper understanding of the role of microglia in preterm white matter damage. One of the major factors that have limited progress is the multifactorial nature of the injury, both in terms of etiological factors and mechanisms of damage. In this context, the development of biomarkers that allow for stratification of patients into homogeneous subgroups is critical. For example, for pre-term born neonates biomarkers are needed that can be applied at birth to identify the infants at risk to develop long-term deficits versus those with a good prognosis. This would allow us to include into clinical trials only 'at risk' neonates and to optimize early interventions and follow up.

Oligodendrocyte response to temporal deprivation of oxygen and glucose affects polarization of microglia: *in vitro* model of perinatal asphyxia

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Temporal deprivation of oxygen and trophic support is one of the main features of perinatal asphyxia, leading to development of subsequent leukodystrophic disorders. One of the very first consequence of the experienced hypoxic-ischemic episode is neuroinflammatory process triggered by activation of microglia residing in the nervous tissue. The reciprocal interaction between oligodendrocytes (myelin generating cells) and microglia (associated with immunological response) are hypothesized to play a major role in potential overcoming the nervous tissue crisis. To verify this hypothesis, the co-culture experiments were established for the purpose of applying *in vitro* model of oxygen-glucose deprivation (OGD). Accordingly, both the oligodendroglial and the microglial cell fractions were separated from the primary cultures of glia isolated from the brains of neonatal Wistar rats. The obtained homogenous monocultures of either oligodendrocyte progenitors

or microglia were subjected to a OGD procedure, in order to mimic *in vitro* the hypoxic-ischemic insult accompanying the perinatal asphyxia. For *ex vivo* studies, the hippocampal organotypic slices were prepared and after being subjected to OGD procedure, were also used for establishing the co-culture systems. Such a schedule allowed us to evaluate interactions between cells, contributing to initiation of mechanisms leading to development of leukodystrophic diseases. The cell phenotype was determined by specific antibodies: ED1, Iba1 and anti-arginase for microglia and anti-NG2, anti-O4, anti-GalC and anti-MBP for oligodendroglia. The migratory potential of the examined cells was assessed by live recording by means of Cell Observer SD (Zeiss). The obtained results indicated that even a short deprivation of oxygen and trophic support affects microglial polarization. This effect is also exerted in a paracrine manner by the OGD-subjected oligodendrocytes. In conclusion, the determined *in vitro* interaction between neural cells might indicate the directions of future pre-clinical studies aimed at modulating cell response to hypoxic-ischemic insult and subsequent eliminating or diminishing results of perinatal asphyxia.

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Mineralocorticoid receptors as potential therapeutic target for neuroprotection after birth asphyxia

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Neonatal asphyxia is one of major causes of acute mortality as well as chronic neurological disability in newborns. Up to 25% of survivors demonstrate permanent neurological deficits such as cerebral palsy, mental retardation, learning disability and epilepsy. However, there is no universally accepted therapy available for asphyxia except for therapeutic hypothermia which, if applied to full term neonates with mild or moderate encephalopathy, may reduce their mortality and disability. Thus, it is essential to explore all potential modifiable natural neuroprotective processes that may provide us with potential targets to prevent or improve the outcome of encephalopathy. There is strong evidence suggesting that mineralocorticoid receptors may be the promising candidates worth investigation.

Glucocorticoids (GC) exert multiple effects within the central nervous system via mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) activation. This defined a one hormone/two receptors system that works in balance, modulating a large spectrum of actions in the central nervous system. MR binds both aldosterone and glucocorticoids, the latter having a ten-fold higher affinity for MR than for the closely related GR. Thus, neuronal MR appears to be fully occupied even at low physiological glucocorticoid levels while GR activation only occurs at high glucocorticoid concentrations, i.e. at the peak of the circadian rhythm or under stress. MR expression is associated with a neuroprotective phenotype, whereas GR activation is implicated in the induction of an endangered neural phenotype and the opposite actions are most evident in hippocampus, where these receptors are predominantly present. MR constitutes a key factor in the arising of higher cognitive functions such as memory, learning and emotion. They are involved in the stress response, long term potentiation, neuroprotection and neurogenesis. It has been shown that MR needs to be present and functional for neuronal survival in the damaged brain regions.

The MR expression as a result of injury possibly represents an endogenous response that may serve as a compensatory mechanism designed to limit the neuronal death. If postnatal manipulations could "overwrite" the deleterious outcome of an adverse prenatal environment, then, there might be a way to exploit the MR and GR levels manipulations for therapeutic benefit to protect brain from the effects of the insult. Especially, the regulation of MR level may be an important target for reduction brain damage resulting from an acute neuronal injury such as neonatal asphyxia.

Epigenetic regulation of microglial phosphatidylinositol 3 kinase (PI3K)/protein kinase B (Akt) pathway alters long-term memory storage in hippocampus

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Microglial cells are the resident macrophages of the central nervous system (CNS). Microglia-mediated neuroinflammation has been shown in various neurological disorders such as Alzheimer's disease and Parkinson's disease. Phosphatidylinositol 3-kinase (PI3K) is known to play a significant role in synaptic plasticity in neurons.

The role of PI3K in microglia has been mainly studied in the context of inflammation and phagocytosis. PI3K and its downstream effectors such as protein kinase B (Akt), cAMP response element binding protein (CREB) and brain-derived neurotrophic factor (BDNF) have been found to influence synaptic plasticity. We therefore hypothesise that the PI3K/Akt pathway in microglia regulates the production of BDNF, which in turn alters synaptic plasticity.

In this study, we investigated the epigenetic regulation and sumoylation of microglial PI3K/Akt pathway involved in synaptic plasticity. It has been shown that sodium butyrate, a histone deacetylase inhibitor (HDACi) upregulated the PI3K expression and the phosphorylation of Akt and CREB in microglia, suggesting that BDNF secretion from microglia may be altered via epigenetic regulation of PI3K. In addition, chromatin immunoprecipitation (ChIP) results showed a significant increase in H3 lysine 9 acetylation (H3K9ac) enrichment at the Pik3ca promoter region which is highly correlated with PI3K gene expression in BV2 microglial cells treated with HDACi, further implying that PI3K is epigenetically regulated.

It has been shown that SUMO modulates the PI3K pathway activation. Knockdown of SUMO1 in BV2 microglia decreased the phosphorylation of Akt and CREB as well as the expression of BDNF. These results suggest that microglial PI3K is epigenetically regulated by histone modifications and post-translationally modified by sumoylation. Electrophysiological recordings in CA1 hippocampal neurons show that when microglia are ablated using clodronate, there is a decline in long-term potentiation (LTP). Adding active PI3K substrate and BDNF onto hippocampal slices post-clodronate treatment rescued the LTP, suggesting the involvement of microglial PI3K/Akt pathway in LTP and therefore, synaptic plasticity. Understanding the mechanisms by which microglial PI3K influences synapses may give us an insight into the ways by which it can modulate synaptic transmission and subsequently synaptic plasticity in learning and memory.

This research was supported by NUS Strategic Research Grant (Memory Networks in Rodent and Primate) DPRT/944/09/14 (R185-000-271-646).

Biography

Dr. S T Dheen, PhD is an Associate Professor and Head of the Department at Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore. He served as the President of Microscopy Society Singapore and is the current President of Singapore Neuroscience Association. He is also a member of Governing Council of International Brain Research Organization (IBRO).

Down syndrome mouse models to unravel pathogenetic mechanisms of behavioural disturbances

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Down syndrome (DS) is a common genetic condition caused by the presence of three copies of chromosome 21 (trisomy 21). Mouse models are crucial to dissect the genetic contribution of trisomy 21 to DS phenotypes including those relevant to AD. I will discuss the mouse behavioural profiles and testing possibilities in the light of what is known about human behaviour in Down syndrome. Also I will present our observations that brain plasticity is effective in the recovery of behavioural disturbances and neural plasticity in a range of DS mouse models which are trisomic for chromosome segments syntenic to human chromosome 21 and in single gene transgenics. Specifically I will focus on the role of DYRK1A (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A), a serine/threonine kinase as a promising molecular target for Down syndrome and autism.

Effect of social stress on circadian rhythms and sleep-wake cycle

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Though it is known that daily rhythms are disrupted in patients suffering from mood disorders, the molecular mechanisms linking aberration in circadian/sleep rhythms and mood disorders is still not well understood. Observations that brain regions associated with mood regulation have robust neural connections, and overlapping molecular pathways, with regions that regulate biological rhythms allow us to investigate the link between these brain regions following expression of depression-like behaviour. We are using a combination of rodent behavioural model of stress together with electrophysiological and molecular approaches to investigate changes in physiological and molecular dynamics between brain regions that encode mood, circadian rhythms and sleep/wake rhythmicity in mice that are resilient (non-depressed) and susceptible (depressed) to social defeat stress.

Cognitive impairments in animal models of schizophrenia

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Schizophrenia is traditionally characterized by the "positive" and "negative" symptoms. The third category, only recently recognized, includes cognitive deficits. According to the MATRICS initiative, cognitive domains that are dysfunctional in schizophrenia include working memory, attention/vigilance, verbal learning and memory, visual learning and memory, processing speed, reasoning and problem solving, and social cognition. The cognitive impairments may appear prior to the onset of psychosis and are often present after remission. While clinically used antipsychotics are efficacious in alleviating hallucinations and delusions, for the cognitive deficits associated with schizophrenia there is no approved pharmacotherapy. This presentation will describe the animal models of cognitive deficits, and discuss some of the effects of novel treatments purportedly improving cognitive disturbances associated with schizophrenia.

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Co-treatment with risperidone and antidepressants in selected behavioral tests

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Schizophrenia impairs mental and social functioning and affects 1% of the world's population. It is known that atypical antipsychotic agents alleviate not only the positive symptoms of schizophrenia but also the negative ones, in contrast to pharmacotherapy with typical antipsychotics. Some clinical reports have suggested that antidepressant drugs are able to augment the activity of atypical antipsychotic drugs, thus effectively improving treatment of the negative and some cognitive symptoms of schizophrenia.

The aim of the study was to evaluate the effect of second generation antidepressants and atypical antipsychotic drug – risperidone, given separately or jointly in selected behavioral tests. Experiments were performed using various behavioral tests: novel object recognition, social interaction and elevated plus maze. The novel object recognition (NOR) in rodents is analogous in some ways to human declarative memory, one of a few cognitive domains which are abnormal in schizophrenic patients, while social interaction test (SIT) mimics the negative symptoms of schizophrenia in animals. Also anxiolytic effect was investigated with the use of elevated plus maze test (EPMT).

The present results showed that co-treatment with an inactive dose of risperidone (0.01 mg/kg) and escitalopram or mirtazapine (5 mg/kg but not 2.5 mg/kg) abolished the deficit of object recognition memory induced by MK-801. In the SIT, co-treatment with an ineffective dose of risperidone (0.01 mg/kg) and mirtazapine (2.5 or 5 mg/kg) or escitalopram only at a dose of 5 mg/kg (but not 2.5 and 10 mg/kg) abolished the deficits evoked by MK-801. Escitalopram (5 mg/kg) and mirtazapine (5 or 10 mg/kg) or risperidone (0.1 mg/kg) induced an anxiolytic-like effect in the EPMT and the combined treatment with an ineffective dose of risperidone (0.05 mg/kg) enhanced the anxiolytic-like effects of escitalopram (2.5 mg/kg) or mirtazapine (1 and 2.5 mg/kg) in this test.

These results suggest that antidepressants may enhance the antipsychotic-like effect of risperidone in the animal tests used for evaluation of some cognitive and negative symptoms of schizophrenia. Moreover, a low dose of risperidone may also enhance the anxiolytic-like action of the studied antidepressants.

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Circuit- and symptom-specific targeted therapy of fragile X syndrome rescues cognitive impairments and normalizes synaptic plasticity and morphology in central amygdala

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Fragile X syndrome (FXS) resulting from the loss of fragile X mental retardation protein (FMRP) is the most common monogenetic cause of inherited mental disability and autism. FXS patients display a wide range of cognitive and social impairments, with very high phenotypic vari-

ability. However, targeted therapeutic approaches tailored to address particular FXS symptoms do not exist.

We elucidate how brain-circuit specific approach aiming at particular molecular mechanism rescue behavioral deficits in symptom-specific manner. To that end, we implement fully automated assessment of cognitive and social impairments in mouse model of FXS, Fmr1 knockouts (Fmr1 KO) treated with nanoparticles (NPs). Further, we combine it with evidence concerning alterations in synaptic plasticity (long term potentiation, LTP) and high-resolution morphology of synapses (electron microscopy).

In both humans and mice lack of FMRP leads to elevated translation of matrix metalloproteinase-9 (MMP-9), an enzyme involved in activity-dependent reorganization of dendritic spines architecture. Notably, abnormal activity of MMP-9-dependent circuits specifically in central amygdala (CeA) disrupts reward learning. We show that targeted, CeA-limited inhibition of hypertranslated MMP-9: (1) rescues cognitive but not social deficits, (2) normalizes severely impaired CeA LTP, and (3) reverses abnormal CeA synaptic morphology in Fmr1 KOs. NPs used for the delivery of the endogenous MMP-9 inhibitor gradually release the compound assuring stable therapeutic levels over several days. Presented results provide critical insights into molecular and neural correlates of FXS. Moreover, they hold promise of obtaining clinically relevant solutions.

Poster session I

|A1|

Respiratory response to hypoxia after dopaminergic D2 receptors blockade in rats lesioned intracerebroventricularly with 6-OHDA injection

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Parkinson's disease (PD) patients apart from motor impairments exhibit respiratory abnormalities attenuating comfort of life. In the present study the effect of intracerebroventricular 6-OHDA injection, mimicking advanced stages of PD, was investigated. Noradrenergic terminals were protected by desipramine administration before surgery. On this ground, respiratory response to 8% hypoxia was analyzed before and after administration of antagonists of dopaminergic D2 receptors: domperidone, which acts only at periphery and haloperidol crossing blood-brain barrier.

