Different brain areas are thought to be integrated into large-scale networks. Recent approaches for investigating structural organization and functional coordination within these networks involve measures of connectivity among brain areas. Transcranial magnetic stimulation (TMS) can be used to analyze the functional state of the cerebral cortex, discovering changes in its excitability, connectivity and plasticity which may have occurred through processes such as learning or recovery from a lesion. We review studies using *in vivo* functional brain connectivity technologies. TMS-EEG studies have begun to describe the nature of the TMS-evoked EEG responses in order to broaden the comprehension of the activation mechanisms of TMS. Several studies have proved the power of TMS-EEG by displaying many data about the excitability or connectivity of the brain. Particularly, it has been proposed that the very first part of the TMS evoked EEG response displays the excitability of the stimulated cortex while its spatio-temporal distribution over the scalp displays the spread of activation to other cortical areas – *via* intra and inter-hemispheric cortico-cortical connections as well as to sub-cortical structures and spinal cord *via* projection fibres – that means the effective connectivity of the stimulated area. Finally effective connectivity may be considered as the union of structural and functional connectivity. These studies provide insights into the relationships between brain structure and function.

S11. A CARE lecture

S11.1

ETHICAL AND LEGAL ASPECTS OF EXPERIMENTING ON ANIMALS

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In my presentation I am going to review the present the state of law concerning research on animals in the European Union and Poland in particular. I am going to refer relate the status of implementation of the EU Directive 2010/63 in Poland and other EU. Next I will review the major changes in standards of the laboratory animal husbandry, process of ethical evaluation and control over scientific experiments that implementation of the new Directive 2010/63/UE of the European Union Council and European Parliament is going to impos. Lastly, I will compare numbers of animals used for animal research in various European countries, the spectrum of species used and the most problematic directions and objects of scientific research on animals from the point of view of the new Directive. In conclusion, I will try to describe the possible impact of the new Directive on animal research in Europe.

POSTER SESSIONS
P1. Neurochemistry and pharmacology

P1.1

EXTRACELLULAR alpha-SYNUCLEIN INDUCES CALPAIN-DEPENDENT OVERACTIVATION OF CYCLIN--DEPENDENT KINASE 5 IN PC12 CELLS

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α-Synuclein (ASN) secreted from neurons into the extracellular space affects the homeostasis of neighboring cells, but the pathophysiology of extracellular ASN remains largely unknown. The aim of the present study was to analyze the role of cyclin dependent kinase 5 (Cdk5) in molecular mechanism of extracellular ASN toxicity. We found that exogenously applied ASN evoked apoptotic cell death in a significant population of dopaminergic PC12 cells. ASN induced rapid and long-lasting calcium influx and activation of calcium-dependent enzymes, including caspase-3, nitric oxide synthase and calpain. ASN-induced calpain activation leads to cleavage of Cdk5 activator p35, and subsequently to formation of p25 and Cdk5 overactivation. Moreover, we showed that exposure of PC12 cells to ASN increased Cdk5 activity by enhancement of its phosphorylation at Tyr15. Calpeptin, an inhibitor of calpains, and inhibitors of Cdk5, Roscovitine and BML-259, prevented ASN-evoked apoptosis and cell death, indicating the involvement of Cdk5 in mechanism of ASN toxicity. Our data showed that alterations in calcium homeostasis and modulation of calcium-dependent enzymes by extracellular ASN may contribute to the early stages of pathogenesis in Parkinson's disease and other synucleinopathies.Supported by a grant from The National Science Centre 2012/05/B/NZ3/02047.

P1.2

IMIPRAMINE-REVERSED ELEVATION OF Hsp72 EXPRESSION IN THE BRAIN OF RATS IN THE CHRONIC MILD STRESS MODEL OF DEPRESSION

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Heat shock proteins HSP70 play a protective role against stress-induced damage of cells. We assessed the expression of inducible Hsp72 in the prefrontal cortex (PFC) and hippocampus (HIP) of male Wistar rats subjected to the chronic mild stress (CMS), the procedure inducing depression-like symptoms, and subsequently

treated with antidepressant drug, imipramine (IMI). Five groups were studied: sham-sal; stress-sal; sham-IMI; stress-IMI and IMI-non-responders and RT-qPCR and Western blotting methods were employed. We found that CMS elevated the Hsp72 protein level in PFC and HIP (by 48 and 41% vs. sham-sal) and this effect was diminished by IMI treatment. Though such alteration in protein level was not paralleled with mRNA expression, interestingly we observed that in rats not responding to IMI treatment the HSP72 mRNA level was increased compared to IMI responders (by 82 and 59% in PFC and HIP, respectively). The different changes in mRNA and protein may reflect post-transcriptional modifications. Our results suggest an existence of a cellular stress reaction in brain of rats subjected to the CMS procedure that is partly cured by IMI treatment. Supported by statutory funds of the Institute of Pharmacology and POIG.01.01.02-12-004/09-00 grant financed by European Regional Development Fund.

P1.3

MicroRNAS EXPRESSION PROFILE OF THE DENTAL GYRUS IN A RAT MODEL OF TEMPORAL LOBE EPILEPSY

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microRNAs are noncoding RNAs acting by degradation or destabilization of target mRNAs. Recent studies have suggested the contribution of miRNAs in neurodegenerative diseases, however its role in epilepsy remains still unknown. The aim of the study was to investigate changes in expression level of miRNAs in dentate gyrus of epileptic animals. Epilepsy was induced in adult Spraque-Dawley rats by status epilepticus evoked by electrical stimulation of the left amygdala (100-ms train of 1-ms biphasic square-wave pulses delivered at 60 Hz, every 0.5 s for 30 min). To determine the frequency of spontaneous seizures animals were constantly monitored with video EEG. Tissue was collected at 7, 14, 30, 90 days after stimulation (n=5). Total RNA enriched in microRNA fraction was isolated from the left dentate gyrus of epileptic and sham operated animals with miRNeasy mini kit (QIAGEN) and profiled using miRCURY LNATM microRNA Array 7th (EXIQON) with the miRBASE version 19.0. Analysis of miRNAs showed significant changes in expression of 66 miRNAs (P<0.05) in stimulated animals as compared to sham operated controls. Nine miRNAs were up-regulated, while 57 miRNAs were down-regulated. *In silico* analysis of miRNAs expression profile revealed potential genes targets for these miRNAs and hierarchical clustering analysis discriminated the epileptic animals from the controls. This data suggest involvement of miRNAs in epileptogenesis or epilepsy.

P1.4

SEARCHING FOR A NEW mGluRs POSITIVE ALLOSTERIC MODULATORS

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Background: The metabotropic glutamate receptors play important neuromodulatory role throughout the brain. Intervention in glutamatergic neurotransmission through group III of mGluRs has been pursued for the treatment of many neurological and psychiatric disorders such as anxiety, schizophrenia, epilepsy, Parkinson disease, addiction. Aim: Identification of novel chemical scaffolds possessing group III of mGluRs positive allosteric modulation (PAM) activity. Methods: The screening study and activity of potential PAM was determined using forskolin-induced cAMP production, in HEK-293 T-REx cell lines stably expressing mGluRs (HTRF cAMP detection kit, Cisbio). Results: We have identified chemical scaffolds possessing mGluRs potential PAM activity. Those compounds alone and in the presence of L-Glutamine decreased the forskolin-induced cAMP production in HEK-293 T-REx mGluRs cells. Conclusions: It have been identified novel chemical scaffolds with mGluRs potential positive allosteric modulator activity. Acknowledgments This study is supported by project UDA-POIG.01.03.010-12-100/08-00 co-financed by European Union from the European Fund of Regional Development.

P1.5

DOES INTERACTION BETWEEN ANTI-INSECT SCORPION TOXIN LqhαIT AND COCKROACH SODIUM CHANNEL IS VOLTAGE DEPENDENT?

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Scorpion toxins (α toxins) binding to receptor site 3 of sodium channel (Nav) inhibit the inactivation of the activated channel. Binding of α toxins selective to mammals is voltage dependent. Interaction of insect selective α toxin (LqhαIT) with insect Na channel is characterized as voltage independent. Results of our electrophysiological experiments performed on cockroach axonal membrane are quite contradictory. Evoked plateau action potentials, recorded after LqhαIT application are shortened when axon is stimulated with high frequency. Such effect can be partially abolished by higher toxin concentration. In voltage clamp, when depolarizing pulses are applied with high frequency the decrease of size of toxin induced late sodium current is observed. To study molecular mechanisms of these phenomena molecular dynamics methods was used. Calcu-

lation was performed for complex system – LqhαIT with domain IV of Nav – with using CHARMM force field. Simulations were performed in present and absent of an electric field. That in silico research shows some correlation between the number of hydrogen bonds linking site 3 of domain IV and LqhαIT and deviations of atoms of segment S4. Studies supported by MNiSW (Poland)-grant N N303 320637; M. Dąbrowski is a beneficiary of PhD grant: Project No POKL.0401.01-00-081/10.