Experiments were performed in spontaneously breathing male Wistar rats. Respiratory tests were made in plethysmography chamber measuring tidal volume (VT), frequency of breathing (f) and minute ventilation (VE). Animals where tested twice, before and after 14 days post 6-OHDA or vehicle injection. After control hypoxic exposure rats received intraperitoneal injection of domperidone or haloperidol and 20 min after hypoxic test was performed. To confirm lesion effectiveness open field test was performed.

6-OHDA treated animals exhibited deficits in motor activity compared to sham operated rats. Animals with PD displayed changes in normoxic breathing. 6-OHDA injection produced increase in VT and decrease in f, without any changes in VE. Magnitude of hypoxic ventilatory response (HVR) was similar in both groups. In healthy animals domperidone injection caused increase in normoxic VT and VE without any effect on reactivity to hypoxia. Haloperidol treatment did not change normoxic breathing but increased VT and VE during hypoxic exposure. 14 days after 6-OHDA injection effect of domperidone treatment was not observed. Haloperidol treatment in PD rats showed augmentation of depressive phase of respiratory response to hypoxia.

Augmentation in depressive phase of hypoxic ventilatory response in lesioned rats may suggest that spared dopaminergic neurons and dopamine play still important role in depressive phase of HVR. Lack of domperidone effect in this PD model indicates that 6-OHDA injection initiates deficits also in the periphery i.e. in carotid body.

|A2|

Application of epidural oscillating field stimulation after Th9 spinal cord injury in rat reduced activated astrocytes formation, and enhanced axonal regeneration and functional recovery

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The massive increase in activated astrocytes associated with glial scar formation and serious axonal damage are the major causes of the failure in functional recovery after acute spinal cord injury (SCI). The aim of this study was to show that specifically designed miniature oscillating field stimulator (OFS; 50 μA) implanted immediately after Th9 compression can effectively promote regeneration of damaged nerve tissue and thus enhance functional recovery after SCI. Three groups of adult Wistar rats were analyzed: control (intact rats with OSF stimulator), SCI (rats with SCI) and treated (SCI rats with OSF stimulator) group. In our experimental design, post-SCI bilateral hindlimb locomotor activity was evaluated using the BBB locomotor rating scale. We observed gradual improvement in locomotor functions in both experimental groups, with more pronounced functional recovery in the treated OFS + SCI group just one week after injury. This effect persisted until the end of experiments, suggesting the potential long-term benefits of OFS stimulation with permanent improvement after SCI. Our study also revealed considerable differences in white matter integrity in animals with an implanted OFS stimulator. Significant differences compared with untreated SCI group were observed in Th8-Th11 spinal segments. Moreover, we detected significantly increased number of neurofilaments and strong reduction in activated forms of astrocytes in the group of stimulated animals compared to the animals without stimulation. Accordingly, we can conclude that OFS stimulation is beneficial in terms of spinal cord regeneration after injury and

that implanting of such OFS stimulator is safe, stable and suitable for future combined therapy which could promote and accelerate functional restoration after spinal trauma.

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|A3|

Treatment with a single dose of atorvastatin reduces inflammation, apoptosis and promotes functional outcome after spinal cord injury

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Two mechanisms of secondary injury – apoptosis and inflammatory response – represent a barrier for tissue regeneration after spinal cord injury (SCI). The goal of our study was to limit the inflammation and apoptosis after spinal cord compression using a single dose of atorvastatin (ATR; 5 mg/kg, *i.p.*) and thus promote axonal outgrowing and motor activity of hind limbs. Adult Wistar rats were divided into five experimental groups: one control group, two Th9-compression (40 g/15 min) groups, and two Th9-compression + ATR groups. The animals survived 1 day and 6 weeks. Strong inflammatory response was noted early after compression. The level of IL-1β 4 h post-SCI was up to 12-fold higher in the blood serum of injured animals compared to the control. ATR applied immediately after injury reduced the increase of IL-1β 4 h after SCI almost to the control level and effectively decreased the excessive infiltration of M1 and M2 macrophages to the lesion site (M1 by 62%, M2 by 41%), caudal (M1 by 52%, M2 by 16%) and cranial (M1 by 66%, M2 by 27%) segment. In addition, at the same time point, ATR markedly decreased the cleavage of caspase-3 in neurons, astrocytes, and oligodendrocytes. Six weeks after compression, spontaneous axonal overgrowing was very low. Immufluorescence analysis revealed that ATR strongly promoted the axons outgrow in cranial and caudal segments. Besides, treatment with ATR increased the expression of neurofilaments in the dorsolateral columns caudally and cranially from lesion site. In addition, early inhibition of apoptosis and inflammation significantly improved the motor activity of the hind limbs tested on day 30 to 42. We can conclude that resulted from ATR application early modulation of the inflammatory response via effects on the M1/M2 macrophages and the inhibition of caspase-3 expression markedly promote the regeneration of spinal cord and functional outcome in later periods of survival.

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|A4|

Is mitochondrial glutaminase involved in endogenous neuroprotection induced by protein kinase C βII?

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The brain has developed several endogenous mechanisms to protect itself from the deleterious consequences of ischemia/reperfusion (I/R) injury. One major goal in research is to explore and utilize such endogenous neuroprotective mechanisms therapeutically. Therefore, using gerbil model of cerebral ischemia, we focus on understanding the processes leading to endogenous neuroprotection. In this model, 5 min-ischemia results in selective death of pyramidal cells in hippocampal CA1 region, while the adjacent region CA2-4, DG remains relatively resistant. Hence, our main interest concerns the CA2-4, DG region and the processes responsible for its ischemia-resistance. So far, we have showed that I/R injury results in translocation of protein kinase C βII (PKC βII) from cytoplasm to mitochondria but only in CA2-4, DG. We claim that described translocation is responsible for endogenous neuroprotection in this region. However, the exact mechanism(s) underlying PKC βII-induced neuroprotection remain(s) unknown. Thus, we hypothesized that the I/R-induced translocation of PKC βII likely results in phosphorylation-dependent activation/inhibition of specific mitochondrial proteins what in turn guarantees neuroprotection by modifying mitochondrial function.

Using pull down method followed by mass spectrometry, we identified mitochondrial glutaminase which is

the main enzyme responsible for glutamate production, as a potential PKC βII partner. Reciprocal co-immunoprecipitation method showed that of two kidney-type glutaminase isoforms, it is GAC (glutaminase C) not KGA (kidney-type glutaminase), which interacts with PKC βII. Moreover, I/R injury leads to increase of GAC level in cytoplasm but only in CA1 region which is most likely the result of its translocations from the mitochondria. This increase is prevented by isozyme-selective PKC βII inhibitor. We speculate that the observed increase of glutaminase is associated with cell death within CA1 region due to the lack of PKC βII there. Moreover, in silico analysis and studies of others have shown that mitochondrial glutaminase might be phosphorylated by PKC. On this basis, we speculate that GAC might be important player in PKC βII-mediated neuroprotection.

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|A5|

The influence of hypercholesterolemia on the components of purinergic signaling in mouse brain endothelial cells

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Current research proves that the occurrence and development of hypercholesterolemia correlate with a series of pathological changes such as diabetes mellitus, atherosclerosis and hypertension, which in turn lead to ischemic stroke, neurodegenerative changes and brain damages. According to the various reports, the main purinergic signaling pathways play a crucial role in controlling the functional integrity of neurons, glial cells and vascular endothelial cells in the CNS. Among the components of the purinergic system, adenine nucleotides play a major role in the inflammatory processes. ATP participates in energy metabolism and has pro-inflammatory activity by stimulating microglia and increasing the release of pro-inflammatory cytokines. The relationship between hypercholesterolemia and the numerous pathological changes that occur in metabolic disorders and brain damages is still unclear. For this reason, we used in our study wild-type C57/BL6 and LDLR–/–/Apo E–/– double-knockout mice to test their role in hypercholesterolemia in the brain. The mutation is associated with elevated plasma cholesterol level and develops atherosclerotic plaques at varying stages. The obtained results allow to confirm the increase activity of e-NTPDase, ecto-5'-NT and eADA enzymes. There were no differences in the intracellular concentration of adenine nucleotides and glycolytic function. The increase of enzymes activity with the unchanged concentration of adenine nucleotides and glycolytic function may be the manifestation of a compensatory mechanism that maintains the energy metabolism of endothelial cells at a constant level in the brain. Therefore, regulation of the inflammatory response by modulation of the extracellular enzymatic activity may be a new therapeutic strategy for metabolic disorders and brain damages.

|A6|

Involvement of cathepsin D inhibition in neuroprotective effects of Necrostatin-1 against oxidative stress-induced cell damage in human neuroblastoma SH-SY5Y cells

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The neuroprotective potential of the necroptosis inhibitor, Necrostatin-1 (Nec-1) has been proven in various experimental models, however the mechanism involved in this effect is still poorly recognized. Thus, in the present study some intracellular processes which could underlie neuroprotection mediated by Nec-1 were investigated in the model of oxidative stress (H_2O_2)-induced cell damage in human neuroblastoma SH-SY5Y cells. The data showed that Nec-1 (10-40 μM) attenuated the cell death induced by H_2O_2 in undifferentiated (UN-) and retinoic acid (RA)-differentiated SH-SY5Y cells with higher efficiency in the former cell type. The engagement of inhibition of caspase-3 activity, calpain activity, apoptosis inducing

factor (AIF) translocation and DNA damage (p-p53 and γ-H2AX) in neuroprotective action of Nec-1 was excluded, although all those parameters were induced by H_2O_2 in SH-SY5Y cells. The extent of protection mediated by Nec-1 was similar to that of the caspase-3 inhibitor, Ac-DEVD-CHO but the effect was not potentiated after combined treatment with both agents, suggesting shared common downstream intracellular mechanisms for apoptosis and necroptosis. Next, the activation of lysosomal protease cathepsin D found in UN- and RA-SH-SY5Y cells after H_2O_2 exposure was significantly attenuated by Nec-1. Moreover, Nec-1 protected cells with similar efficiency as the cathepsin D inhibitor, pepstatin A but that effect was not further exaggerated by combined treatment with both agents. In conclusion, our data showed neuroprotective potential of Nec-1 against oxidative-stress induced neuronal cell damage associated with cathepsin D inhibition.

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|A7|

The effects of fingolimod on the expression of genes involved in ceramide metabolism in a murine model of Alzheimer's disease

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Accumulating evidence suggests the engagement of sphingolipid metabolism and signaling in the early stages of Alzheimer's disease (AD). Analysis of postmortem AD brains reveals a shift towards synthesis of the pro-apoptotic ceramide at the cost of sphingosine-1-phosphate – pro-survival signaling molecule.

We analyzed mRNA levels of enzymes engaged in sphingolipid metabolism in a mouse mutant (V717I) overexpressing amyloid β precursor protein (AβPP). We observed notably different responses of hippocampal gene expression to AβPP at 3, 6, and 12 months. At 3 months ceramide synthases and ceramidase were up-regulated in response to AβPP, suggesting early imbalance in life/death signaling and accelerated metabolic turnover of ceramide and sphingosine. In turn, possible accumulation of ceramide at 6 and 12 months was suggested by reduced sphingomyelin synthases expression.

Fingolimod (FTY720), a Federal Drug Administration-approved modulator of S1P receptors (S1PRs) is altering gene activities through S1PR-binding G proteins that relay the signal to transcription factors. In the hippocampus of (V717I) AβPP-expressing mice fingolimod (1 mg/kg, *i.p.*) reduced ceramide synthases at 3 months, but the potential significance of fingolimod-induced changes at 12 months was less clear (up-regulated ceramide synthase, ceramidases, sphingomyelin synthase, and sphingomyelinase) probably resulting in accelerated metabolism between ceramide-sphingosine and ceramide-sphingomyelin. Thus, fingolimod is capable to modulate the time-course of sphingolipid metabolism alterations resulting from disease-associated (V717I) AβPP, but there is urgent need for further elucidation of its significance as a potential disease-modifying drug in AD.

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|A8|

The possible role of glycans in translational regenerative medicine

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Cell surfaces are coated with a large variety of glycoconjugates uniquely arranged in the cellular membrane as glycoproteins and glycolipids. It is well recognized that glycans play an essential role in a myriad of biological events including cellular adhesion and migration, organism development, disease progression, or the modulation of immunological responses.

It is widely appreciated that the relative positioning of glycans, which can be regulated by their display on glycoprotein scaffolds within the three-dimensional geometries or the spatial arrangement of glycoconjugates, can

profoundly influence the avidity and specificity of glycan-oligomerized receptor interactions.

After we do manage to identify, isolate and then trigger the appropriate differentiation of stem cells, these cells still must be implanted into the patient and accepted into the environment created by the native body cells. The success of the stem cell transplantation is therefore dependent on the effective and functional integration of transplanted cells into the patient's body systems and into the specific environment of extracellular matrix, composed of polysaccharide chains of glycosaminoglycans, often attached to proteins and forming large proteoglycans, and usually heavily glycosylated fibrous proteins, produced and secreted by the surrounding native cells. Cell divisions, differentiation, cell-cell communications, and synthesis and secretion of the regulatory factors including cytokines, are all regulated by the interactions between ECM and complex glycosylation present on the cell membrane glycoproteins and glycolipids.

Such information about the cell surface complex glycosylation that would allow functional integration of the transplanted stem cells with the ECM of the host environment will be essential to truly successful, effective transplantations of regenerative and therapeutic intent. Methods of investigating the dynamics of cellular glycosylation and of the cell-extracellular environment will be discussed.

|A9|

Hydrogen sulfide mediated protection against oxidative stress after spinal cord injury

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Reactive oxygen species (ROS) and oxidative stress play a crucial role in pathophysiology of spinal cord injury (SCI). Recently, it has been suggested that hydrogen sulfide (H_2S) is an effective agent for attenuation of a variety of pathophysiological processes. Up to now, the relationship between H_2S and oxidative stress in spinal cord after trauma remains unknown. The aim of the present study was to explore the therapeutic potential of H_2S against oxidative stress in rodent model of SCI. Adult Wistar rats were divided into three experimental groups: (1) control group, (2) Th9-compression (40 g/15 min) group, and (3) Th9-compression (40 g/15 min) + GYY4137. GYY4137, the slow-releasing H_2S donor was applied in single dose (133 µmol/kg, *i.p.*) immediately after SCI. The animals survived 24 hours. The effect of H_2S was examined by several parameters. The activity of antioxidant enzymes (superoxide dismutase, catalase) and the level of glutamate was measured 3 h, 6 h and 24 h after surgery in the blood serum, and the expression of neural markers was identified by real-time PCR and immunohistochemistry 24 h post-injury in Th8-Th10 segments. We have found significant increase of superoxide dismutase activity 6 h after the Th9 compression. Acute GYY4137 treatment markedly decreased the superoxide dismutase activity at 3 h and at 6 h, and considerably reduced the catalase activity in the blood serum 24 h post-injury. Immunohistochemical and PCR analyses indicated that early application of GYY4137 prevented the neurons from oxidative stress. This drug effectively reduced apoptosis, inflammation and glutamate neurotoxicity. Our data indicate that H_2S scavenges superoxide early after SCI, while catalase exert its effect in scavenging the hydrogen peroxide in later post-SCI period. GYY4137, as a slow-releasing H_2S compound is a potential therapeutic agent which could be used in treatment of SCI.

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|A10|

Ischemia/reperfusion-induced translocation of protein kinase C βII to mitochondria results in endogenous neuroprotection. A potential neuroprotective strategy

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Currently, using gerbil model of cerebral ischemia, we focus on understanding the processes leading to endogenous neuroprotection. In this model, cerebral ischemia results in selective death of pyramidal cells in hippocampal CA1 region, while the adjacent region CA2-4, DG remains relatively resistant. Recently, we have provided evidences that ischemia/reperfusion-induced translocation of PKC βII to

mitochondria is an important mediator of a protective signaling mechanism exactly in CA2-4, DG. However, the exact mechanism(s) underlying PKC βII-induced neuroprotection remain(s) unknown. Thus, we hypothesized that the I/R-induced translocation of PKC βII likely results in phosphorylation-dependent activation/inhibition of specific mitochondrial proteins what in turn guarantees neuroprotection by modifying mitochondrial function.

Using pull down method followed by mass spectrometry, we identified NDUFS1, the 75 kDa subunit of respiratory complex I, as a potential PKC βII partner. This protein-protein interaction was confirmed by co-immunoprecipitation method as well as by proximity ligation assay. *In vitro* phosphorylation assay and Phos-tag SDS-PAGE revealed that PKC βII specifically phosphorylates NDUFS1. Moreover, NDUFS1 sequence analysis based on PKC βII substrate preferences revealed four potential phosphorylation sites: Ser363, Thr595, Ser627 and Thr715. Protein-protein docking, conducted with MOE software, has narrowed down those results, to only two amino acids, that is Ser363 and Thr715.

On this basis, we speculate that PKC βII-mediated phosphorylation of NDUFS1 might be involved in PKC βII-induced neuroprotection. The contribution of NDUFS1 in this process likely preserves mitochondrial function and attenuates production of reactive oxygen species during I/R.

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|A11|

Characterization of rebound depolarization in medial prefrontal cortex pyramidal neurons *in vitro*

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Rebound depolarization (RD) is a form of membrane depolarization triggered in some neurons following hyperpolarization. Typically, a series of action potentials are evoked during RD plateau. RD converts an inhibitory signal arriving to the neuron into an excitation signal, which is subsequently synaptically transmitted to other cells. The nature of RD in cortical neurons has been tested for several years without satisfactory explanation. The purpose of our study was to identify the mechanisms that trigger RD in synaptically isolated layer V medial prefrontal cortex pyramidal neurons in slices obtained from adult rats. The key finding of our study is that following temporary hyperpolarization, two currents are concomitantly activated: 1) a low-threshold, persistent inward Na^+ current that evokes RD; and 2) an outward K^+ current through Ca^{2+}-dependent K^+ (type BK) channels that opposes Na^+-dependent depolarization. These currents conceal each other in resting conditions, not allowing the emergence of RD. RD occurred when the outward K^+ current through BK channels was abolished by the extracellular application of paxilline, by removing Ca^{2+} from either the extra- or intracellular solution, by activation of phospholipase C or protein kinase C. Furthermore, RD could be evoked by activation of several neurotransmitter receptors, among others by GABAB receptors. To conclude, our results explain how RD arises in medial prefrontal cortex pyramidal neurons.

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|A12|

Alzheimer's disease early stage miRNA biomarkers in human plasma

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Alzheimer's disease (AD) is the most common age-related dementia. One of the major challenges in the AD field is deciphering the molecular signatures in peripheral tissues,

characteristic of early stages of the disease in patients with mild cognitive impairment due to AD (MCI-AD).

Using qRT-PCR we evaluated microRNA (miRNA) profiles in blood plasma collected from 15 MCI-AD patients, whose neuropsychological diagnoses were confirmed by cerebrospinal fluid (CSF) biomarkers, 20 AD patients and 15 non-demented, age-matched individuals (CTR). The TargetScan, MirTarBase and KEGG analysis of the differential miRNAs was done.

In the first screening, 179 plasma miRNAs were compared between AD and CTR, and between MCI-AD and CTR. 23 differentially expressed miRNAs reported earlier as AD biomarker candidates in blood were confirmed in the current study and 26 novel differential miRNAs between AD and CTR were detected. The potential of these 15 miRNAs to be used as biomarkers was further verified in independent AD, MCI-AD and CTR groups. Finally, 6 miRNAs (3 novel in AD context and 3 reported) were selected as the most promising biomarker candidates differentiating early AD from controls with the highest fold changes (from 1.32 to 14.72), consistent significance, specificities from 0.78 to 1.00 and sensitivities from 0.75 to 1.00 (patent pending, PCT/IB2016/052440). Bioinformatics analysis indicated putative protein targets of the differential miRNAs involved in key cell processes such as cell cycle, apoptosis and cancerogenesis.

The miRNA panel is promising for diagnostics of early AD and indication of complex signaling pathways contributing to the pathology.

|A13|

Neuroprotective and immunomodulatory properties of WJ-MSC cultured in 3D hydrogel scaffolds on postischemic organotypic hippocampal slices

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Mesenchymal stem cells (MSC) exhibit neuroprotective, angiogenic and immunomodulatory properties. Their availability, high plasticity and possibility for expansion have made MSC-based therapy one of the most commonly used in regenerative medicine. In order to provide optimal microenvironment *in vitro*, 3D scaffolds were designed with structural and functional properties to protect transplanted cells from recipient immune system, as well as facilitate structural logistic for host transplant interaction.

WJ-MSCs isolated from human umbilical cords were cultured under 21% O_2 and 5% O_2 conditions. The aim of *in vitro* study was to test the effect of different oxygen concentration and dimensional conditions on proliferation, viability and gene expression profile of WJ-MSC. In *ex vivo* studies an experimental model of oxygen glucose deprivation was used in order to mimic an ischemic injury. MSC-induced neuroprotection was evaluated after 24 h in OHC co-cultured with WJ-MSCs in 2D or 3D conditions. WJ-MSC from control (2D) and 3D scaffolds were characterized with qRT-PCR for the expression of growth factors and cytokines after 24 h of co-culture.

WJ-MSCs have a linear growth rate and are able to migrate beyond the 3D hydrogel scaffolds structures. The increased expression of e.g. BDNF, GDNF, VEGF-A, bFGF as compared with 2D cultures has been observed. WJ-MSCs have shown a strong neuroprotective effect on injured hippocampal slices. Moreover, WJ-MSC cultured on 3D scaffolds revealed the increased expression of several neurotrophins (BDNF, NGF), growth factors (bFGF, EGF) and decreased expression of pro-inflammatory cytokines, e.g. IL-1β, together with higher expression of anti-inflammatory TGF-β.

The results have indicated that different conditions of microenvironment (oxygen concentration and 3D scaffolds) affect analyzed stem cells properties. Moreover, the analyzed scaffold models, together with modulating oxygen level allow building up biomimetic conditions for *in vitro* stem cells culture and serving as a promising material for future use in MSC-based therapy.

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|A14|

Cytoprotective role of kynurenic acid in the experimental model of liver disease – seeking for a solution to avoid encephalopathy

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The latest research on the peripheral effect of kynurenic acid (KYNA) have shown that it reduces the development of oxidative stress and has an anti-inflammatory and cytoprotective effect, so it causes biological actions which may inhibit acute liver failure (ALF) development.

The aim of this work was to assess the influence of KYNA on the inflammatory process and oxidative stress in thioacetamide (TAA) induced ALF in rats.

The research was conducted on male Wistar rats. The level of liver damage was estimated basing on histopathological image analysis as well as alanine and aspartate aminotransferase (ALT, AST) activity. The influence of KYNA on the synthesis of interleukin 10 (IL-10) and cachectin (TNF-α) as immunological activation markers was also investigated. The level of oxidative stress was assessed basing on the measurement of concentration of heme oxygenate induced form (HO-1), the level of lipids peroxidation products – malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) and myeloperoxidase activity (MPO). Additionally, oxygen radical absorbance capacity (ORAC) was quantified based on the decrease in fluorescence of the so-called molecular probe. The level of oxidative protein damage was assessed based on measurement of concentration of thiol groups (-SH) in the liver homogenates. The concentration of KYNA in tissue homogenates was assessed with HPLC methods.

KYNA had positive effect on all studied biochemical parameters and decreased level of proinflammatory TNF-α.

KYNA shows a protective effect, inhibiting development of TAA-induced ALF, which could be useful in prevention of encephalopathy.

|A15|

The heterozygous mutation of *LRRK2* gene – p.Asp1437His in Polish family

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, with the prevalence of about 1% in people over 60 years of age. PD results from degeneration of dopaminergic neurons in the substantia nigra pars compacta.

The exact pathomechanism of the disorder still remains unknown, however is assumed that the sporadic cases, constituting the majority, are caused by a combination of genetic and environmental risk factors. Approximately 15% PD cases are familial, monogenic. Genetic background of familial PD, especially of late onset has not been very well analysed in Polish population.

The aim of our study was to characterize the familial case of PD caused by pathogenic variant, missense mutation p.Asp1437His in *LRRK2* gene.

The large PD family, 10 affected members in four generations, with autosomal dominant disease inheritance was included in the analysis. General and neurological examination was performed for all affected, living family members. The Whole exome sequencing (WES) was performed for the proband. All identified variants were confirmed by Sanger sequencing, and cosegregation of genotype-phenotype was performed for all available 3 family members.

WES analysis performed for family proband showed the presence of heterozygous missense mutation – p.Asp1437His in gene *LRRK2*, presented in all affected family members under analysis. All examined patients - carriers of mutations had symptoms of typical PD.

In presented family PD was confirmed to be caused by mutation in the *LRRK2* gene – p.Asp1437His. Mutations in this position, substitution of p.Asp1437 to His or Ser have already been described and their role in PD pathogenicity proven. To our knowledge this is the first family with this mutation in Poland. Only a few such cases were described but detailed analysis of available data revealed similar symptoms in all patients – age of onset about 50 years old and asymmetrical slow progressive parkinsonism.

|A16|

Blood-based microRNA diagnostic panel for Alzheimer's disease: perspective after a decade of research

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the main cause of dementia in the elderly. Among critical needs are AD biomarkers preferably present in easily available tissues such as blood. One of the most promising approaches concentrates on circulating microRNAs (miRNAs). We evaluated AD biomarker potential of circulating miRNAs reported in the last decade by addressing data reproducibility, AD specificity and also mechanistic links of the differential miRNAs with AD pathology.

The study concept was based on the assumption that AD molecular signature in the blood may include molecules originating from the brain and the CSF, as well as from peripheral tissues. We used a 'bottom-up' approach. First, we identified repeatedly reported differential blood-based miRNAs. Next, we revised reports of the differential miRNA profiles in the CSF and the brain and searched for the overlap between the miRNA alterations in these specimens. Finally, we computationally predicted genes and pathways regulated by the selected miRNAs. Data were retrieved from Web of science, miRTarBase and WebGestalt appropriately.

Our analysis shows that out of 137 miRNAs found to be altered in 34 AD blood studies, 36 have been repeatedly found, and out of 166 miRNAs reported as differential in 26 AD CSF studies, 13 have been repeatedly found. Only 3 miRNAs have been consistently reported as altered in three analyzed specimens: blood, CSF and the brain (miR-146a, miR-125b, miR-135a). Nonetheless, all 36 repeatedly differential miRNAs in AD blood are promising as components of the diagnostic panel. We found that AD-implicated miRNAs target many cancer-related transcripts and overlap, but in opposite direction of regulation, with miRNAs implicated in cancer indicating epigenetic tradeoff between the two diseases.

Minimally invasive blood-based miRNA panel detecting selected circulating miRNAs may facilitate AD diagnostics. The miRNA panel can report multiple pathways contributing to AD pathomechanisms, enabling the design of personalized therapies for AD.

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|A17|

No modification in blood GFAP and NGF levels but changes in BDNF concentrations after exercise to volitional exhaustion in sedentary individuals exposed to acute normobaric hypoxia

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The physiological state of the central nervous system (CNS) can be determined indirectly in humans by measuring serum concentration of neuropeptides which are synthesized in the brain and can cross the blood-brain barrier (BBB).