P1.6

MEMANTINE AND MK-801 CAUSED ALTERATIONS OF WORKING MEMORY IN THE MORRIS WATER MAZE TEST WITH SHORT AND LONG DELAYS

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The Morris Water Maze (MWM) is a behavioral method for testing allothetic memory. The aim of study was to asses immediate effect of NMDA receptors blocking, by MK-801 or memantine, on working memory. Memory was tested in the MWM test with two various delays. Male adult Long-Evans rats were injected intraperitoneally with 5 or 20 mg/kg b.w. memantine or 0.1 mg/kg b.w. MK-801 or saline (1 ml/kg) 30 minutes before training in three consecutive days. The follow-up effect (without injection) was tested on day 9. Daily session consisted of training and testing trials with 5 or 15 min interval. Maximum time of a trial was 60 s. Location of hidden platform was alternated every day. Rats treated with high dose of memantine showed poor working memory, manifested as long latency of swim to the platform. This effect was present in trials with both delays. Low memantine group performed task as well as control. Working memory was affected by MK-801 just in trials with a long delay. Test with long delay was more sensitive on low dose of MK-801 than on low dose of memantine. The study shows a role of MK-801 in induction of psychosis-like behavior in an animal model of schizophrenia. Grant MNiSW 8165/B/P01/2011/40 and IGA MZ CR NT13386.

P1.7

INTERACTION OF TNFalpha AND INTERLEUKIN-6 IN SPINAL HYPEREXCITABILITY OF NOCICEPTIVE NEURONS

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Treatment with biologicals neutralizing TNFα was shown to reduce inflammatory hyperalgesia in experimental (Böttger et al. 2008) and human arthritis (Hess et al. 2011). While in the periphery TNFa induced long-lasting sensitization of joint afferents (Richter et al. 2010) the role of spinal TNFα in inflammation-evoked spinal hyperexcitability is possibly indirect because we detected IL-6 after TNFα application in the spinal supernatant. We tested whether spinal effects of TNFα depend on IL-6. We recorded from nociceptive spinal neurons with input from the knee joint in anesthetized rats. We used either normal rats or animals with acute Kaolin/Carrageenan inflammation in the knee as a model of arthritis. The leg was mechanically stimulated at the knee, ankle and paw with innocuous and noxious intensity. During development of inflammation spinal application of an antibody to TNFR1 but not to TNFR2 prevented spinal hyperexcitability. Hyperexcitability during acute inflammation was not reduced by etanercept. When TNFα was applied to the surface of the spinal cord responses to stimulation of the leg increased. Spinal co-application of TNFα and spg130 lead to significant smaller responses to stimulation than application of TNFα alone. In summary TNFα induces spinal hyperexcitability *via* activation of TNFR1 receptors and subsequent release of spinal IL-6 which is overtaking the maintenance of spinal hyperexcitability.

P1.8

ROLE OF Wnt/β-CATENIN PATHWAY IN DIFFERENTIATION OF MOUSE ES CELLS TO CORTICAL PROGENITORS

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The Wnt/β-catenin was reported to promote both pluripotency maintenance and differentiation. Treatment with Wnt and Nodal antagonists-Dkk1 and Lefty-1 or Wnt antagonist-IWP2 in serum-free floating culture of embryoid body-like aggregates (SFEBq) promoted ES differentiation to neural lineages with high efficiency. Surprisingly treatment with Wnt pathway agonist-Wnt3a down-regulated stem cells surface markers (GCTM2, CD9) in hES cells and evoked morphology characteristic of differentiation. We used Wnt inhibitors (iCRT3 and IWP2) and Nodal inhibitor (SB431542) alone or in combination to investigate the differentiation of mouse ES cells to cortical progenitors. SFEBq aggregates were differentiated and analyzed for the expression of the markers of cortical progenitors. The expression of Pax6, Sox1 and Foxg1 in SFEBq without inhibitors peaked on day 7 of differentiation while IWP2 and iCRT/SB431542 aggregates exhibited a delayed expression of cortical markers with highest expression on day 11 of differentiation. Moreover, the addition of Wnt3a on day 7 to 11 increased cell proliferation and

sustained the expression of cortical progenitors markers. Taken together we observed an influence of Wnt regulation on neuronal differentiation and on proliferation of cells at later stage of differentiation.

P1.9

HYPERBARIC TREATMENT RESULTS IN DECREASED ROS PRODUCTION IN NEONATAL HYPOXIA-ISCHEMIA RAT MODEL

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Encephalopathy caused by birth asphyxia results in significant mortality and long-term morbidity. In our previous studies we proved that HBO reduces brain damage in experimental model of birth asphyxia by almost 60%. The aim of present study was to evaluate the effect of hyperbaric oxygen (HBO) on reactive oxygen species (ROS) production and antioxidative enzymes activities – catalase (CAT) and glutathione peroxidase (Gpx) in 7-day old rat brain after hypoxia-ischemia (H-I). In the experimental model of H-I the left (ipsilateral) common carotid artery ligation is followed by 75 min hypoxia. HBO (2,5 ATA) was applied 1, 3 or 6 h after H-I for 60 min. Treatment was repeated for 3 following days. DCF test showed that H-I causes almost 4-fold increase in ROS production in ipsilateral hemisphere, while HBO reduced it by 40%, 24% and 18%, applied 1, 3 and 6 h after H-I, respectively. H-I resulted in 32% increase in catalase activity, probably as a compensation to high ROS concentration. HBO treatment reduced this increase to 4, 5 and 16%, respectively, which is probably a consequence of reduced oxygen radicals production. Similar pattern was observed in activity of Gpx. Our results suggest that HBO reduce synthesis of ROS (which manifests in decreased DCF fluorescence) and also decrease antioxidative enzymes activity. This may be one of the mechanism of HBO neuroprotective and diminishing brain injury effect.

P1.10

EXPRESSION OF Kiss-1/GPR54 mRNA IN THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS IN OBESE AND DIABETIC MALE RATS

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Obesity is now dramatically on the rise and is a major risk factor for diabetes. Besides primary metabolic health problems occurring in people with obesity and diabetes there are numerous secondary problems including disruptions of the reproductive system. Kisspeptins and its receptor GPR54 play a key role in regulation of reproduction and integration of metabolic and reproductive systems. We hypothesized that obese and/or diabetic male rats would have altered Kiss-1 and/or GPR54 mRNA levels in the hypothalamic-pituitary-gonadal (HPG) axis. Rats were fed with high fat diet (HFD) for 5 weeks to induce obesity (DIO group). Injections of STZ were performed to induce diabetes type 1 (STZ group) or diabetes type 2 (HFD/STZ group). Control animals (C group) were fed with lab chow diet. Real-time PCR was performed. We have found that: (1) Kiss-1 and GPR54 expression in HPG axis was related to the rat metabolic status; (2) both STZ and HFD/STZ rats had elevated GPR54 mRNA level in the hypothalamus and (3) STZ rats had decreased Kiss-1 mRNA levels in the pituitary and decreased GPR54 levels in the testis. We have concluded that observed changes may contribute to reproductive failure in animals with diabetes. Supported by grant NCN 2011/01/B/NZ4/04992.

P1.11

CFA-INDUCED INFLAMMATION INFLUENCES NEUROCHEMICAL PROPERTIES OF TRIGEMINAL GANGLION NEURONS

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Despite of many years of research, the evidence for interactions between the peripheral nervous system and the immune system remains incomplete. Our recent studies have shown that the proinflammatory cytokines interleukin (IL)-1β, IL-6 and tumor necrosis factor-α (TNF) play a critical role in the pathogenesis of the immune response within the peripheral endings of trigeminal ganglion neurons. The goal of the current project was to determine whether peripheral inflammation leads to changes in BDNF expression in trigeminal ganglia. In order to examine the effects of peripheral inflammation on the regulation of BDNF content in trigeminal ganglion neurons *in vivo*, a model of CFA-evoked inflammation in the TMJ of C57BL mice was employed. BDNF levels in trigeminal ganglia ipsilateral to the CFA-induced inflammation were compared with BDNF levels in the control ganglia from the contralateral (intact) side, on day 3 and 7 after induction of inflammation. A standard sandwich ELISA methodology was used to compare levels of BDNF in the trigeminal ganglion that supplies the tissue that has been affected by the inflammatory process with BDNF levels in the contralateral ganglion. These *in vivo* data further support the notion that BDNF is a likely key player of trigeminal inflammatory pain.

P1.12

SPATIOTEMPORAL CHARACTERIZATION OF mTOR KINASE ACTIVITY FOLLOWING KAINIC ACID INDUCED STATUS EPILEPTICUS AND ANALYSIS OF RAT BRAIN RESPONSE TO CHRONIC RAPAMYCIN TREATMENT

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Mammalian target of rapamycin (mTOR) is a protein kinase that senses nutrient availability, trophic factors support, cellular energy level, cellular stress, neurotransmitters and adjusts cellular metabolism accordingly. Recently, several groups reported that seizures increase mTOR activity, and such increased activity in genetic models can contribute to spontaneous seizures. However, the current knowledge about the spatiotemporal pattern of mTOR activation induced by proconvulsive agents is rather rudimentary. Also consequences of insufficient mTOR activity on a status epilepticus are poorly understood. Here, we investigated these two issues. We showed that mTOR signaling was activated by kainic acid (KA)-induced status epilepticus through several brain areas as well as revealed two waves of mTOR activation: an early wave (2 h) that occurs in neurons and a late wave that predominantly occurs in astrocytes. Unexpectedly, we found that pretreatment with rapamycin, a potent mTOR inhibitor, gradually (1) sensitized animals to KA treatment and (2) induced gross anatomical changes in the brain. Supported by Polish National Science Center OPUS grant (2012/05/B/NZ3/00429).