One of the best studied protein produced by neurons and involved in protection against their neurodegeneration is brain derived neurotrophic factor (BDNF). However, it is know that astrocytes and microglia are also able to produce and release neuropeptides: glial fibrillary acidic protein (GFAP), and nerve growth factor (NGF), respectively. Moreover, these tissues are known to be involved in supporting of the physiological functioning of neurons.

The most well-known environmental factors modifying the CNS activity are physical exercises and/or hypoxic condition.

Therefore we investigated whether exercise performed on ergocycle to volitional exhaustion under normobaric hypoxia conditions (equivalent of 2000 m – 16.6% O_2 – H2000, and 3000 m – 14.7% O_2 – H3000) affects release of aforementioned neurotrophins in seven sedentary volunteers.

In normoxic condition, in response to exercise the serum BDNF level decreases. The same effect was seen in low (H 2000) hypoxic conditions. However, the opposite effect occurred in moderate hypoxic conditions (H3000). There was no significant changes in serum GFAP and NGF levels before and after exercises in all investigated conditions.

Our study provide evidence that exercise-induced activation of neurons depends on severity of acute hypoxia. Both exercise and acute hypoxia does not affect physiological state of astrocytes and microglia.

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|A18|

TOMM40 variants and oxidative damage regulation in Alzheimer's disease

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Alzheimer's disease (AD) is a progressive disorder leading to deterioration of cognition in older adults, with improper metabolism of biothiols, such as homocysteine (Hcy) and glutathione (GSH). Antioxidant GSH protects cells and mitochondria from oxidative stress and decreases formation of 8-oxo-2'-doexyguanosine (8-oxo2dG), excised from DNA by N-glycosylase of 8-oxoguanine 1 (OGG1). GSH enters mitochondria via translocase of the outer mitochondrial membrane homolog 40 (TOMM40) whose variants are associated with AD.

The objectives were to analyze the rs1052452 and rs2075650 polymorphisms in TOMM40 locus and the levels of Hcy and GSH, oxidative damage (8-oxo2dG) and reparative enzyme (OGG1) in plasma of AD patients and controls.

230 individuals were recruited: 88 subjects with AD, 80 control volunteers without (UC) and 62 control persons with (RC) positive family history of AD, all above 60 years of age. The plasma concentrations of OGG1 and 8-oxo2dG were determined by ELISA, whereas Hcy and GSH were assessed by HPLC/EC method. The TOMM40 genotypes were determined by HRM and capillary electrophoresis.

Minor variants: rs10524523-L and rs2075650-G occurred more frequently in AD vs. UC ($p < 0.0001$; Fisher exact test). About 2/3 of AD patients had increased Hcy levels ($p < 0.01$ vs. UC and $p < 0.001$ vs. RC), while GSH ($p < 0.01$ vs. UC) and 8-oxo2dG ($p < 0.01$ vs. UC and $p < 0.001$ vs. RC) were significantly reduced (Mann-Whitney test). In carriers of major variants: rs10524523-S/VL, and rs2075650-A we observed the misbalance of Hcy and GSH as well as reduced DNA repair capacity (8-oxo2dG and OGG1).

It seems that TOMM40 rs2075650 and rs10524523 polymorphisms may be risk factors of developing AD, whose frequent variants are complemented by improper biothiols turnover and less efficient mechanisms of DNA repair.

|A19|

3,3'-diindolylmethane protects hippocampal cells against oxygen and glucose deprivation via inhibition of apoptosis and autophagy

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Although stroke is the 2nd leading cause of death worldwide, there are no effective therapies that may protect the brain against ischemia-induced damage. Our previous study has shown that plant-derived 3,3'-diindolylmethane (DIM) protects neurons undergoing hypoxia via inhibition of AhR/ARNT signaling pathway. However, there are no data examining the effects of DIM on neuronal cells subjected to oxygen and glucose deprivation (OGD). Little is also known about the effects of DIM on ischemia-induced apoptosis and autophagy. Therefore, the aim of the present study was to investigate neuroprotective capacity of DIM in mouse hippocampal cells exposed to OGD at different developmental stages with special concern on apoptosis- and autophagy-dependent effects.

Our experiments were performed on mouse primary hippocampal cell cultures. On 2, 7 and 12 day *in vitro* (DIV) the cells were treated with DIM (0.01-10 μM) and subjected to 6 h of OGD. Caspase-3 and lactate dehydrogenase (LDH) activities as well as protein expression levels were measured after 18 h of reoxygenation. We have shown that 6 hours of OGD increased LDH release by 24%, 165% and 140% at 2, 7 and 12 DIV, respectively. OGD also caused 30% enhancement in caspase-3 activity, but only at 7 and 12 DIV. DIM inhibited the ischemia-induced LDH and caspase-3 activities at early and later developmental stages. DIM strongly decreased the expression of pro-apoptotic proteins such us FAS and Caspase-3, and autophagy-related Beclin-1. DIM also reversed the ischemia-induced decrease in the level of Nucleoporin 62.

These data demonstrated strong anti-autophagic and anti-apoptotic capacity of DIM in hippocampal cells subjected to oxygen and glucose deprivation thus providing prospects for the designing of new therapeutic strategies targeting different types of cell death on early and later stages of neuronal development.

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|A20|

The effect of α-synuclein on initiation of inflammatory reaction in the murine model of Parkinson's disease

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Parkinson's disease (PD), one of the most common neurological disorders, is characterized by the loss of dopaminergic neurons in substantia nigra and striatum (ST). The progression of PD is characterized by an inflammation, especially the activation of the microglia. The data suggests that increased level of α-synuclein (ASN), a small protein which is the major component of Lewy bodies, can induce microglia activation. Activated microglial cells release proinflammatory and potentially cytotoxic substances like cytokines.

The aim of this study was to investigate the role of increased ASN monomers concentration as a major pathogenic factor causing microglia response, and changes in the expression of inflammatory cytokines [interleukin 1α (IL-α), IL-10, IL-12 as well as tumor necrosis factor α (TNF-α)] in ST. We also examined the level of ionized calcium binding adaptor molecule 1 (Iba-1) and transglutaminase 2 gene (TG2, an enzyme involved in aggregation of ASN) in ST.

Male and female C57Bl/10 Tar 9 month-old mice were used in this study. Human recombinant ASN was bilaterally administered into ST (single treatment – 4 µg per structure, 8 µg per brain). Mice were decapitated 4 or 12 weeks post injection. The changes in the level of inflammatory factors in ST were evaluated using real time PCR and enzyme-linked immunosorbent assay (ELISA).

We observed increased level of a microglia marker – Iba1 protein after ASN injection into ST. We noticed also some differences in the levels of IL-1α, IL-10, IL-12 and TNF-α mRNA. The ASN administered intracerebrally into ST increases striatal expression of *TG2* gene, which may lead to enhanced ASN aggregation.

Our results support the hypothesis of pro-inflammatory impact of ASN monomers. Injection of ASN into ST induces microglia activation. Our research provides further evidence for the involvement of ASN in the inflammatory response in the CNS.

|A21|

Impact of VGVAPG peptide on nitric oxide synthases in mouse cortical glial cells *in vitro*

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Degradation products of elastin, elastin-derived peptides (EDPs) are detectable in cerebrospinal fluid of both group of healthy subjects and patients with ischemic and hemorrhage stroke. To date, it has been well described that main effects of EDPs are mediated through the interaction with an elastin binding protein (EBP), identified as an enzymatically inactive spliced variant of Glb1 gene. Nitric oxide (NO) is synthesized from L-arginine by enzymes named NO synthases (NOS). To date three isoforms have been described: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). NO modulates neuronal survival and differentiation. Moreover, a number of studies have shown that NO regulates neurotransmitter release and different aspects of synaptic dynamics, such as differentiation of synaptic specializations, microtubule dynamics, architecture of synaptic protein organization, and modulation of synaptic efficacy. Therefore, the aim of this research was to investigate the impact of the elastin-derived peptide, the Val-Gly-Val-Ala-Pro-Gly (VGVAPG) on nitric oxide synthases in mouse cortical glial cells *in vitro*. The cultures of cortical astrocytes were prepared from Swiss mouse embryos on 17/18th day of gestation period. The cells were cultured in phenol red-free DMEM/F12 medium supplemented with 10% FBS and were exposed to 10 nM or 1 µM of VGVAPG peptide for 6 h. Moreover, siRNA gene silencing technique was applied. Afterwards, mRNA was collected and gene expression was measured by qPCR method. The results showed that after 6 h of exposure to VGVAPG peptide gene expression of nNOS, iNOS and eNOS were decreased. However, after use of Glb1 gene siRNA we observed an increase in expression of nNOS, iNOS and eNOS. Our data suggests that some of the effects caused by VGVAPG peptide depend on EBP. However, more research underlying mechanism of VGVAPG peptide action in nervous system is needed.

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|A22|

Neuroprotective effects of cystamine in the murine model of Parkinson's disease

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Parkinson's disease (PD) is a common neurodegenerative movement disorder characterized by a progressive and selective loss of dopaminergic neurons in substantia nigra and an accumulation of intraneuronal Lewy bodies containing misfolded α-synuclein. Neurodegeneration is coincident with a decrease in dopamine, the dopamine transporter and the dopamine metabolites levels in PD brain. Current treatment of PD are only symptomatic and do not stop neuronal loss. Recently enormous amount of work has been conducted to identify molecules that could be used as neuroprotective drugs. One of them is cystamine – the inhibitor of transglutaminases activity. Transglutaminases are involved in the formation of cellular α-synuclein aggregates, therefore blocking of its activity may prevent the PD progression.

Male C57Bl/10 Tar mice 1 year-old were used in this study. Cystamine (40 mg/kg) was injected intraperitoneally for 14 days, beginning 13 days prior to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 40 mg/kg) intoxication. The changes in the mRNA level of inflammatory factors (Iba-1 and interleukin 1a) in striata were examined using real-time PCR. Neurotransmitters levels (dopamine and its metabolites) in striata were evaluated by high-performance liquid chromatography (HPLC).

Our study demonstrated that chronic administration of cystamine before MPTP intoxication improved striatal levels of dopamine and its metabolites (homovanillic acid and 3,4-dihydroxyphenylacetic acid), as compared to MPTP-treated groups. We observed also an inhibition of inflammatory reaction induced by MPTP (lower expression of microglia marker and proinflammatory interleukin).

Cystamine preserves nigrostriatal function after MPTP intoxication and may have the treatment efficacy in PD. However, further research must be conducted to provide more evidence of protective role of cystamine in PD.

|A23|

Myriocin alters transcription of NAD+ dependent enzymes in animal model of Alzheimer's disease

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The last data indicated that enzymes involved in metabolism of bioactive sphingolipids: ceramide and sphingosine-1-phospate (S1P) are very promising targets for the therapy of neurological disorders. In this study we examined in animal model of Alzheimer's disease the effect of myriocin (MYR, the inhibitor of serine palmitoyl transferase – key enzyme of *de novo* ceramide synthesis) on brain mRNA levels of NAD+ dependent enzymes: sirtuins and PARP-1 which play crucial role in the regulation of energy metabolism, oxidative stress, cell survival and death.

FVB/APP+ transgenic mice with London APP (V717I) mutation were used in this study. Mice without mutation (FVB/APP–) were used as a control. Animals were divided in three age groups (3, 6 and 12-month; 3M, 6M, 12M) and received MYR (*i.p.* 1 mg/kg b.w.) or DMSO (vehicle) for 2 weeks. Brain cortex was isolated, biochemical and qPCR methods were applied.

Our results indicate several significant changes in SIRTs gene expression in APP+ mice vs APP–.

We observed that *Sirt4* gene expression was downregulated in cortex of 3M APP+ mice. Mutation in APP transiently elevated *Sirt5* gene expression in 6M mice brain cortex. However, *Sirt1*, *Sirt5* and also *Parp1* gene expression was significantly downregulated in 12M APP+ mice brain cortex. MYR administration significantly upregulated cortical *Sirt1*, *Sirt3*, *Sirt5* gene expression in 6M and Parp1 in 12M APP+ mice. Concomitantly, administration of MYR elevated gene expression of anti-apoptotic BCL-2 protein, which was downregulated in 3 and 6M APP+ mice brain cortex.

Our data indicate that MYR could be an potent modulator of SIRTs and PARPs gene expression and may have important implications in therapeutic strategy of neurodegenerative disorders.

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|A24|

Modulation of the crosstalk between co-cultured glioma and microglial cells to alter PSA-NCAM and Siglec-E expression by dexamethasone

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According to the guidelines for glioma therapy, dexamethasone (Dex) is considered as the main steroid routinely used in the management of tumor-induced edema. There are growing evidences that Dex, in addition to the strong therapeutic potential, may interfere with function of resident immune cells and promote glioma-induced weak immune surveillance. The aim of this study was to evaluate the effects of Dex on microglia surface Siglec-E expression and its potential ligand, polysialylated neuronal cell adhesion molecules (PSA-NCAMs). We used ESdM (embryonic stem cell derived microglia) and glioma GL261 cells grown in monoculture or in co-culture exposed to 10 μM of Dex for 24 hours. Expression of Siglec-E and immune activity markers, e.g. IL-1β, IL-10 and Iba-1, were analysed in microglia using flow cytometry. The effect of Dex on sialylation of NCAMs was assessed in both, microglia and glioma cells. In response to Dex the expressions of IL-1β and Iba-1 in microglia were reduced, and these effects were potentiated when co-cultured with glioma cells. In opposite, Dex did not alter IL-10 level in microglia in monoculture, while in co-culture the level was increased by about 30%. In monoculture, the level of PSA-NCAM increased significantly in GL261 cells, but not in microglia, in presence of Dex. In co-cultures, both naïve and treated cells showed enhanced sialylation of NCAM when compared to monocultured cells. These results suggest that Dex-induced alterations in Siglecs expression may be involved in the regulation of glioma-immune communication.

|A25|

Analysis of *PINK1* gene polymorphisms in Parkinson's disease

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Parkinson's disease (PD) is a chronic and progressive neurological disorder characterized by rigidity, bradykinesia and resting tremor. It affects at least 1% of individuals above the age of 60 and 5% above the age of 85. The pathogenesis of PD depends on both environmental and genetic factors like mutations and polymorphisms in PARK genes e.g. *PINK1* (PARK6). *PINK1* gene encodes PTEN-induced kinase 1 and mutated form of its protein is associated with damage of neurons by stress-induced apoptosis and mitochondrial dysfunction.

The aim of the study was to analyze the frequency of two polymorphisms of PINK1 – G1018A and A1562C, in PD patients and control group. The duration of disease and response to levodopa treatment was taken into account.

To this study we enrolled 57 patients (mean age was 57) with PD and 57 healthy controls (mean age was 60). Genotyping was performed using the HRM method. The obtained results were confirmed by sequencing.

The study has shown no significant differences in the frequency of occurrence of G1018A and A1562C polymorphisms between the study group and the controls. However, the frequency of A allele of G1018A and C allele of A1562C was higher in PD patients than in the control group. GA genotype of G1018A and both AC and CC genotypes of A1562C was more frequent in PD group compared to controls. These patients were characterized by a good response to levodopa treatment and the duration of the disease more than five years.

It seems that there is an association between the frequency of occurrence of both PINK1 polymorphisms G1018A and A1562C in PD patients. Further study of *PINK1* gene may bring new information into the pathogenesis and contribute to improving the quality of patients' life.

|A26|

Lipoxygenases and other stress response proteins in experimental models of Alzheimer's disease. Searching for promising targets in neuroprotection

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It is proposed that oxidative stress, amyloid β oligomers (AβO) and alterations of ceramide metabolism/level may play a crucial role in pathogenesis and in mechanism of Alzheimer's disease (AD).

This study focused on the expression of genes encoded lipoxygenases and other prooxidative/antioxidative enzymes and proteins in experimental models of AD. The study were carried out on *in vivo* model: FVB/APP+ transgenic (Tg) mice with London APP mutation (V7171), and control FVB/APP-mice; and *in vitro* model: PC12 cells transfected with human wild type APP (APPwt) or bearing double Swedish mutation (APPsw) and control PC12 cells transfected with empty vector. The PC12 cells were subjected also to additional stress evoked by ceramide. The effect of selected pharmacological compounds was investigated. Molecular biology and biochemical methods were applied.

Our study carried out on Tg mice demonstrated alterations of gene expression and protein level for prooxidative enzymes such as 12- and 5-lipoxygeneses (12-LOX, 5-LOX) and nitric oxide synthase (NOS) in hippocampus and brain cortex. The anti-oxidative enzymes: SOD1 and SOD2 expression was not changed. However, mRNA and protein level of mitochondrial apoptosis inducing factor (AIF), crucial player in antioxidative defense and regulator of cells survival or death, was significantly upregulated, which may suggest its protective function in this conditions. Moreover, the transcription of subunits of all ETC complexes was not altered. The further analysis of molecular processes carried out on cell cultures indicated that ceramide induces AIF release from mitochondria and affects transcription of mitochondria sirtuins. Ceramide in APP transfected PC12 cells enhanced gene expression for BACE1 and subunits of secretase γ and significantly decreased cells viability. Agonist of S1P receptor, SEW 278 protects the cells against ceramide and Aβ toxicity.

Our study suggest that 12-LOX and 5-LOX and S1P receptor 1 should be promising targets for improvement of AD therapy.

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Poster session II

|B1|

The role of oxygen tension in the MeHgCl *in vitro* embryotoxicity

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Methylmercuric chloride (MeHgCl) is a neurotoxic and embryotoxic agent, but the role of oxygen tension for toxic effect of MeHgCl exposition was not evaluated yet.

Human induced pluripotent stem cells (hiPSC) were cultured in 21% O_2 and 5% O_2 oxygen tension. This model was used in our study as an alternative to ethically controversial embryonic stem cells, to test the MeHgCl embryotoxicity *in vitro*. hiPSC was exposed for 5 days to the MeHgCl (0-1 µM), after this time: viability, ROS level and gene expression of POU5F and NANOG by qRT-PCR were analyzed.

In control populations 5% O_2 we have shown higher expression of POU5F1 and NANOG genes as compared to 21% O_2, which was confirmed by immunocytochemistry at the protein level. The viability was decreased by MeHgCl in both oxygen conditions, but for 0.5 µM it was significantly lower only in the 21% O_2 ($p < 0.001$). The decreased viability was accompanied by a significant increase in ROS level ($p < 0.05$) for all tested doses only in 21% O_2. The significant difference in the ROS level as compared between tested oxygen conditions was confirmed for 0.5 and 1 µM ($p < 0.05$) dose of MeHgCl. The response to the MeHgCl regarding the expression of pluripotency markers on the mRNA level was opposite in different oxygen conditions: NANOG and POU5F1 were significantly ($p < 0.001$) up-regulated in the 5% O_2 while down-regulated in the 21% O_2.

In this report, we have shown that oxygen tension plays an important role in the MeHgCl toxicity, and should be taken into account as an important parameter in the *in vitro* embryotoxicity studies.

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|B2|

Investigating a mechanism of spontaneous, aging-related head twitching in rats: study of mRNA expression of monoaminergic receptors and their signaling proteins in hippocampus

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Aging is a natural biological process that is associated with physiological decline – both physically and cognitively. This impairment is caused by alterations in mechanisms of intra- and inter-cellular signaling in brain. Above process may be reflected by the appearance and increase of spontaneous head twitches during aging in rats.

We aimed to investigate the expression of signaling-related genes (monoamine receptors, α and β subunits of G proteins, small G proteins, adenylate cyclases, kinases, phospholipases, matrix metalloproteinases) in the hippocampus (HIP) of Wistar rats during aging. Three groups of animals were assessed: young control rats, old rats (at age of 26-30 months) divided into two subgroups, HSHT – with high number and LSHT – with low number of spontaneous head twitches. First, we used the TaqMan Low Density Arrays to identify particular genes with changed expression in aging. Then, obtained result were validated on larger group of rats by means of the PCR reactions with single TaqMan probe for identified gene.

We identified 16 candidate genes whose expression was changed in old rats (one-way ANOVA, *post-hoc* Tukey test, $p < 0.05$). For further detailed analysis 8 especially interesting genes were chosen. Finally, we found the decrease of mRNA expression of Htr1a, Htr5b, Mapk8/Jnk1, Mmp2, Prkcb genes in both HSHT and LSHT groups vs. control group (by 20-45%, $p < 0.01$), whereas the increase of mRNA expression of only Adrb2 gene was observed ($p < 0.01$). Moreover, we showed no differences in mRNA expression of above genes between HSHT and LSHT groups. Summarizing, these

results revealed mainly the decrease in mRNA expression of selected signaling genes during rat aging. However, we found no correlation between changes in mRNA expression of assessed genes and the number of spontaneous head twitches during senescence of rats.

|B3|

The metabotropic glutamate receptors group II (mGluR2/3) agonists postconditioning reduces brain damage in the model of birth asphyxia in 7-day-old rats

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Hypoxic-ischemic encephalopathy (HIE) results in permanent damage of central nervous system that may result in neonatal death or developmental disorders. It was shown recently that group II metabotropic glutamate receptors (mGluR2/3) activation before or after ischemic insult results in neuroprotection but the exact mechanism of this effect is not clear. The aim of present study was to investigate whether mGluR2/3 activation after hypoxia-ischemia reduces brain damage and if the reduction of the expression of pro-apoptotic factors is one of the mechanisms involved.

We used an animal model of hypoxia-ischemia (H-I) on 7-day old rat pups in which the left common carotid artery was isolated and cut between the ligatures. Thereafter the pups were subjected to hypoxia (7.4% oxygen in nitrogen for 75 min). Control pups were sham-operated. Animals were injected intraperitoneally with specific mGluR2 (LY 379268) and mGluR3 (NAAG) agonists 1 h or 6 h after H-I (5 mg/kg). The weight deficit of the ischemic hemisphere was measured. The damage in the hippocampal CA1 region was examined by Cresyl violet staining. Infarct area was measured using TTC staining. The activity of caspase 3 and 9 was measured.

Our results show that application of mGluR2/3 agonists after H-I results in neuroprotection. Both applied agonists decreased weight loss in ischemic hemisphere at both times of application (from 40% in H-I to 15-20% in treated). Both mGluR2/3 agonists applied 1 h or 6 h after H-I decreased the damage of neuronal cells and the disorganization of CA1 region of hippocampus and reduced the brain infarct area. mGluR2/3 agonists reduced increased by H-I activation of caspase-3 and caspase-9.

The results show that activation of mGluR2 or mGluR3 in a short time after H-I insult triggered neuroprotective mechanisms and reduced apoptotic processes initiated by HI in developing brain.

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|B4|

Influence of microbiotic supplementation (*Lactobacillus rhamnosus* JB-1™) on behavioural and brain metabolic changes in rat model of chronic unpredicted mild stress

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Changes in gut microbiota have been shown to have an impact on concentration of certain neurotransmitters in the rodent brain. Thanks to the brain-gut-microbiome axis it is possible to regulate those levels which may have a therapeutic effect in depressive disorders. The purpose of this study was to assess behavioural and metabolic (glutamate – Glu and gamma-aminobutyric acid – GABA) changes after microbiotic diet in an animal model of chronic unpredictable mild stress (CUMS) with the use of elevated plus-maze (EPM) test and ELISA tests.

Healthy male Wistar rats were treated with seven weeks CUMS protocol. Simultaneously, half of them was fed with *Lactobacillus rhamnosus* JB-1™ (JB-1, $N = 12$) and the second half was given placebo (PBS, $N = 12$). To verify stress model each rat underwent the 5-min EPM test of anxiety: the number of animal entries into open and enclosed arms and the time spent in the arms were measured. After diet rats were decapitated, the brains were sectioned into hippocampus and cortex. Levels of glu-

tamate and GABA were assessed from the brain extract using ELISA tests.

In the behavioural tests, the JB-1 group demonstrated longer exploration time than placebo group (43 vs. 18 s, $p = 0.037$). The JB-1 diet resulted in mitigated anxiety, and the placebo group displayed deepened anxiety expressed by almost complete avoidance of exploration. The results of the ELISA test confirmed statistically significant differences for glutamate: higher concentration in JB-1 vs. placebo group in hippocampus (8.9 ±1.3 vs. 7.6 ±0.8 µmol/g, $p = 0.008$) and in cortex (10.0 ±0.9 vs. 9.1 ±0.3 µmol/g, $p = 0.02$). There were no significant differences between groups for GABA assessed from brain extracts.

Our study shows that chronic stress induces anxiety-like behaviour. Enriching gut microbiome with JB-1™ bacteria strain cause glutamate increase, both in hippocampus and cortex, and improves animals' behaviour.

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|B5|

Farnesoid X receptor in the brain of rats with tioacetamide-induced acute liver failure

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Farnesoid X receptor (FXR), a key regulator of bile acids (BA) homeostasis, is highly expressed in the liver and intestines. As a consequence of liver failure, an increase of BAs in the systemic circulation and further impairing the blood-brain barrier (BBB) might be observed. The very latest reports demonstrated FXR expression in human and mice brain.

The purpose of this study was to examine BA concentration in the plasma and brain of rats with thioacetamide (TAA) induced acute liver failure (ALF). We also aimed, to our knowledge for the first time, analysis of the expression of FXR in the brain (cortex, hippocampus, cerebellum) of ALF rats, and primary cultures of neurons and astrocytes.

ALF resulted in a significant increase and unaltered total BA level in plasma and brain tissue, respectively. A quantitative RT-PCR analysis revealed that ALF highly reduced expression of fxr and shp mRNA in the rat liver. Cerebral levels of fxr and shp mRNA were intrinsically low, however, the decrease and/or decreasing tendency in the TAA group was also observed. Immunofluorescence imaging of FXR in primary cultures revealed its content mainly in neurons. Subsequently, the FXR immunofluorescence in the brain and co-localization with neuron/astrocyte markers confirmed FXR dominant localization in neurons. Importantly, FXR staining was the highest in Purkinje cells of the cerebellum.

Summing up, these results confirmed and extend previous reports in terms of determining the cell-specific localization of FXR in the brain. The main finding is the demonstration that TAA did not result in the increase of brain BA level and brain FXR, and that the decrease observed in the level of mRNA is not distinctly reflected in the protein level. Above observations may be associated with subtle BBB impairment documented in the TAA model of ALF.

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|B6|

Nanosilver-induced autophagy in brain of rat subjected to prolonged oral exposure

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Silver nanoparticles (AgNPs) are widely used in the production of medical and commercial products because of their anti-microbial activity. Together with the rapid development of nanotechnology and the widespread use of AgNPs, the risk of human exposure rapidly increases. Therefore, understanding of the mechanisms of their cell-specific cytotoxicity is of importance. There is evidence that exposure to AgNPs leads to the oxidative stress in many organs, including brain, by inducing reactive oxygen species (ROS). AgNP-induced oxidative stress may cause accumulation of damaged proteins, which in turn, may provide signals for the activation of the process of autophagy. In the present study we investigated ultrastructural and biochemical markers of autophagy in the brain of rats exposed to a low dose of AgNPs. Small (10 nm) citrate-sta-

bilized silver nanoparticles were administered once daily via the gastric tube at a dose of 0.2 mg/kg b.w. per day for 14 days. Appropriate control groups of rats received silver citrate or saline, respectively. Ultra-thin sections of the rat brain were examined by transmission electron microscopy.