P1.13

EFFECT OF SILDENAFIL ON SEIZURE THRESHOLD AND ACTIVITY OF SOME CLASSICAL ANTIEPILEPTIC DRUGS IN THE 6-Hz SEIZURE TEST IN MICE

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Sildenafil citrate, a selective phosphodiesterase 5 (PDE5) inhibitor, is the first oral drug used in the therapy of erectile dysfunction. Presence of PDE5 in the brain and ability of sildenafil to cross the blood-brain barrier cause that this drug influences many central nervous system-related effects. Animal studies revealed that sildenafil has both pro- and anticonvulsant potential. It also influences activity of antiepileptic drugs in models of seizures in mice. The aim of the present study was to evaluate the effect of sildenafil on the psychomotor seizure threshold and activity of some classical antiepileptic drugs, i.e., valproic acid, phenobarbital, clonazepam and ethosuximide, in the 6 Hz seizure test in mice. We noted that sildenafil raises seizure threshold and enhances anticonvulsant action of the studied antiepileptic drugs in this test. Since sildenafil increases valproic acid concentration in mouse brain, interaction between these drugs had undesirable pharmacokinetic character. Sildenafil did not change concentration of the other studied antiepileptic drugs and thus noted interactions seem to be pharmacodynamic. Our results show that using sildenafil in epileptic patients should be safe and beneficial. Moreover, its combination with antiepileptic drugs might have both favourable and negative results and therefore it should be controlled in the clinical practice.

P1.14

INFLUENCE OF ANTIDEPRESSANTS ON LPS-INDUCED BRAIN-DERIVED NEUROTROPHIC FACTOR IN HIPPOCAMPUS OF FEMALE RATS SUBJECTED TO CHRONIC SOCIAL INSTABILITY STRESS

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It is widely accepted that chronic stress leads to the development of, and is associated with, mood disorders. Exposure to stress may intensify consequences of neuroinflammation which is considered as a crucial mechanism leading to CNS injury involving the neuroanatomical alterations in hippocampus – structure play a significant role for mechanism of action of antidepressants. Chronic treatment with some antidepressants up-regulate expression of BDNF which is the key neurotrophic factor promoting cell survival and neuroplasticity. The study was carried out to investigate the influence of desipramine, fluoxetine or tianeptine given chronically on the lipopolysaccharide (LPS) effect on the expression and the level of BDNF in hippocampus of female rats subjected to chronic stress. In the hippocampus of LPS-treated rats subjected to chronic stress, BDNF mRNA and protein levels were reduced, in comparison to the stress-group. The LPS effect was protected by treatment with studied antidepressants. The protection of BDNF against the deleterous synergistic effect induced by inflammation and chronic stress may have significance for therapeutic effects of long-term treatment with antidepressants.

P1.15

CHL1 GENE EXPRESSION OF HUMAN BLOOD LYMPHOCYTES AS SSRI RESPONSE BIOMARKER

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Previous findings show lack of proven biomarkers for predicting antidepressant drug response. Recent genome-wide expression study indicates CHL-1 gene as potential depression treatment biomarker. Aim of study is to examine possibility of applying CHL-1 gene with a group of Polish depressive patients as SSRI response biomarker. Peripheral blood samples were collected from well clinically characterized naïve (N), treatment resistant (TR) depressive patients and healthy (H) volunteers. Lymphocytes were isolated and cultured with two selected drugs – paroxetine and mirtazapine. Cell proliferation assay was done after 72 h of incubation. The total RNAs from lymphocytes without drugs were extracted and cDNAs were synthetized. Levels of CHL-1 gene expression were checked by real-time PCR method. There are significant differences between chosen phenotypes: high and low sensitivity to mirtazapine for H, TR and N; high and low sensitivity to paroxetine for H and N. There are significant differences between mirtazapine sensitivity for whole groups of H and TR. There are not significant differences of CHL-1 gene expression levels between groups. Findings indicate different phenotypes occurrence for SSRI mediated growth inhibition sensitivity. Conclusions about CHL-1 gene as a SSRI response biomarker are still unclear. Suported by Era-Net-Neuron "PADRE" grant.

P1.16

DUAL ENDOTHELIN RECEPTOR ANTAGONIST REDUCES SUBCELLULAR ORGANELLES DAMAGE IN CEREBRAL CORTEX NEURONS IN EXPERIMENTAL MODEL OF CARDIAC ARREST

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In the present study we investigated the effect of dual endothelin receptor (ETR) antagonist bosentan on postischemic structural abnormalities of subcellular organelles in neurons of cerebral cortex and on endothelin binding sites in the brain after 10 min of cardiac arrest in male Wistar rats. After seven days, cortical neurons in bosentan-treated animals revealed diminished morphological changes compared with non-treated rats, including less advanced nuclear pore formation, disaggregation of ribosomes, abnormalities of Golgi network and better preservation of long neurotubules. The analysis of 125I-endothelin-1 binding in brain documented a decrease in endothelin-1 maximum density of receptors (Bmax) and equilibrium dissociation constant (KD) up to 1 week in untreated animals. In bosentan-treated animals above values increased postischemically. In conclusion, the results indicate that bosentan works towards salvage of cytoskeleton and other organelles of cortical neurons after cardiac arrest through a modulation of ETR signaling.

P1.17

Arc FUNCTIONAL NEIGHBOURHOOD IN THE NEURONAL CELL NUCLEUS

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Arc protein is a versatile factor connecting memory formation, plasticity changes and neuronal activity. Through regulation of actin polymerization, Arc contributes to synapse expansion and may furthermore influence synapse strength *via* management of AMPA receptor turnover. The function of Arc in the neuronal cell nucleus is poorly understood. In this work we performed structural, functional and biochemical analysis to identify Arc`s nuclear interactome. Using confocal microscopy we investigated Arc`s functional neighborhood and found that it occupied internal parts of the nucleus, closely to hnRNPs. This observation were confirmed with electron microscopy, which demonstrated that Arc localizes mainly at the peripheral areas of chromatin. Furthermore, pull-down-based biochemical experiments suggested that Arc interacts with splicing machinery. Collectively, our data suggest that nuclear Arc is involved in the gene expression phenomena.

P1.18

INHIBITION OF mGluR1 AND mGluR5 IMPAIRS MEMORY CONSOLIDATION AND RECONSOLIDATION BY DISREGULATION OF LOCAL PROTEIN SYNTHESIS

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It is known that mGluRs group I (mGluR1 and mGluR5) are involved in memory consolidation and reconsolidation probably by local protein synthesis regulation. One of the consequences of group I mGluRs activation is the synthesis of new proteins that play important role in memory consolidation and reconsolidation – NCAM and CaMKII, and also FMRP, that plays an important role in the mRNA transport and regulation of mRNA translation in the nerve endings by a negative-feedback mechanism. The aim of this study was to investigate the effect of mGluR1 and mGluR5 inhibition on synthesis of NCAM, CaMKII and the regulatory protein FMRP. In our experiments the passive avoidance task in one-day chicks was used and mGluRs 1/5 were inhibited by intracerebral injection of specific inhibitors, LY367385 and MPEP respectively. The expression of chosen proteins was determined in nerve endings

isolated from chick brain. Our results show, that inhibition of each receptor around the time of training resulted in decrease of NCAM and CaMKII synthesis, as well as in synthesis of FMRP. However, the inhibition of mGluR1/5 around the time of reminder resulted in increase in NCAM and CaMKII synthesis, although synthesis of FMRP was significantly decreased. These results suggest that both receptors are involved in memory formation by regulation of termination of protein synthesis through control of FMRP synthesis.

P1.19

NEUROPHARMACOLOGICAL PROFILE OF AQUEOUS EXTRACT FROM *CALEA ZACATECHICHI*

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Abstract withdrawn

P1.20

ULTRASTRUCTURAL AND BIOCHEMICAL CHANGES IN BRAIN OF RAT CHRONICALLY EXPOSED TO SILVER NANOPARTICLES

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Silver nanoparticles (AgNPs) demonstrate strong antimicrobial activity resulting in their wide-spread use in different applications, including medical ones. Despite of the fact that human exposure to nanosilver is constantly increasing, there is no many research dedicated for investigating their potential neurotoxic effects. Most of the previous studies on mechanisms of nanosilver neurotoxicity have used *in vitro* models. The aim of the present study was to determine whether this small-sized commercially available AgNPs induce ultrastructural and biochemical changes in brain of male adult rats. Rats were exposed orally to 10 ± 4 nm nanosilver in size for 14 days. Using transmission electron microscopy (TEM), nano-sized granules were detected in brains of exposed rats. Besides, ultrastructural changes in cortical and hippocampal neurons were found. TBARs level was measured to assessed lipid peroxidation in homogenates from exposed animals and immunochemical analysis was used to measure the level of selected tissue markers of oxidative stress in brain. Supported by grant nr NN401619938.