Analysis of electronmicrographs revealed swollen mitochondria with disturbed cristae, as well as myelin-like bodies (concentrically layered structures containing fragmented membranes and mitochondria inside) exclusively in the brain of AgNP-exposed rats. These structures represent the initial stage of macroautophagosomes formed in the process of autophagy in response to stress. Mitochondrial membrane potential, as well as expression of autophagy markers such as beclin 1, MAP LC3-II, Rab 7, cathepsin β, were measured. We noticed significant reduction of mitochondrial membrane potential in AgNP-exposed rats. Moreover, exposure to AgNPs increased relative protein concentration of beclin 1 and MAP LC3-II, whereas the concentration of Rab7 and cathepsin β was not changed. The results indicate that activation of autophagy may be the cellular response against AgNPs-toxicity. However, this process is presumably ineffective being limited to the initial stage.

|B7|

Tetrabromobisphenol A depolarizes cerebellar granule cells in primary culture: role of the ionotropic glutamate receptors and voltage-gated sodium channels

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Available literature data indicate that the brominated flame retardant tetrabromobisphenol A (TBBPA) may depolarize synaptosomes isolated from the rat brain cortex, but there is no relevant information concerning intact neurons in culture. Depolarization may be an important element of TBBPA neurotoxicity. Therefore, in the present study, using oxonol VI, a fluorescent probe sensitive to changes in plasma membrane potential, we investigated the effects of TBBPA on the plasma membrane potential of cerebellar granule cells (CGC) in the primary culture. The second aim was to reveal the mechanisms of the expected depolarization. Because CGCs are glutamatergic neurons, predictable mediators of depolarization could be ionotropic glutamate receptors, i.e. NMDA and AMPA receptors. This was tested using selective antagonists of these receptors, MK-801 and CNQX, respectively. According to the working hypothesis tested here, an additional mechanism independent of glutamate receptors may also participate in the TBBPA-induced CGC depolarization. Sodium-gated sodium channels were pre-selected for testing, and this hypothesis was verified using tetrodotoxin (TTX). The control experiments showed that 100 mM KCl and 100 μM glutamate induced instant rise in oxonol VI fluorescence, which reflects the depolarization of neurons. TBBPA in concentration greater or equal to 7.5 μM, induced a gradual, concentration-dependent increase in fluorescence, which was significantly inhibited in the presence of MK-801, while the tendency to inhibit it by CNQX did not reach the level of statistical significance, and TTX given separately had no significant effect. The application of combination of MK-801, CNQX and TTX was required for an almost complete prevention of TBBPA-induced depolarization. These results show for the first time depolarization of CGC by TBBPA, and disclose the role of ionotropic glutamate receptors and voltage-gated sodium channels in this phenomenon.

|B8|

Oligodendrocyte response to temporal deprivation of oxygen and glucose affects polarization of microglia: *in vitro* model of perinatal asphyxia

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Temporal deprivation of oxygen and trophic support is one of the main features of perinatal asphyxia, leading to development of subsequent leukodystrophic disorders. One of the very first consequence of the experienced hypoxic-ischemic episode is neuroinflammatory process triggered by activation of microglia residing in the nervous tissue. The reciprocal interaction between oligodendrocytes (myelin generating cells) and microglia (associated with immunological response) are hypothesized to play a major role in potential overcoming the nervous tissue crisis. To verify this hypothesis, the co-culture experiments

were established for the purpose of applying *in vitro* model of oxygen-glucose deprivation (OGD). Accordingly, both the oligodendroglial and the microglial cell fractions were separated from the primary cultures of glia isolated from the brains of neonatal Wistar rats. The obtained homogenous monocultures of either oligodendrocyte progenitors or microglia were subjected to a OGD procedure, in order to mimic *in vitro* the hypoxic-ischemic insult accompanying the perinatal asphyxia. For *ex vivo* studies, the hippocampal organotypic slices were prepared and after being subjected to OGD procedure, were also used for establishing the co-culture systems. Such a schedule allowed us to evaluate interactions between cells, contributing to initiation of mechanisms leading to development of leukodystrophic diseases. The cell phenotype was determined by specific antibodies: ED1, Iba1 and anti-arginase for microglia and anti-NG2, anti-O4, anti-GalC and anti-MBP for oligodendroglia. The migratory potential of the examined cells was assessed by live recording by means of Cell Observer SD (Zeiss). The obtained results indicated that even a short deprivation of oxygen and trophic support affects microglial polarization. This effect is also exerted in a paracrine manner by the OGD-subjected oligodendrocytes. In conclusion, the determined *in vitro* interaction between neural cells might indicate the directions of future pre-clinical studies aimed at modulating cell response to hypoxic-ischemic insult and subsequent eliminating or diminishing results of perinatal asphyxia.

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|B9|

Comparison between LSD and 25-I-NBOMe effects on brain neurotransmitters and WDS response in rats

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Recently the novel psychoactive substances (NPS) have become popular as recreational drugs of abuse. Hallucinogens, a class of NPS, powerfully alter perception and mood but do not produce dependence and addiction. Indoloamine and phenylethylamine hallucinogens are potent agonists of serotonin receptors. 4-Iodo-2,5-dimethoxy-N-(2-methoxybenzyl)phenethylamine (25-I-NBOMe) is a N-benzylmethoxy derivative of the 2C family of hallucinogens that mimic LSD effect. Serious toxicity and fatalities have been described. The knowledge on central nervous system (CNS) effect of 25-I-NBOMe is very limited. Therefore, the aim of this study was to find out the effect of 25-I-NBOMe and LSD on dopamine (DA), serotonin (5-HT) and glutamate extracellular levels in the rat frontal cortex and striatum. Furthermore, the ability of both drugs to evoke wet dog shakes (WDS) as indication of hallucinogenic activity in rats was also examined. Rats were treated with single doses of 25-I-NBOMe (1, 3, 10 mg/kg *s.c.*) or LSD (0,1 mg/kg *i.p.*). The release of DA, 5-HT and glutamate was measured using microdialysis in freely moving animals. WDS movements were observed during microdialysis experiment. 25-I-NBOMe at all studied doses increased the release of DA, 5-HT and glutamate in frontal cortex and striatum; however, the dose of 3 mg/kg was the weakest in evoking glutamate release in the rat frontal cortex. The effect of the 25-I-NBOMe at the dose of 3 mg/kg was correlated with the lower WDS response to this dose. LSD potently increased cortical glutamate level, was less effective in increasing DA, while decreased 5-HT release. LSD only slightly increased striatal DA release. The effect of LSD on WDS response was weaker in comparison to 25-I-NBOMe. The differential effect of 25-I-NBOMe and LSD seems to result from various receptor affinity profile of both agents: 25-I-NBOMe is efficacious 5-HT2A/2C receptor agonist while LSD shows agonist activity at 5-HT1A, 2A/2C and D2 receptors.

|B10|

Role of IGF-1 in oligodendrocyte progenitor differentiation and maturation: *in vivo* and *in vitro* studies in rat model of neonatal hypoxia-ischemia

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Maturation of oligodendrocyte progenitor cells (OPCs) is influenced by multiple factors. Among them, there is the insulin-like growth factor-1 (IGF-1), a neurotrophic factor involved in regulation of cell proliferation and differentiation during normal brain development. The aim of our study was to investigate, whether changes in IGF-1 concentration may contribute to altered oligodendrogenesis resulting from neonatal hypoxia-ischemia.

To address the issue, we performed rat models of neonatal hypoxia-ischemia (HI). The *in vivo* model was based on dissection of the left common carotid artery and exposition of 7-days old animals to 7.5% oxygen for 60 min. The *in vitro* model was a temporal oxygen and glucose deprivation (OGD) performed on primary monocultures of oligodendrocytes and microglia. The concentration of IGF-1 in the brain hemispheres (injured and intact) and cell lysates (control and OGD) collected in different time points after the insult was measured by means of ELISA tests. Subsequently, to determine, whether IGF-1 may affect oligodendrocyte differentiation directly, we cultured OPCs in medium supplemented with IGF-1 inhibitors or IGF-1 at concentrations 10 ng/ml and 50 ng/ml. Results were evaluated by immunocytochemical analysis with antibodies against Ki67 for proliferating cells, Olig2 for preoligodendrocytes and CNP-ase for immature oligodendrocytes.

As indicated by the obtained results, from the day 3 after insult the endogenous IGF-1 level was elevated in injured brains, while in isolated OPCs, immediately after OGD, the level of this factor was decreased (8.59 ±2.06 vs. 7.65 ±1.92 pg IGF-1/mg total protein). Supplementation cell cultures with IGF-1 resulted in an increased differentiation of OPCs after OGD procedure.

Changes in IGF-1 amounts in the nervous tissue after HI might contribute to the resulting white matter disorders developing in newborn children who experienced perinatal asphyxia. Pharmacological modulation of IGF-1 secretion by neural cells could be reasonable solution in studies aimed at searching for therapies alleviating consequences of perinatal asphyxia.

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|B11|

Dynamics of the stem cell glycosylation follows the status of the cell migration

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Challenges facing cell-based regenerative medicine include generation of immuno-compatible biomaterials that will facilitate long-term, functional integration of transplanted cells with the targeted host tissue. Cell surface glycome consists of complex glycans functionalizing cell membrane glycoproteins and glycolipids. It quickly responds to any changes in cellular environment and growth conditions, and it therefore regulates cell-cell and cell-extracellular matrix interactions, represents cellular phenotype, status of cell differentiation, and defines interactions with the immune system.

Our objective was to follow the dynamics of the cell membrane reorganization and trafficking, and the cellular differentiation-related glycosylation of human mesenchymal stem cells (MSCs) subjected to the pulsed electrical field (PEF) within ranges used in experimental and in therapeutic conditions.

Cultured adult human MSCs derived from bone marrow remaining after the hip replacement were treated with varying conditions of the PEF for 0, 3, 6 and 9-hours, their migration routes were recorded, and the dynamics of their membrane reorganization and trafficking was followed using biotinylated and fluorescence-labeled plant lectins recognizing cellular differentiation-related glycans.

Substantial reorganizations within the ER, Golgi apparatus and the cell membrane were observed already within the first three hours of the lowest PEF values. With time and PEF increase, major membrane reorganizations were highlighted by the signals of lectins binding glycans associated with the cell stemness maintenance or loss, including certain manno-oligosaccharides and α2,6 sialic acid. Main features included directional ER and Golgi polarization and capping, appearance of focal domains within membranes of individual cells, and polarized cell-cell and cell-substratum focal contact points. The observed polarization followed mostly the direction of cell migration, often associated with the leading edge protrusion.

|B12|

ZIKA virus alters the DNA methylome in human neural progenitor cells

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Zika virus (ZIKV) has recently become an epidemic in many countries as it has been shown to cause neurological complications including microcephaly in newborns of infected mothers. In this study, human neural progenitor cells (hNPCs) were infected with ZIKV Asian strain (PRVABC59) and subjected to high-throughput DNA methylation analysis to unravel the molecular basis of ZIKV-induced microcephaly. The results revealed that ZIKV infection altered the DNA methylation status of several CpG sites in hNPCs. Furthermore, pathway analysis identified that genes from several signaling pathways including the Hippo signaling pathway were differentially methylated in ZIKV-infected hNPCs. The Hippo signaling pathway, which regulates diverse cellular process including mitochondrial and centrosome function, is a crucial determinant of organ size. Consistent with this, our results revealed that ZIKV infection reduced the activity of mitochondrial respiratory chain complexes, altered the expression of several centrosomal-related microcephaly (CRM) genes and decreased the expression of neural stemness markers in hNPCs. In summary, our results show that ZIKV epigenetically alters the Hippo signaling pathway leading to mitochondrial dysfunction and decreased expression of CRM genes which limit the expansion of hNPCs, and depletes progenitor pool, thereby resulting in microcephaly. The findings of this study unravel a novel molecular basis for ZIKV-induced microcephaly.

|B13|

MTHFR C677T polymorphism and homocysteine concentration in migraine patients

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Migraine, which belongs to primary headaches, is one of the most common neurological diseases. It occurs in two main clinical subtypes: migraine with aura (MA) and migraine without aura (MO). There is a close relationship between migraine and vascular diseases, especially cerebrovascular. The increased risk of ischemic stroke in patients with MA may be caused by a number of factors, both modifiable and non-modifiable, e.g., homocysteine (Hcy) concentration and polymorphisms in MTHFR gene encoding methylenetetrahydrofolate reductase, an enzyme essential for Hcy metabolism.

The aim of the study was to analyze MTHFR C677T polymorphism, Hcy plasma concentration and clinical features of migraine.

The study included 80 female migraine patients (MA: 40, MO: 40) and 80 healthy women as controls. Mean age of participants was 35 ±12 years. The high resolution melting (HRM) analysis and Sanger sequencing were used for genotyping, while high performance liquid chromatography with electrochemical detection (HPLC/EC) was used to determine Hcy plasma level.

The TT MTHFR C677T genotype was more common in MA group as compared to MO and controls. MA and MO patients with the TT genotype had longer and more frequent migraine attacks than patients with the CC genotype. Moreover, the TT genotype, both in migraine patients and healthy controls was associated with the higher Hcy level.

The TT MTHFR may be a risk factor for MA. It may influence the clinical feature of migraine probably due to increased the Hcy level.

|B14|

Altered volumes of the cerebellar lobes in spontaneously hypertensive rats – an animal model of attention-deficit/hyperactivity disorder

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The cerebellum plays an important role in the motor control and also cognitive and/or emotional functions which are disrupted in children with attention-deficit/hyperactivity disorder (ADHD). It was also reported that in the spontaneously hypertensive rats (SHRs) – an animal model of ADHD, the anatomical abnormalities in the different areas of brain were observed in the juvenile animals (5-weeks-old) and they disappear in mature animals (10-weeks-old). Therefore, the main aim of the present study was to compare the volume of the cerebellar lobes in the SHRs and Wistar Kyoto rats (WKYs; used as a control group) in two developmental periods: juvenile and mature.

10-µm-thick frozen brain sections from juvenile (5-weeks-old) and maturing (10-weeks-old) male SHR and WKY rats, were processed by immunohistochemistry using neuronal nuclear antigen as a neuronal marker. The volumes of the anterior and posterior (superior and inferior) lobes were compared using Cavalieri method.

The results show that the volumes of all cerebellar lobes in 5-week-old SHR were significantly lower than those of age matched WKY rats. Moreover, anterior and posterior lobes (and its part i.e. inferior lobe) were also lower in 10-week-old SHR rats compared to their age matched counterparts. There was no statistical difference in the volume of superior posterior lobe in 10-weeks-old WKY and SHR rats. Additionally, the volumes of almost all cerebellar lobes significantly increased with age in both strains.

Summarizing, these data demonstrate that differences in the volume of cerebellar lobes between both strains were observed before and after puberty. Thus, these data might partly explain why many teens with ADHD still have symptoms when they are adults. However, further studies are required to clarify the role of cerebellar lobes in the ADHD pathogenesis during adulthood.

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|B15|

Post-translational modifications and not alterations at the transcriptional level are responsible for memory consolidation and reconsolidation impairment after TRP channels inhibition

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Transient receptor potential (TRP) channels are a group of ion channels permeable to sodium, calcium and magnesium. These channels may be involved in calcium-dependent neuronal mechanisms, including learning and memory formation. From 7 TRP sub-families, canonical (TRPC) and vanillioid (TRPV) were reported to modulate these processes.

The aim of this study was to investigate the involvement of TRP channels in memory consolidation and reconsolidation and in expression of proteins engaged in memory formation.

The passive avoidance task in one-day chicks was used. TRP channels were inhibited by bilateral injection of non-specific TRP channels antagonist SKF96365 (30 µmol/hemisphere) or specific antibodies (0.2 µg/hemisphere). Injections were made into a chick brain region responsible for early stages of memory formation – intermediate medial mesopallium (IMM) immediately after training/reminder. Animals were tested at different times after training/reminder. The expression of NCAM, FMRP and TRPC3/5 and TRPV1/3 was analyzed by real time PCR and Western blot.

The injection of SKF96365, anti-TRPC3 and anti-TRPV3, anti-TRPV1 antibody after training/reminder resulted in amnesia when tested 2 h or 24 h later. Application of anti-TRPC5 antibody did not produce significant amnesia. Injection of SKF96365 immediately after training resulted in increase in NCAM and FMRP expression, while application after reminder increased NCAM and decreased FMRP expression in the IMM. Analysis of mRNA content for analyzed proteins showed no differences.

Our results show significant involvement of TRP channels in memory consolidation and reconsolidation. Decrease in

FMRP expression reduces its effect on translation termination which results in the overexpression of specific proteins engaged in these processes – in this case as increase in the NCAM levels. Both, decreased protein expression after training and increased after reminder disrupts memory formation. No changes at the mRNA level were observed which suggests that transcription is not involved in these processes.

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|B16|

Methods of image analysis as tools intended for the recording of the cell surface glycosylation dynamics

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In regenerative medicine, stem cell surface glycans are an irreplaceable source of information of the cell functional and differentiation status, and, very likely, of the compatibility between the transplanted cells and the host environment. Analysis of changes in the cell surface glycan profiles may provide a valuable information that can be used to assess the competence of transplanted stem cells. Such analysis is often performed via visual assessment, what can be tedious, time-consuming, and prone to several types of errors.

The aim of our study was to develop and test a novel approach to monitoring a cell surface glycosylation, i.e. to use combined methods of image analysis for the purpose of automatic segmentation of stained glycans, including also features of cellular nuclei. Similar approach, currently used in the research and clinical laboratories, facilitates a quick and reliable analysis of results, e.g. through an automatic counting of stained cellular nuclei. In the presented study, images obtained through staining of selected glycans using six fluorescence-labelled plant lectins in mesenchymal stem cells, following stimulation in pulsed electric field in various conditions, were used.

Obtained results show that the automatic, semi-quantitative analysis of stained glycans is possible, and that presented approach, using easily programmable methods of digital image processing and analysis, can be useful in the laboratory practice.

|B17|

Expression of glutamate transporters and glutamate NMDA receptor in brain of rats exposed developmentally to a low dose of silver nanoparticles

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Increasingly popular silver nanoparticles (AgNPs) are used in many medical and consumer products. Their strong antimicrobial properties make them useful in protection against bacterial and fungal contamination. Unfortunately, it has been confirmed that AgNPs exert neurotoxic effects in many experimental models. It has been proved that AgNPs have the ability to enter the brain and accumulate in this organ. Thus, it is important to investigate the mechanisms of their neurotoxicity, especially in developing organisms which are more vulnerable to toxins.

We exposed 3-week-old rats to a low dose (0.2 mg/kg. b.w.) of small AgNPs for three weeks. A silver citrate-exposed group was established as a positive control of ionic silver effects. All analyses were performed in two time points, at postnatal day 35 (PND 35) and PND 90, to assess short- and long-term effects of exposure during development. We measured concentration of silver in serum and brain of exposed rats using ICP-MS method. As it was predicted, the level of silver rose significantly at PND 35 in both silver-treated groups compared to negative control. At PND 90 concentration of silver declined to control value in serum whereas increased further in brain of exposed animals. Using Western blot analysis and qPCR method, we examined the expression of NR1 and NR2 subunits of glutamatergic NMDA receptor and glutamate transporters GLT-1, GLAST, EAAC1. Expression of both NR1 and NR2, as well as GLT-1 and GLAST increased in AgNP-exposed group, whereas expression of EAAC1 decreased compared

to control. The results of the current study demonstrate that glutamatergic neurotransmission, which underlies the processes of cognition, memory and learning, may be strongly affected by AgNPs in developing brain.

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|B18|

Epigenetic regulation of microglial phosphatidylinositol 3-kinase (PI3K) by miR-21-5p and HDAC3 may be involved in memory and Alzheimer's disease

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Microglial cells are the resident macrophages of the central nervous system (CNS). Microglia-mediated neuroinflammation has been shown in various forms of dementia such as Alzheimer's disease. Phosphatidylinositol 3-kinase (PI3K) is known to be involved in synaptic plasticity in neurons and has been implicated in Alzheimer's disease.

We found that microglial PI3K is involved in synaptic plasticity and memory, suggesting that it may also be involved in memory disorders such as Alzheimer's disease. miR-21-5p was upregulated in amyloid beta-activated microglia and is predicted to target PI3K suggesting that PI3K signaling is attenuated in the brains of Alzheimer's disease patients, and is epigenetically regulated by miR-21-5p.

Histone deacetylase (HDAC) inhibition by sodium butyrate brings about an increase in the expression of PI3K and its downstream effectors. In this study, we found that RGFP966, a selective HDAC3 inhibitor, was able to upregulate the expression of PI3K and the activity of its downstream genes such as Akt, CREB and BDNF. HDAC3 has been implicated in Alzheimer's disease and inhibition of HDAC3 is able to restore amyloid beta-induced plasticity deficit in hippocampal CA1 pyramidal neurons suggesting role for microglial PI3K in Alzheimer's disease.

Rat hippocampal slices incubated with clodronate cause microglia to become dystrophic. This brought about a decline in long-term potentiation (LTP) in hippocampal CA1 neurons indicating the involvement of microglia in neuronal LTP. Incubation with amyloid beta caused a similar decline in LTP suggesting that microglia are similarly dystrophic in the presence of amyloid beta. This decline was rescued with the addition of active PI3K or BDNF protein. Our results suggest the involvement of microglial PI3K pathway in learning and memory and also Alzheimer's disease. Understanding the mechanisms by which microglial PI3K is regulated may give us an insight into the ways it can modulate memory be a potential target for therapeutic interventions.

|B19|

The effectiveness of short-lasting intensive rehabilitation of hospitalized SCA1 patients

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Spinocerebellar ataxia type 1 (SCA1) is a rare neurodegenerative disorder of autosomal dominant inheritance, caused by the expansion of CAG trinucleotides in ATXN1 gene. SCA1 is clinically heterogeneous. The postural instability is the most common symptom distracting daily routines of the patients. As there is no effective pharmacological treatment, the rehabilitation is the only way to improve ataxia signs.

The aim of the study was to assess the alterations of ataxia signs with an emphasis on balance performance after short-lasting intensive rehabilitation in symptomatic SCA1 patients.

Twenty two SCA1 patients, aged 50 ±9 years, CAG repeat number 47.2 ±2.8 (42-53), were evaluated prior to intensive 2-week rehabilitation and immediately after it was finished. The rehabilitation was designed for trunk and extremities coordination treatment including: proprioceptive neuromuscular facilitation, individual physiotherapy, exercises in the swimming pool. Severity of ataxia was clinically evaluated with the Scale for Assessment and Rating of Ataxia (SARA). The sway of center of pressure (COP) was measured on a force plate in standing position with eyes opened and closed. The radius, velocity and area of

COP obtained in SCA1 patients were compared with the posturography results of 25 healthy volunteers.

Ataxia symptoms were significantly reduced only in patients with shorter CAG repeats (SARA: $p < 0.05$). The sway of COP improved significantly after rehabilitation in the whole studied group only when their eyes were opened (COP radius: $p = 0.04$; number of fast alternating COP oscillations higher than 1 cm in the anterior-posterior direction: $p = 0.02$).

Directed intensive rehabilitation improves balance performance. The CAG repeat number influences the outcome of the rehabilitation in SCA1 patients. We postulate that pathologically prolonged polyglutamine tracts in ataxin-1 decrease the neuroplasticity facilities. The plasticity of the nervous system seems to be the crucial compensating mechanism for the neurodegenerative processes in SCA1.

|B20|

Novel machine learning and statistical learning approaches in neurology

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Scientists and clinicians are familiar with statistical/mathematical approaches to data analysis. Given a particular research/clinical hypothesis, statistical tests are applied to the data to see if any relationships can be found between different variables or parameters. Nowadays, in the era of internet and new medical technologies/bioinformatics, large-scale datasets from experimental investigations, clinical trials and cohort studies, electronic databases/health records and national health registries are becoming increasingly available in biomedical researches. The urgent need of novel algorithms and techniques/methods that can help in better understanding of genotype-phenotype relationships, find factors that can predict/estimate disease risk/progress, discover profiles of patients who better respond to a treatment/rehabilitation and discover or define/classify disease categories. The situation has led to a revolution in statistical sciences. In the XXI century, "traditional" statistical approach cannot assimilate and integrate new "omics" data of genomics, transcriptomics, proteomics, metabolomics or (neuro)imaging and others. Modern statistical artificial intelligence (AI) tools can be used for biomedical applications and healthcare. Popular novel AI techniques include machine learning (ML) and statistical learning (SL) for structured data as well as natural language processing (NLP) for unstructured data. Statistical ML/SL-based models and receiver operating characteristic (ROC) curve or decision curve analysis (DCA) are a new natural extension of classical statistical approaches.

The purpose of the study is to outline the potential benefits of statistical ML and SL algorithms/models, ROC/DCA analysis in neurological disease studies, both research and practical clinical points of view. The novel statistical approaches can help to reduce diagnostic and therapeutic errors that are inevitable in the human clinical practice not only for neurology but also cardiology or oncology. The most novel and modern technologies, such as Smartphone or Virtual Reality, with ML/SL are promoted for the patient healthcare, e.g. "Using Smartphones and Machine Learning to Quantify Parkinson Disease Severity" (JAMA Neurol doi: 10.1001/jamaneurol.2018.0809).

|B21|

Brain asymmetry in health and neurological disorders, including selected aspects based on VR solutions

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Brain asymmetry related to brain behavior (laterality) has been well recognized since the nineteenth-century discoveries by Paul Broca. Recently, the knowledge about laterality has been considerably deepened by neuroscience studies. Laterality is observed across a wide range of species, both vertebrates and invertebrates. Laterality, in biological (neuro)psychology is described as the development of specialized functioning in each hemisphere of the brain, and in each side of the body being controlled. The best-known example of laterality is handedness. It is common practice to classify persons as: right-handed, left-handed, or two-handed. Neurobiological studies of this phenomenon indicate the dominance of the rightwards: handedness ~90%, footedness ~80%, eyedness ~70% and earedness ~60% of human population. Even though molecular and genetic bases of this asymmetry are not well understood, its existence is supported by abundant converging evidences from *in vivo* and post-mortem

neuroanatomy, neurochemistry, neuropsychology, neuroimaging, electrophysiological and behavioral studies. There is also evidence that the asymmetries are affected by conditions that alter the anatomical and functional integrity of the brain, such as brain damage, some neurological diseases and aging.