P1.21

INTERACTION BETWEEN INDOCYANINE GREEN AND GADOBUTROL IN CEREBELLAR GRANULE NEURONS CULTURES

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In experiments we used indocyanine green (ICG), and gadobutrol (Gad) contrast dyes. There is no information about parallel application of ICG and Gad, therefore we decided to study their toxicity using primary cerebellar granule cells culture (CGC). The aim of study was to assess the minimal ICG concentration which evokes neurotoxicity. 30 min exposition to 75 and 125 μM ICG resulted in neurotoxicity. We observed imbalance in calcium homeostasis (extra- and intracellular) after addition of ICG, which can be one of the mechanisms of ICG neurotoxicity. We measured absorbance and NMR spectra for 25–125 μM ICG concentrations in three solvents. Gad contrast media mixed with ICG were also measured and neurotoxicity of this mixture was examined. The shape of absorption and NMR spectra show differences between water, water with 2.3 mM Ca^{2+} and Locke25 for all analyzed ICG concentrations. Other possible mechanism of ICG neurotoxicity can be dose dependent oligomerization of ICG. We did not observe any toxic effect of Gad on CGC. Protective effect on surviving neurons after treatment of ICG, dependent on Gad dose and sequence of its administration (before > simultaneously > after addition of ICG) was observed. However, the mechanism of this phenomenon remains not clear. Supported by grant 2011/03/B/ST7/02576.

P1.22

REGULATION OF THE IMMEDIATE-EARLY GENE EXPRESSION IN THE MOUSE FOREBRAIN BY ACUTE ADMINISTRATION OF ANTIDEPRESSANTS

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The sites of action of antidepressants in the brain responsible for their psychotropic effects are not fully elucidated. Our study was undertaken to compare acute effects of antidepressants representing diverse modes of molecular action on regional brain activity, reflected by expression of immediate-early genes (IEG). *In situ* hybridization was used to analyze expression of the IEG Fos, Egr1 and Arc in the mouse forebrain after a single injection of tranylcypromine (MAO inhibitor), mianserin (acting on 5-HT, NA and histamine re-

ceptors but not on monoamine levels) and tianeptine (with unknown molecular targets). Tranylcypromine and tianeptine produced a widespread IEG induction in the neocortex and striatum (where mianserin down-regulated IEG). The similarity of the tianeptine and tranylcypromine effects suggests that elevation of monoamine levels is an important mechanism by which tianeptine affects forebrain function. Moreover, all three drugs elicited IEG induction in several brain regions implicated in the regulation of mood in humans: anterior cingulate and insular cortex, basolateral amygdala and paraventricular thalamic nucleus. The common activation of these regions by different types of antidepressants suggests that they may be the sites where neuroplastic changes take place upon chronic drug treatment, leading to the long-term psychotropic effect.

P.2 Motor control

P2.1

SUBCORTICAL EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS)

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Subcortical effects of tDSC were tested on direct and transsynaptic (monosynaptic) activation of reticulospinal and rubrospinal neurons in anesthetised rat and cat preparations. Rubrospinal neurons were activated by stimuli applied in the red nucleus (RN) and reticulospinal neurons *via* collaterals of reticulospinal neurons stimulated in the medial longitudinal fascicle (MLF), or collaterals of corticospinal neurons stimulated in the ipsilateral pyramidal tract (iPT). Responses of these neurons were facilitated by anodal polarization of the brain in the cat and by cathodal polarization in the rat. Facilitation was expressed by an increase in the amplitude and a decreased in the latency of EMG responses in the rat and of the descending volleys in the cat. tDCS did not facilitate actions of iPT neurons alone, but it increased the probability of activation of reticulospinal neurones by joint actions of ipsilateral and contralateral PTs, especially together with the MLF. All the facilitatory effects of tDCS outlasted its application up to 2 hours.

P2.2

THE CORRELATIONS OF PROPRIOCEPTION-RELATED PARAMETERS IN TWO GENERATIONS OF MEN

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Motor abilities depend on activity of proprioceptors. The literature data suggest that proprioception is genetically determined. To test this hypothesis, 30 pairs of fathers and their sons were studied. The estimation of a force generated by a knee extensors, the control of knee position, balance at a dynamographic platform were studied before and immediately after the fatiguing running. Significant correlations between the results for groups of fathers and their sons in relation to the three abilities were found. The correlation coefficients between the recurrence of positioning the knee were 0.197 and 0.491, whereas the coefficients between the recurrence of the knee extension force were 0.695 and 0.752, before and after the fatiguing exercise, respectively. For the body balance, the strongest correlation between the average deflection point of the center of feet pressure in the front-to-back axis amounted to 0.650 and 0.731, whereas for the speed of moving the center of gravity in the sagittal plane amounted to 0.546 and 0.703 before and after exercise, respectively. Because the significant correlations were obtained for pairs of relative people these observations indicate genetic determinants of proprioception.

P2.3

THE MEDULLARY RETICULO-CEREBELLAR PROJECTION TO THE CEREBELLAR CAUDAL VERMIS

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The projections from reticular formation (RF) to the pyramis (Pr; related to innervation of axial and proximal forelimb muscles) and uvula (Uv; interconnected with vestibular system) were studied using two fluorescent retrograde tracers. Bilaterally labeled RF neurons, parent for the reticulo-cerebellar projection, were found in the magno- (Lcmc) and parvocellular (Lcpc) pars of caudal lateral nucleus (*n*=7797), oral lateral nucleus (Lo; *n*=3893), and in smaller number in the lateral reticular nucleus (LR; *n*=1326) and gigantocellular nucleus (RGc; *n*=1319). The projection is seven times greater to Pr than to Uv. Connections from Lcmc to Pr originate from entire nucleus, except for the dorsolateral region, and to Uv arise from two separate neuronal populations, the ventromedial and dorsolateral, at rostral levels. While entire Lcpc supplies Pr, its rostral dorsolateral region connects Uv. The ventral LR region projects to Pr, whereas two groups of neurons in the caudal part project ipsilaterally to Uv. In Lo, neurons supplying Pr are present ventrolaterally, but these connecting Uv cluster in the dorsolateral and dorsomedial regions. Central core of the caudal RGc sends fibers exclusively to Pr. The RF projections differ regarding regions of origin and laterality, probably due to different function of Pr and Uv.

P2.4

THE COMPARISON OF MOTOR UNITS CONTRACTILE PROPERTIES IN MEDIAL GASTROCNEMIUS MUSCLES OF THE NORMAL AND ADHD RATS

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The motor unit (MU) contractile properties in medial gastrocnemius muscle of young Wistar Kyoto rats, the model of attention deficit hyperactivity disorder (ADHD), and normal Wistar rats were studied. Functional isolation of motor units was achieved by electrical stimulation of single axons from ventral roots of L4–L5 spinal nerves. The motor units parameters: the force of twitch and tetanus, the contraction and the half-relaxation times and the properties of action potentials were analyzed for 47 and 60 MUs in ADHD and normal rats, respectively. The three types of MUs (FF, FR and S) in both groups were found. The distribution of FF, FR and S MUs for normal rats was: 26.7, 55.0 and 18.3% whereas the distribution of FF, FR and S MUs for ADHD rats was: 27.6, 44.7 and 27.6%. There were no statistical differences in motor units force and twitch time parameters. However, the twitch-to-tetanus ratio was higher for MUs in ADHD animals. Moreover, differences between shapes of twitch recordings were found and a ratio of the contraction to the half-relaxation times was lower for MUs in ADHD animals. The mean number of turns of MU action potentials was lower in all types of MUs of ADHD rats.

P2.5

CHANGES IN CONTRACTILE PROPERTIES AND ACTION POTENTIALS OF MOTOR UNITS IN THE RAT MEDIAL GASTROCNEMIUS MUSCLE DURING MATURATION

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The development of motor unit (MU) properties after the elimination of polyneuronal innervation of muscle fibers has not been studied so far in contrast to the embryonic and neonatal development which are well known. Three groups of Wistar rats were investigated – 1 month old, 2 months old and the adult – 9 months old (1 m.o., 2 m.o. and 9 m.o. rats, respectively). The basic contractile properties and action potentials of MUs in the medial gastrocnemius muscle were analysed. In 1 mo rats the three main physiological types of MUs were already separated. It was visible that various MU's contractile properties reached adult values at different stage of development. The twitch time parameters were similar for all studied age groups whereas the force was increasing, especially in the second month of life. The fatigue index for FF MUs as well as the twitch-to tetanus ratio for all three types of MUs in 1 and 2 m.o. rats were higher than for 9 m.o. rats. The amplitude and time parameters of MUAPs, except their latency, were not changing during the studied period of development.