In our study, we review evidences using different models/approaches of/to the hemispheric asymmetry and/or synaptic/neuronal/muscle plasticity, as well as motor learning in healthy subjects and neurological patients. In fact, it seems that the phylogenetic and neurobiological bases for cerebral asymmetry in humans are likely to be found in motor systems rather than in perceptual ones. Most neuroscientists argue that these two asymmetries have been linked to the specialization of the left hemisphere of the brain for language, speech and motor/postural control. In clinical practice altered lateralization has been associated with such conditions/properties as: dyslexia, attention deficit, stress, hyperactivity disorder, and schizophrenia or stroke. In clinical neurology, an important issue is the significance and influence of lateralization on effects of therapy/rehabilitation of patients with cognitive-motor deficits/dysfunctions. Selected aspects of the above issues will be highlighted based on our latest posturographic studies, which are being developed and applied using virtual reality (VR) technologies.

|B22|

Brain reward system activation with nicotine and caffeine – potential implications for reward-aimed behavior in the rat

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Coffee drinking is frequently accompanied by cigarette smoking. Simultaneous consumption of both these psychostimulants can induce intensifying effect on the brain reward system. Although growing body of evidence suggests the involvement of phosphorylated form of extracellular signal-regulated kinase (pERK), phosphorylated form of cyclic AMP-response element binding protein (pCREB), and DeltaFosB signaling pathways, the exact mechanisms of this phenomenon have not been elucidated. In this study we determined the effects of nicotine and caffeine stimulation on the activation of pERK, pCREB and DeltaFosB in the main structures of the brain reward system: nucleus accumbens (NAc), ventral tegmental area (VTA), amygdala (Amg), hippocampus (Hip), medial prefrontal cortex (mPfr) and dorsal striatum (CdP) using different patterns of both stimulants administration. The adult male Wistar rats were treated with nicotine and caffeine administered separately or in combination. The activation patterns of the studied factors were assessed by stereological method after immunofluorescent staining and documented with confocal microscopy. Our results reveal differences in activation of the studied markers among the various structures of the brain reward system depending on the psychostimulants' administration regime. This can suggest that specific regulatory mechanisms in the reward system depend on the nature of the stimulus, specific brain area and functional context, which altogether determines the reward- and addiction-aimed behaviors.

|B23|

Effect of salbutamol on antiepileptic drugs efficacy in maximal electroshock-induced seizures in mice

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β2-adrenergic receptors agonists are commonly used in the treatment of respiratory tract as well as in obstetric disorders. One of the most commonly administered β2-adrenergic receptors agonist is salbutamol, a short and fast acting substance. Because patients with epilepsy may have many concomitant diseases, we aimed to analyze the effect of salbutamol, a selective β2-adrenergic receptor agonist on antiepileptic drugs (AEDs) efficacy: valproate (VPA), carbamazepine (CBZ), phenytoin (DPH) and phenobarbital (PB) in mice with maximal electroshock (MES) induced seizures, the animal model of tonic-clonic seizures. The effect of AEDs and salbutamol given alone or in

combination on animals motor coordination and memory was additionally examined. Moreover, the free serum level of AEDs after salbutamol injection was analyzed.

Single salbutamol intraperitoneal injection did not change, whereas 7 days salbutamol administration decreased seizure threshold in MES induced seizures in mice. Furthermore, salbutamol injected intraperitoneally for 1 day and for 7 days lowered PB antiepileptic efficacy in MES induced seizures in mice, but did not change the effect of other tested AEDs: VPA, CBZ and DPH. Butoxamine, a selective β2-adrenergic receptor antagonist reversed salbutamol's influence on PB antiepileptic activity in MES induced seizures in mice. Salbutamol after 1 day and 7 days of intraperitoneal administration did not change PB free serum concentration in mice. Salbutamol given alone or in combination with tested AEDs did not affect animals motor performance and memory after single or 7 days administration.

Our results show that salbutamol decreases the antiepileptic efficacy of PB. Patients with epilepsy receiving β2-adrenergic receptors agonists in the therapy should be carefully monitored.

|B24|

Application of two-component integrated calibration method to simultaneous determination of glutamate and aspartate in biological samples

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One of the main neurotransmitters is glutamate which plays important role in visual, auditory and sensory stimuli transmission. It is believed that glutamate is crucial for the formation of memory trace, and thus the processes of learning as well as for proper functioning and support of many processes, e.g. brain detoxification or improvement of excretion of harmful metabolites. Aspartic acid has a big influence on neurons – stimulates them and facilitates the creation of memory traces – it facilitates learning and improves concentration.

The main aim of the present research was the application of integrated calibration method (ICM) to two-component analysis (2C-ICM) of glutamate and aspartate firstly in synthetic samples and then in cerebrospinal fluids collected by microdialysis from free-moving animals. All analyzes were carried out with the use of high-performance liquid chromatography (HPLC) with electrochemical detection. The ICM is a new calibration approach based on the integration of the interpolation and extrapolation methods into a single analytical procedure, resulting in a series of analyte concentrations. At the laboratory stage of the 2C-ICM method, ten calibration solutions were prepared. Based on the measured analytical signals, eight two-point calibration curves were constructed and two series of six estimations for the both analyte concentrations were calculated. Based on the same analytical signals it was possible to calculate results according to basic version of ICM and 2C-ICM. The conducted research proved that ICM adapted to the two-component analysis enables verification of the accuracy of analytical results and the diagnosis of occurrence of systematic errors for both analytes.

The obtained results showed that the application of the 2C-ICM method is an effective and very useful analytical tool in analysis of biological samples in a context of neurotransmitters determination in pharmacology areas.

|B25|

Alterations in the expression of purinergic receptors in adolescent rats following embryological exposure to valproic acid

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Autism is a neurodevelopmental disorder characterized by symptoms related to deficits in social interaction and impaired communication as well as anxiety and stereotypical behavior. Most recently extracellular ATP and adenosine related neurotransmission have been suggested to participate in the pathogenesis of autism. However, the relevance of particular purinergic receptor subtypes to anatomical, pathological and etiological changes characteristic for autism remain speculative.

Therefore, the present study aimed to examine the expression of adenosine and purinergic receptors in the brain of rats prenatally exposed to valproic acid (VPA, 400 mg/kg) on the 12.5th day of gestation which is one of the most used animal models of autism.

In order to verify the behavioral aberrations in experimental animals we performed behavioral tests and found that rat pups prenatally exposed to VPA vocalized less compared to control animals, however they showed longer mean time duration of vocalization. In adolescence, VPA offsprings showed no impairment in locomotor activity; but they exhibited increased anxiety. Transmission electron microscopy of cerebral synaptosomes isolated from VPA treated animals revealed significant abnormalities in their ultrastructure. The large majority of synaptosomes contained an unidentified electron-dense matrix material and the post-synaptic densities were fragile and malformed comparing to control group. We have also found that prenatal exposure to VPA generates a rearrangement of selected ionotropic and metabotropic purinergic receptors gene expression. While mRNA level for adenosine A1 and A2a as well as purinergic P2X5, P2X7, P2Y1 receptors were significantly decreased in the brain cortex, the expression level of adenosine receptor A3 in cerebellum and P2X1 receptor in cortex were elevated.

We demonstrated that embryonic exposure to VPA altered purinergic receptors expression in adolescent rats. These findings indicate that the purinergic receptors may be involved in the synapse structure and function changes and molecular mechanisms of autism spectrum disorders.

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|B26|

The organotypic hippocampal slice culture model to examine nanoparticles uptake, accumulation and toxicity

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Potential benefits of modern nanobiotechnology result in developing novel biomedical applications. Due to the rapid growth of nanotechnology, new and better nanomaterials are still being sought. Especially in neurobiology field, new nanomaterials with low cytotoxicity and multifunctionality are still expected. Neurobiological research and nanoparticle application are fundamental and crucial for new diagnostic or imaging purposes like cancer visualization, guided surgery and therapeutics methods, e.g. to transfer drugs. The brain is one of the most important targets for possible toxic effects of nanoparticles after medical exposure like bioimaging or targeted therapy, what is widely reported in literature.

The organotypic hippocampal slice culture is a very useful platform for studies on neuroprotection. We have used rat hippocampal organotypic slices as an *ex vivo* model of lanthanide-doped up-converting nanoparticles (UCNPs) uptake, accumulation and possible toxic effect. The UCNPs are a new generation of nanomaterials which have ability to convert near infrared (NIR) radiation to visible or UV light. This unique properties make them very useful and attractive tool in biomedical applications.

We optimalized various methods to deliver nanoparticles to hippocampal slices. We demonstrated three delivery methods of UCNPs to hippocampal slices on membrane or slices free in medium ("swimming-slices") per 2 hours directly after isolation. Nanoparticles transfer is very difficult and complicated because of technical aspects and morphological structure of the brain. We have tested different sizes and concentration of nanoparticles in various variants of exposition time with hippocampal slices. We have confirmed presence of UCNPs inside hippocampal slices by transmission electron and confocal microscopy. It means that they are able to penetrate into the brain tissue. Furthermore, we used this microscopic techniques to examine morphological and ultrastructural changes including potential cytotoxic effect after nanoparticles treatment. We also used fluorescent dye propidium iodide (PI), showing red fluorescence in dead cell to determine cell viability.

|B27|

Gemfibrozil decreases kynurenic acid production in rat brain *in vitro*

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Fibrates used to decrease serum triglycerides level were reported to provide beneficial effects in neurodegenerative disorders. Additionally, low fat diet known as 'ketogenic diet' became an important armamentarium in the treatment of refractory epilepsy.

Kynurenic acid (KYNA) is an endogenous antagonist of ionotropic glutamatergic receptors. KYNA production in the brain from L-kynurenine (L-KYN) is conducted by kynurenine aminotransferases (KATs), from which the predominant role plays KAT II. Interestingly, long term exposure to ketogenic diet was reported to enhance brain KYNA production in animals.

In this study the influence of gemfibrozil on KYNA production as well as KAT I and KAT II activity in rat brain cortex *in vitro* was investigated. Additionally, the molecular docking of gemfibrozil to KAT I and KAT II structure and reanalysis of previously published microarray experiments concerning the effect of gemfibrozil on KAT-coding genes expression were performed.

In rat cortical slices *in vitro* gemfibrozil at the concentration of 0.1 mM, 0.5 mM and 1 mM decreased KYNA synthesis to 80% ($p < 0.05$), 79% ($p < 0.05$) and 58% ($p < 0.001$) of control value, respectively. KAT I activity was lowered by gemfibrozil at 0.1 mM, 0.5 mM and 1 mM concentration to 67% ($p < 0.05$), 37% ($p < 0.05$) and 23% ($p < 0.05$) of control value, respectively. The activity of cortical KAT II was decreased by gemfibrozil at the concentration of 0.1 mM, 0.5 mM and 1 mM to 68% ($p < 0.05$), 46% ($p < 0.001$) and 17% ($p < 0.001$) of control value, respectively. Molecular docking results suggest that gemfibrozil may bind to the active site of KAT I and KAT II. Gemfibrozil administration did not change KATs expression in the brain in available data.

Our study demonstrates that gemfibrozil decreases KYNA synthesis in rat brain in vitro through KAT I and KAT II inhibition.

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|B28|

The role of maternal immune activation on transcriptional alteration of mitochondrial proteins

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Epidemiological evidence implicates maternal infection as a risk factor for autism spectrum disorder and schizophrenia. Studies on animal models have revealed maternal immune activation (MIA) to be a profound risk factor for neurochemical and behavioral abnormalities in the offspring. Recently, evidence has accrued that mitochondrial dysfunction is closely associated with autism.

Here, we explore the alteration of gene expression of proteins regulating mitochondrial dynamics/biogenesis and subunits of electron transport chain (ETC) complexes in rodent MIA model induced by exposure of pregnant rats to lipopolysaccharide (LPS; 0.1 mg/kg, intraperitoneally) on gestational day 9.5. The molecular biology methods were applied.

Our data demonstrated downregulation of expression of ETC complex I subunit (mt-Nd1), complex III subunit (mt-Cyb) and complex IV subunit (mt-Co1) in brain cortex of young/adult MIA offspring. In hippocampus of MIA rats only mRNA level of complex IV subunit was reduced. We also demonstrated that MIA altered transcription of proteins that regulate mitochondrial fusion-fission and biogenesis. The reduced mRNA level of mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2), with concomitant elevation of dynamin related protein 1 (Drp1) was observed in the cortex and cerebellum, whereas the mitochondrial fission 1 (Fis1) gene expression was altered only in hippocampus and cerebellum of MIA rats. Our data also demonstrated the down-regulation of transcription factors responsible for mitochondrial biogenesis: Pgc1α (Ppargc) in brain cortex and Tfam in both brain cortex and hippocampus of young/adult MIA offspring. However, the level of Tfam in cerebellum was increased.

The observed changes of gene expression formain proteins regulating mitochondrial dynamics/biogenesis and ETC subunits may help in understanding of the role of mitochondrial dysfunction in synaptic stress and may lead to novel therapeutic strategies for the treatment of neurodevelopmental disorders through the protection of synaptic transmission by targeting to mitochondrial deficits.

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Anti-inflammatory effect of histone deacetylase inhibitor, sodium butyrate, after neonatal hypoxia-ischemia

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Neonatal hypoxic-ischemic (HI) encephalopathy still remains one of the most important causes of neonatal mortality and/or long-term neurological sequelae. Histone deacetylase inhibitor (HDACi) – sodium butyrate (SB) has been shown to be neuroprotective in adult brain injury models. Potential explanation for the inhibitor action involves among others reduced inflammation, which is important pathogenic factor. We therefore anticipated that SB will provide a suitable option for treatment of brain injury in immature animals.

The aim of our study was to test the hypothesis that one of the supposed mechanisms of protection afforded by SB after neonatal hypoxia-ischemia may be also associated with anti-inflammatory action. We examined the effect of SB on the production of inflammatory factors including analysis of the microglial and astrocytic cell response. We also examined the effect of SB on molecular mediators that are crucial for inducing cerebral damage after ischemia.

Seven-day-old rat pups were subjected to unilateral carotid artery ligation followed by 60 minutes of hypoxia (7.6% O_2). SB (300 mg/kg) was administered in a 5-day regime with the first injection given immediately after hypoxic exposure. The damage of the ipsilateral hemisphere was evaluated by hematoxylin-eosin staining (HE). Microglial and astroglial cells were identified by immunohistochemistry. Effects of SB on HI-induced inflammation (cytokines and chemokine) were assessed by Luminex assay. Expression of molecular mediator (COX-2) were assayed by Western blot.

SB treatment reduced brain damage, as assessed by HE staining, suppressed the production of inflammatory markers – IL-1β, chemokine CXCL10 and blocked ischemia-elicited up-regulation of COX-2 in the damaged ipsilateral hemisphere. Furthermore, administration of SB promoted the conversion of microglia phenotype from inflammatory M1 to anti-inflammatory M2.

SB appears to exert a beneficial effect in neonatal hypoxia-ischemia injury *via* suppression of HI-induced cerebral inflammation.

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