P2.6

SEX DIFFERENCES IN MOTOR UNIT CONTRACTILE PROPERTIES OF THE RAT SOLEUS MUSCLE

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The soleus muscle has unique physiological characteristics, as a typical slow-twitch muscle, composed predominantly of slow motor units (MUs). In this study we examined electrophysiologically functionally isolated MUs of male and female adult Wistar rats. It was revealed that the mean mass of the soleus muscle in males was approximately by 80% bigger than in females, however, a relation of the muscle-to-body mass was higher for females. No differences were observed with respect to the MU twitch forces, but the maximum tetanus forces were substantially lower for female rats, what significantly influenced higher twitch-to tetanus ratios in females. The contraction and the half-relaxation times were significantly longer in female MUs, what might be due to differences in muscle architecture. The force-frequency curve of slow MUs in males was shifted rightwards with respect to females, indicating that the same relative level of a tetanic force could be achieved at a considerably higher stimulation frequency in males. The maximum force-time area per pulse was significantly higher for males, and the analysis of MU action potentials revealed about four times higher amplitudes in male rats. In conclusion, numerous sex differences in MUs of the rat soleus muscle are not directly influenced by differences in body and muscle size.

P2.7

ENHANCING PROPRIOCEPTIVE INPUT TO MOTONEURONS DIFFERENTIALLY AFFECTS EXPRESSION OF NEUROTROPHIN 3, BDNF AND TrkB/TrkC RECEPTORS IN RAT HOFFMANN--REFLEX CIRCUITRY

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The importance of neurotrophin 3 (NT-3) for motor control prompted us to ask whether direct low-threshold electrical stimulation of the tibial nerve aimed at activation of Ia fibers, could

increase the pool of NT-3 and its receptor TrkC in the Hoffmann-reflex circuitry of the soleus (Sol) muscle. The effects were compared with those on BDNF and its TrkB receptor. Cuff-electrode over the tibial nerve was used to deliver continuous bursts of stimuli in awake rats. Functional mapping of neuronal activation with c-Fos showed that a number of spinal neurons was activated by Ia stimuli. Stimulation produced a strong increase of NT-3 protein (ELISA), in L3- 6 spinal segments and in Sol with minor effect on BDNF level in L3-6. Protein level of NT-3 and BDNF corresponded to the changes of NT-3 mRNA and BDNF mRNA expression in L3-6 segments but not in Sol muscle. TrkC and TrkB mRNA tended to decrease in L3-6 but in the Sol muscle TrkB mRNA decreased and TrkC mRNA strongly increased showing sensitization of the Sol muscle to NT-3 signaling. The possibility of increasing NT-3/TrkC signaling in the neuromuscular system, with minor effects on BDNF/TrkB signaling, by selective stimulation of peripheral nerve, which in humans might be applied in non-invasive way, offers an attractive therapeutic tool. Supported by N N 401 0480 33, BIOIMAGINE grants.

P2.8

SINUSOIDAL MECHANICAL RESPONSE OF DIFFERENT TYPES OF MOTOR UNITS

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Aim of the work was to verify if the sinusoidal modulation of the stimulation rate of individual rat MUs may provide reliable sinusoidal responses of the recorded force with an acceptable harmonic distortion (HD). After MU classification, the isolated axons of the spinal ventral roots were electrically stimulated with rates sinusoidally changing, from a minimum to a maximum value, with different frequencies of modulation for slow (0.4–1.0–2.0–4.0 Hz) and fast (1.0–2.0–4.0–7.0 Hz) MUs. From the twitching raw signal an interpolated force curve was obtained for each frequency to generate, from the sample by sample difference with the theoretical sine, the specific error signal. At each input frequency, HD was calculated as the percentage ratio between the total power of the error signal and the total power of the theoretical sine. Nine MUs, characterized as S (n=3), FR (n=3) and FF (n=3), were studied. The range of HD for S, FR, FF units was 0.9–5.1%, 0.3–3.5%, 0.3–4.1%, respectively. These HD values indicate that the sinusoidal responses of muscle functional unit were reliable and further suggest the possibility to use this method for single MU transfer function identification.

P2.9

AN ALTERNATIVE METHOD FOR FAST/SLOW MOTOR UNIT DIVISION, TESTED IN VARIOUS PROGRAMS OF ALTERED PHYSICAL ACTIVITY IN THE RAT, AND IN THE CAT MUSCLE

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Plasticity of motor unit (MU) contractile properties observed as a result of various forms of altered physical activity or neurological disorders often leads to difficulties in their division into fast and slow types on a basis of standard physiological criteria (sag disappearance, changes of the contraction time). A method to recognize fast or slow MU types on a basis of a profile of tetanic contraction evoked at 20 Hz frequency has been proposed. We evaluated its efficiency in the male and female rats, after several types of a physical training (locomotor training, whole-body-vibration), and after various spinal cord injuries (transection or hemisection). Analogical method was used to distinguish fast and slow MUs in the cat muscle. Functionally isolated MUs of the medial gastrocnemious muscle were investigated, and the 20 Hz Tetanus Index was calculated for each MU as a ratio of the peak force in a response to the last stimulus within the unfused 20 Hz tetanus to the peak force following the first stimulus. For fast MUs values of this index were lower than 2.0, and for slow MUs higher than 2.0 in either group investigated. However in the cat muscle, composed of MUs with considerably longer twitch-time characteristics, the stimulation frequency, which enabled us to receive comparable results was lower (15 Hz) and border value of the index amounted to 5.0.

P2.10

THE INFLUENCE OF BETA-ALANINE SUPPLEMENTATION ON CONTRACTILE PROPERTIES OF MOTOR UNITS IN RAT MEDIAL GASTROCNEMIUS

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Beta-alanine (BA) supplementation increases muscle carnosine concentration resulting in better muscle performance. *In vitro* experiments on isolated muscles and single muscle fibers indicated that carnosine improved excitation-contraction coupling and slowed fatigue. We investigated effects of BA supplementation on muscle carnosine levels and *in vivo* motor units (MUs) contractile properties in rat medial gastrocnemius muscle (MG). Ten male Wistar rats were randomly assigned to control (n=5) or BA (supplemented with 1% BA in the drink-

ing water for 10 weeks; *n*=5) groups. Contractions of 258 MUs were evoked by electrical stimulation of ventral root filaments. MUs were classified into fast fatigable (FF), fast resistant (FR) and slow (S) according to the standard criteria. Twitch force (TwF), maximum tetanic force (TetF) and force profile during the fatigue test were analyzed. BA supplementation enhanced carnosine levels in white and red portion of MG muscle by 94% and 56%, respectively. After BA supplementation TwF in FF and TetF in FR MUs increased, and force was better maintained from 20th to 130th s of the applied fatigue test in FR MUs. In conclusion, BA supplementation primarily improves contractile performance of FR MUs.

P2.11

THE APPLICATION OF THE MECHANOMYOGRAPHIC (MMG) SIGNAL TO ANALYSIS OF MOTOR UNITS RECRUITATION PROCESS EVOKED BY NMES

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The MMG signal properties were analyzed during electrical stimulation of the tibialis anterior muscle. A group of 4 healthy volunteers were stimulated with train of pulses of increasing intensity. Two different pulse waveforms were tested: a rectangular monopolar and rectangular bipolar with inter-pulse interval (IPI). The EMG, force and the MMG signals were recorded. The MMG signal was recorded transcutaneously by using 3D accelerometer located over the muscle belly. The muscle response recorded for particular stimulus intensity and waveform were averaged. In the analysis four time intervals of MMG has been analyzed: P1 in range 2–12 ms, P2 in range 12–27 ms, P3 in range 27–65 and relaxation phase (RP). As the motor units (MUs) respond in all-or-none manner the recruitment process can be analyzed on the base of the MMG signal changes generated by increasing stimulation intensity. The obtained results have revealed significant difference in P3 phase of MMG courses dependent on the stimulus shape. The computer model-based analysis showed that the response recorded in P1 phase is generated by easy excitable fast MUs, while in P3 phase is determined by number of activated slow MUs. Concluding, the MMG signal profiles can provide the information concerning an influence of electrical stimulation pulses waveforms on recruitment of different types of motor units.

P2.12

THE MOTOR UNIT CONTRACTILE PROPERTIES OF RAT MEDIAL GASTROCNEMIUS MUSCLE SUBJECTED TO THREE MONTHS COMPENSATORY OVERLOAD

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Compensatory overload of medial gastrocnemius muscle was induced by bilateral tenotomy of synergists (lateral gastrocnemius, plantaris and soleus). Operated muscles were regularly voluntarily activated as after surgery rats were kept in wheel-equipped cages and additionally were exercised on a treadmill. 3 months after the surgery the final electrophysiological experiments were carried out. 106 motor units (MUs) of the overloaded muscle (OMG) and 154 MUs of the untreated muscle (MG) were studied. Results showed that OMG mass and its relation to the body mass were higher in comparison to MG. In OMG higher percentage of slow (S) and fast fatigable (FF) MUs and lower contribution of fast resistant (FR) MUs as compared to MG were noted. Changes in MUs contractile properties of OMG included: lower twitch force and higher tetanus force (resulted in lower values of the twitch-to-tetanus ratio), higher post-tetanic potentiation in all MUs types and shorter half-relaxation time for S MUs. Changes in fatigue resistance concerned FF and S MUs: in OMG the fatigue index for FF was lower, but for S higher as compared to MG. In conclusion, the 3-month period of the muscle overload induced changes in MUs contractile properties accompanied by transformational processes.

P2.13

LIMITED RECOVERY OF LOCOMOTOR FUNCTIONS AFTER LATERAL HEMISECTION OF THE SPINAL CORD IN RATS

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Lateral hemisection of the spinal cord at the low thoracic level in rats causes severe deterioration of hindlimb locomotor movements followed by the substantial improvements of locomotor functions. However the rate and the level of this improvement remain disputable. In this study we investigated the time course of locomotor recovery analyzing spatial indices of locomotion obtained with CatWalk Gait Analysis System. The animals started to be tested in the CatWalk System two weeks after the injury, when hindlimb plantar stepping recovered. Within first 2 weeks hindlimb locomotor function recovered substantially, and the analyzed locomotor indices reached plateau about one month after injury. Nevertheless, most of the indices, like speed of locomotion, hindlimb base of support, hindlimb abduction did not reached the level obtained before the injury. Within next few months some of them remained at the same level, but 5 months after the hemisection locomotion again started to deteriorate, as was manifested by decrease of locomotor velocity and increase of hindlimb base of support. This study shows that after lateral hemisection of the spinal cord at the low thoracic level the recovery of locomotor functions is limited and that 5 months after the injury the secondary deterioration of locomotion is observed.

P2.14

EFFECTS OF PROGRESSIVE WEIGHT LIFTING TRAINING ON MOTOR UNIT CONTRACTILE PROPERTIES

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Strength training increases muscle strength and contractile speed. The purpose of the study was to examine basic motor unit (MU) contractile properties of medial gastrocnemius muscle after 5 week progressive strength training in adult rats. Three and half mo rats were randomly assigned to the two groups: untrained control and progressive weight lifting (PWL). Conditioned by food reward PWL rats performed "squat-rise to toes-squat" type exercises 5 days/week in the custom made apparatus, which intensity grew from 70 up to 85% of one-repetition maximum during applied training period. MUs were functionally isolated by ventral root filament splitting, and classified according to the fatigue index and "sag" property into slow (S), fast resistant to fatigue (FR) and fast fatigable (FF). For analysis, the peak force of the maximum tetanic contraction and the peak force and the contraction and relaxation times of the twitch were studied. The peak tetanus force increased in S and FR MUs. The twitch contraction time was shortened and the twitch-to-tetanus force ratio decreased in FF and FR MUs. These initial data indicate that a short-term PWL increases force of S and FR MUs and speed of contraction of fast MUs within fast-twitch skeletal muscle.

P2.15

THE DIFFERENCES IN ORGANIZATION OF MOTOR NUCLEUS OF THE MEDIAL GASTROCNEMIUS MUSCLE IN MALE AND FEMALE RAT

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The aim of this study was to recognize the sex differences in the architecture of the medial gastrocnemius (MG) motor nucleus in the same age rats. The retrogradely labeled motoneurons in the MG motor nucleus were studied following a bath of proximal stump of the transected MG nerve in the horseradish peroxidase. The rostrocaudal distribution of motoneurons along the spinal cord and on transversal sections as well as size and density of motoneurons in the motor nucleus were determined from serial microscopic images. It was shown that length of the motor nucleus in L4–L6 segments was 37% greater in males. Three types of motoneurons with different soma diameter were revealed: α one (27.5–40.0 μm), α two (greater than 40.0 μm) and γ (smaller than 27.5 μm). The density of α one and α two motoneurons was 15% higher in females. However, sex differences between the number of α one and α two motoneurons were more significant in group of α one motoneurons. The number of α one motoneurons in the motor nucleus was 24% higher in males than females (41 *versus* 33 motoneurons). The density of α motoneurons was 31% higher in females. It is concluded that length of the MG motor nucleus is greater in males, but the density of α and γ motoneurons was higher in females.

P2.16

CHANGES IN ELECTROPHYSIOLOGICAL PROPERTIES OF RAT HINDLIMB SPINAL MOTONEURONS EVOKED BY THE WHOLE BODY VIBRATION

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The whole body vibration training was performed on adult male Wistar rats. The experimental group subjected to a whole body vibration consisted of seven rats, while the control group of nine rats. The training program included 5 weeks and was applied by 5 days a week. Each daily session consisted of four 30-s runs of vibration at 50 Hz. Intracellular properties of motoneurones were investigated during experiments on deeply anesthetized animals. It was demonstrated that a whole body vibration evoked adaptations in excitability and firing properties of fast-type motoneurons, exclusively. A significant decrease in rheobase current and a decrease in the minimum and the maximum currents required to evoke steady-state firing in motoneurons were revealed. These changes resulted in a leftward shift of the frequency-current relationship, combined with an increase in slope of this curve. These results showed that fast motoneurons of rats after vibration have the ability to produce series of action potentials at higher frequencies in a response to the same intensity of activation. Obtained data provided direct evidence on motoneuronal plasticity following a whole body vibration.

P2.17

L1 OVEREXPRESSION IN RATS WITH COMPLETE SPINAL CORD TRANSECTION DOWNREGULATES PHOSPHACAN AND UPREGULATES SYNAPTOPHYSIN, GAP43 AND Adcy1 EXPRESSION

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Recovery after spinal cord injury requires neuronal remodeling which is regulated by cell adhesion molecules (CAMs) and chondroitin sulfate proteoglycans (CSPGs). CSPG may be potentially both inhibitory and supportive of regenerative plasticity. To verify whether chronic (5 weeks) L1 CAM overexpression in transected spinal cord of the rat, proven to promote recovery in mice, affects CSPG phosphacan and markers of synaptic plasticity, adeno-associated viral vector encoding L1 protein (AAV5-L1) was injected into L1 lumbar segment, immediately after transection at Th10/11. Control group received AAV5-EGFP. AAV5-L1 transduced neurons and astrocytes below the lesion, resulting in 170-fold increase in L1 mRNA level at low thoracic segments (Th<L) and 260-fold increase in L1-2, where L1 protein level was increased by 20%. L1 overexpression significantly reduced phosphacan immunostaining around neurons in L1-2 segments and upregulated synaptophysin, adenylate cyclase 1 and growth-associated protein 43 transcripts. We conclude that L1 overexpression, promotes structural remodeling by inhibiting phosphacan expression and upregulating molecules indispensible for axonal growth and synaptic function. Support: Polish-German S007/P-N/2007/01 & NCN GP2241 (for R.P.) grants.

P2.18

THE MOLECULAR MACHINERY THAT ORCHESTRATES NEUROMUSCULAR JUNCTION DEVELOPMENT

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Neuromuscular junctions (NMJs) in mammalian skeletal muscle undergo a postnatal topological transformation from a simple oval plaque to a complex branch-shaped structure. We previously demonstrated that podosomes, actin-rich adhesive organelles, promote the remodeling process and showed a key role for one podosome component, LL5β. To better understand molecular mechanisms of postsynaptic maturation, we purified LL5β protein complex from myotubes and showed that three regulators of the actin cytoskeleton – Amotl2, Asef2 and Flii – interact with LL5β. These and other LL5β-interacting proteins are associated with conventional podosomes in macrophages and podosome-like invadopodia in fibroblasts, strengthening the close relationship between synaptic and non-synaptic podosomes. We then focused on Amotl2, showing that it is associated with synaptic podosomes in cultured myotubes and with NMJs *in vivo*. Depletion of Amotl2 in myotubes leads to increased size of synaptic podosomes and corresponding alterations in postsynaptic topology. Depletion of Amotl2 from fibroblasts disrupts invadopodia in these cells. These results demonstrate the role Amotl2 plays in synaptic remodeling and support the involvement of podosomes in this process.

P2.19

SELECTED BEHAVIORAL AND MOTOR PARAMETERS IN Bcl-2 KNOCKOUT MICE

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Bcl-2 knockout mice doesn't produce Bcl-2 protein – one of the major protein factors regulating apoptotic processes involved in development of the nervous system. Bcl-2 deficiency result in increased cell death of motoneurons, sensory and sympathetic neurons (Michaelidis et al. 1996). Only very sparse data on impact of this mutation on motor activity and behavior are available. In the present study we examined the exploratory activity of knockout mice in the open field task, and checked motor related behaviors by means of RotaRod, elevated runway test, strength grip test, and balance rod test. Preliminary data suggest lack of prominent alteration of analyzed parameters, but more experiments are planned to look for possible more subtle changes.

P2.20

STRUCTURAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF MUSCLE COMPARTMENTS IN RAT MEDIAL GASTROCNEMIUS

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Rat medial gastrocnemius is composed of the proximal and distal compartments. To diversify these subvolumes, glycogen depletion technique based on a stimulation protocol one of the two primary nerve branches to the muscle was applied. The area of compartments, number and diameter of muscle fibers in the two distinct subvolumes on five muscle levels (10, 25, 40, 75 and 90% of muscle length) were determined. It was shown that the two smallest, opposite serial sections: close to the knee (10% of muscle length) and close to the Achilles tendon (90% of muscle length) were occupied by only one compartment, i.e. proximal and distal, respectively. In the largest section (40% of muscle length), the proximal compartment constituted 27–38% of the muscle area. Maximal number of muscle fibers in the proximal compartment was 4536–6698, while in the distal one 4773–6241. The mean muscle fibers diameter in the proximal and the distal subvolumes ranged: 36.9–54.3 μm and 46.5–63.8 μm, respectively. Additionally, in electrophysiological ex-

periments the forces evoked by common or separate stimulation of L4 and L5 ventral roots in whole muscle and in one of compartments were measured. The ratio of forces evoked at L5/L4 ventral roots stimulation amounted to 2.18 in the proximal compartment, whereas 64.67 in the distal compartment.

P2.21

SPINAL CORD TRANSECTION LEADS TO ATTENUATION OF DETERMINANTS OF GABAERGIC AND GLYCINERGIC TRANSMISSION NOT ACCOMPANIED BY CHANGES IN INHIBITORY INPUTS TO MOTONEURONS

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The data on the responses of inhibitory circuits to the spinal cord transection are conflicting. We examined the segmental distribution of determinants of GABAergic and glycinergic transmission in adult rats five weeks after complete spinal cord transection at Th9-10. Concentrations of the GABA and glycine (Gly) in segments below the lesion were evaluated in rats that did not receive any treatment. Decreases in GABA (24%) and Gly (26%) were found only in the lumbar L1-2 segments. Two other groups of spinal rats received microinjections of PBS (SP-PBS) or AAV- EGFP transgene (SP-EGFP) to L1-2. Both led to GABA decrease (43% in L1-2 and 23% in L3-6 segments) and a decrease in mRNA for GAD67 (43% in L1-2 in both groups and 10% in L3-6 segment of SP-PBS vs 49% in L3-6 of SP-EGFP rats). The respective decreases in mRNA for Gly transporter GlyT2 were 68 vs. 72% in L1-2 and 29 vs. 76% in L3-6 segments. These changes were not accompanied by changes in the density of GABAergic/glycinergic network and inputs to motoneurons identified with GAD67/GlyT2 immunostaining. We conclude that albeit spinalization does not reduce inhibitory inputs to lumbar motoneurons it leads to long-term impairment in presynaptic determinants of inhibitory neurotransmission which may attenuate inhibitory signaling. Support: NN401 324739 grant.

P3. Cortical and subcortical neuronal networks

P3.1

EXPRESSION AND STRUCTURAL LOCALIZATION OF NEUROTROPHIN RECEPTOR TrkB IN THE DEVELOPING BRAIN OF THE *MONODELPHIS* OPOSSUM

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Neurotrophins are important regulators of neuronal function in the developing and adult brain. We studied expression of TrkB receptors in the postnatally (P) developing brain of the opossum (*Monodelphis domestica*). The Western blot analysis showed presence of the full-length catalytical isoform of TrkB and three truncated kinase-lacking isoforms in the opossum brain. Expression of the full-length TrkB receptor was present in the newborn opossum, whereas truncated forms of TrkB receptors were almost undetectable at this period. The level of the full-length TrkB protein gradually increased with a developing opossum brain, reaching maximum at P12–20. The highest levels of expression of the full-length TrkB correspond to the time of cortical layers generation. The level of truncated TrkB rapidly increased at P20 and started to dominate since P35 (when opossums open eyes). Immunohistochemical staining for TrkB receptors showed that the majority of labeled neurons were placed in the olfactory bulb, cerebral cortex, hippocampus, thalamus, hypothalamus, cerebellum and various brainstem structures. Interestingly, TrkB receptors were predominantly expressed in neurons. Lack of TrkB receptors in glial cells, especially astrocytes and oligodendrocytes, provides the evidence that TrkB receptors can play functionally different role in marsupials than in eutherians. Supported by the NSC grant No 2011/01/B/NZ4/01575.

P3.2

CHOLINERGIC ENHANCEMENT OF MEMBRANE POTENTIAL FLUCTUATIONS IN NEURONS FROM POSTEROMEDIAL THALAMIC NUCLEUS IN RAT

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Higher order posteromedial thalamic nucleus of the rat (PoM) receives cholinergic (Ach) and noradrenergic (NA) neuromodulatory projections originating from the brain stem. With a whole-cell patch-clamp method we investigated influence of these neuromodulators on membrane potential of PoM neurons in the thalamic slices. Ach (carbachol) or NA (norepinephrine) agonists were added to the bath to mimic the activation of appropriate neuromodulatory system. Both agonists induced slow depolarization of membrane potential by about 9 mV. However, carbachol but not norepinephrine substantially enhanced amplitude of membrane potential fluctuations in the frequency range from 8 to 500 Hz (reaching more than two-fold elevation between 25–180 Hz). These carbachol induced fluctuations were not blocked neither by manual membrane repolarization to the control level, nor by the blockage of GABA-A receptors. Our results suggest that this increase of fluctuation strength might result mainly from activation of muscarinic receptors. This research and SN were supported by the European Union Regional Development Fund through the Foundation for Polish Science within the frames of International PhD Program in Neurobiology.

P3.3

ARE GAP JUNCTIONS OR MINERALOCORTICOID RECEPTORS INVOLVED IN GENERATION OF POSTERIOR HYPOTHALAMIC THETA IN RAT?

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There is a large body of research indicating that occurrence of hippocampal (HPC) theta dependent on the integrity of ascending pathway originating in the brainstem reticular formation. Anatomical studies indicate that reticular influences are relayed *via* the posterior hypothalamus, specifically the posterior hypothalamic (PH) and supramammillary (SuM) nuclei. In addition, neurons localized in these nuclei discharge rhythmically and in phase with HPC theta. Recently we have shown that local theta activity could be generated in anesthetized rats in PH and SuM nuclei. Recorded signal was produced independently of simultaneously occurring HPC theta and had a cholinergic profile. In the preset study we extended pharmacological observation; specifically, carbenoxolone (CBX – 75 μg/μl; gap junction (GJ) blocker and mineralocorticoid receptor agonists) was administrated into posterior hypothalamus in urethanized rats. Injection of CBX, induced well synchronized theta activity. The effect of CBX was not antagonized by injection of GJ opener – trimethylamine (45 μg/μl) – but was abolished by antagonist of mineralocorticoid receptor, spironolactone (10 μg/1 μl). These results demonstrate for the first time that PH theta is mediated not by GJ but by mineralocorticoid receptors. Studies supported by NCN grant no. 2011/01/B/NZ4/00373.

P3.4

THETA MODULATED CELLS IN ANTEROMEDIAL AND REUNIENS THALAMIC NUCLEI IN FREELY MOVING RAT

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Theta rhythm is considered to play a critical role in spatial and nonspatial mnemonic functions of the limbic system. The anterior thalamic nuclei, a central component of Papez' circuit, form the core of an "extended hippocampal system" that is vital for memory. The nucleus reuniens, the largest of the midline nuclei of the thalamus, has been neglected in contemporary neuroscience, despite being a major source of thalamic afferents to the hippocampus and parahippocampal structures. Moreover, there are suggestions that it plays key roles in memory consolidation. In both anteromedial and reuniens thalamic nuclei, in freely moving rats, we have recorded subsets of theta modulated cells that differ in terms of their electrophysiology. In anteromedial thalamic nucleus we detected bursting and non-bursting neurons that were strongly entrained by theta oscillations and synchronized their activity in the 6–11 Hz range. In nucleus reuniens we recorded theta modulated cells which show unusual pattern of firing. Except that the bursts of neuronal activity were strongly entrained by the theta rhythm they also phasically differed in their intensity.

P3.5

RELAXIN-3/RXFP3 SIGNALLING IN RAT PARAVENTRICULAR NUCLEUS: NOVEL ELEMENT IN NEURAL CONTROL OF FEEDING

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Relaxin-3 (RLX3) is recently discovered peptide of the insulin superfamily. The highly-conserved relaxin family peptide receptor 3(RXFP3) signalling system is associated with stress response and feeding behaviour. Central RLX3 injections stimulate feeding *via* RXFP3 in the paraventricular nucleus (PVN). We hypothesize that RLX3 exerts its orexigenic effect through inhibition of PVN oxytocin neurons activity. To investigate the influence of selective RXFP3 agonist RXFP#-A2, on the activity of magnocellular PVN neurons whole cell patch clamp recordings were performed on the rat brain slices. We have shown that selective RXFP3 agonist, reversibly inhibits electrical activity of magnocellular PVN neurons. Characterization of the recorded neurons was based on their electrophysiological properties and identification of their neurochemical content. Responsiveness of RLX3 neurons to stress factors and their impact on feeding behaviour allow us to hypothesize that this peptide is a bridge between chronic stress and overeating. Future patch clamp experiments with RLX3 antagonist, tract-tracing and behavioural studies will allow to further characterize the role of RLX3 in stress inducted overeating.

P3.6

5-HT1A RECEPTORS ARE INVOLVED IN THE DEVELOPMENT OF THETA OSCILLATIONS IN HIPPOCAMPAL FORMATION *IN VITRO*

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Hippocampal formation (HPC) is a limbic structure that generates a synchronized EEG activity, termed the theta rhythm. The theta rhythm is a sinusoidal activity with a frequency band ranging from 3 to 12 Hz. Many years of research conducted with the use of the model of HPC slice preparations allowed to determine the specific role of the cholinergic and GABAergic systems in the production of this EEG pattern. In addition, the literature data indicate that serotonergic input may be involved in the desynchronization of hippocampal theta. To verify this suggestion a micro-EEG recording were performed on HPC slices obtained from 20 male Wistar rats. All experiments were monitored by a Local Ethical Commission. Field potentials and extracellular recordings were made from the CA3c hippocampal field during the bath perfusion of a 5-HT1A receptor antagonist, (S)WAY-100135 in a range of concentrations in the following μM ratio: 1:3: 10:30 and 100. Preliminary results showed that besides epileptiform discharges, oscillatory activity in theta band was observed only in the slices perfused with 10 μM (S) WAY-100135. This demonstrates that the synchronization of neuronal networks needs the appropriate and precise level of excitation which can also be achieved by the manipulation of HPC 5-HT1A receptors activity. This study was supported by the NSC grant No.2011/01/N/NZ4/01722.

P3.7

ACETYLCHOLINE RECEPTORS UNDERLYING THE CHOLINERGIC MODULATION OF CORTICOTHALAMIC TRANSMISSION FROM LAYER 6 INPUT TO POSTEROMEDIAL THALAMIC NUCLEUS

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We have previously shown that cholinergic agonist carbachol modulates synaptic transmission of layer 6 corticothalamic input to posteromedial nucleus of the thalamus. In rat brain slices application of carbachol depresses synaptic responses to stimulation of corticothalamic fibers but enhances their frequency facilitation. Here we show that carbachol acts *via* activation of muscarinic receptors, because application of muscarinic antagonist (scopolamine or atropine) abolished both: depression of corticothalamic EPSPs and increase of frequency facilitation. In contrary, high concentration (100 μM) of specific nicotinic agonist DMPP (dimethylphenylpiperazinium) neither depressed corticothalamic responses nor enhanced the frequency facilitation. Surprisingly, low concentration of DMPP (10 μM) increased corticothalamic EPSPs, but did not change the frequency facilitation of corticothalamic transmission. This research and SN were supported by the European Union Regional Development Fund through the Foundation for Polish Science within the frames of International PhD Program in Neurobiology.

P3.8

AGING DOES NOT ALTER THE DISTRIBUTION AND THE DENSITIES OF PARVALBUMIN-, CALRETININ- AND CALBINDIN-POSITIVE NEURONS IN THE MOUSE SOMATOSENSORY CORTEX

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Gabaergic interneurons are involved in regulating developmental plasticity of visual, auditory and somatosensory cortices, as well as in learning and memory processes. It has been suggested that age related worsening of brain function and plasticity could be, at least in part, attributed to alterations in gabaergic system. Gabaergic interneurons constitute a heterogeneous cell population classified into groups based on their calcium binding protein content. In the present study, we evaluate with immunohistochemistry technique the effect that aging has on the three of them, namely Parvalbumin (PV)-, Calbindin (CB)- and Calretinin (CR)-expressing neurons, in the somatosensory cortex of young (3 month old) and middle-aged (1 year old) mice. We found that the distribution across layers differs between neuron types. PV-positive neurons were observed across the thickness of the cortex, with highest densities in layer IV. CB-positive neurons were much less numerous than the latter, and were observed in layers II/III and V/VI. Layer IV was practically devoid of CB-positive neurons. CR-positive neurons were scarcely distributed across all layers, with lower densities in layers V/VI. We did not observe the effect of age in neither of the neuron type examined. It suggested that age-dependent decline of somatosensory cortex plasticity does not depend on gabaergic neuron loss.

P3.9

MELANOPSIN MODULATION OF SLOW OSCILLATORY ACTIVITY IN THE SUBCORTICAL VISUAL SYSTEM

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Slow oscillatory activity (SOA) has been described in several structures of the subcortical visual system, including the olivary pretectal nucleus (OPN) and lateral geniculate nucleus (LGN). Since rhythmic spiking in the OPN is dependent on contralateral retinal output, we set out to investigate whether rod-cone and/or melanopsin photoreceptors are important for SOA generation in the OPN and LGN. Two different approaches were used: extracellular single-unit recordings in rats combined with intraviteral injections of photoreceptor blockers, and extracellular multi-electrode recordings from wild-type or genetically modified mice, lacking either melanopsin (Opn4-/-) or

rods and cones (rd/rd cl). The first of these methods indicated a major contribution of melanopsin to SOA generation, since this was abolished in the OPN after intravitreal 2-APB injection. This melanopsin contribution was confirmed by the second approach, with Opn4-/- mice having the lowest percentage of oscillatory cells and also the shortest average period in both structures. This is the first study to show SOA in the mouse visual system and to characterize its retinal origins, revealing melanopsin expressing retinal ganglion cells as the main driving force underlying this activity.

P3.10

DUAL OREXIN-A ACTION ON IONIC CURRENTS IN INTERGENICULATE LEAFLET NEURONS OF THE THALAMUS

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The intergeniculate leaflet (IGL) is an important component of mammalian biological clock, passing information to the suprachismatic nuclei (SCN) – master generator of biological rhythms. Its main function is to integrate photic with non-photic information, coming from retina and nonspecyfic brain systems, respectively. Arousal mediated by orexinergic system modulates various cellular mechanisms in different nuclei throughout whole brain. The aim of our experiments was to investigate the ionic mechanism underlying orexin-A (OXA) modulation of single IGL neurons. *In vitro* whole-cell patch clamp recording were performed on acute brain slices from 2–3 weeks old male Wistar rats. OXA (200 nM), tetrodotoxin (0.5 μM) and artificial cerebro-spinal fluid with changed ionic composition were applied by bath perfusion. Patch pipette were filled with intrapipete solution with 0.1% biocytin. Brain slices were stained with the use of anti-NPY antibodies and ExtrAvidinCy3, before the examination by confocal laser microscope. Our previous study showed depolarizing effect of OXA on IGL neurons. This patch clamp experiments revealed the major role of Na^+ currents in this activation, possibly *via* nonselective cation channels. Moreover, our investigation proved the effect of OXA on K^+ currents of single IGL neurons. Further research with the use of selective channel blockers is needed to evaluate the exact mechanism of OXA in IGL.

P3.11

CALCIUM INVOLVEMENT IN NUCLEUS INCERTUS ACTIVATION FOLLOWING ADMINISTRATION OF OREXIN A

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The nucleus incertus (NI) is a group of GABAergic neurons located in the midline tegmentum. It is the main source of the neuropeptide relaxin-3, which has been shown to be involved in appetite control, modulation of arousal and stress responses, as well as hippocampal theta rhythm. This is similar to the profile of orexins – neuropeptides expressed in the lateral and perifornical hypothalamus. Orexin neurons innervate numerous brain areas, including the brainstem, and activate g-protein-coupled receptors OXR1 and OXR2. This can lead to cell membrane depolarization through a number of possible mechanisms, including increases in intracellular calcium levels. We performed whole-cell patch clamp recordings from NI neurons in rat brain slices. To examine mechanisms of orexin receptor activation, recordings were made using TTX and calcium channel blockers: nickel chloride and nifedipine. We found the depolarizing effect of orexin A on NI neurons was reduced in the presence of calcium channel blockers. These findings help better understand the nature of interactions between the two peptide systems. Combined with further research, they could shed light on the possible involvement of the NI relaxin-3 system in the interplay between arousal, feeding and spatial memory.

P3.12

NORADRENERGIC, SEROTONINERGIC AND DOPAMINERGIC ACTIVITY IN THE RAT HIPPOCAMPAL SLICES DURING OREXIN-INDUCED THETA RHYTHM

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Orexins are two neuropeptides appearing to be particularly interesting in terms of hippocampal (HPC) EEG development. Recent studies conducted in our laboratory demonstrated that orexin A induce theta oscillations in HPC slices in certain concentrations. The lack of data concerning the influence of orexins on the changes in the activity of other neurotransmission systems encouraged our research. Thus the aim of the study was to investigate the changes in the activity of monoaminergic neurotransmitter systems in isolated HPC slices, which were used in EEG recordings. Experiments were performed on 50 slices obtained from 6 Wistar rats in accordance to Local Ethical Commission. Slices were subjected to neurochemical analysis immediately after the section, after the 45 min incubation in artificial cerebrospinal fluid (ACSF), and after incubation in ACSF with Orexin A for additional 45 min. Analysis of the concentration of monoamines and their metabolites was determined using high-performance liquid chromatography with electrochemical detection. To determine the changes in the activity of NE, 5-HT and DA systems, we calculated their utilization indexes, i.e. ratio of metabolite to its parent amine. Preliminary results suggest an increase in the particular neurotransmitter systems activity in hippocampal slices in that experimental conditions.

P3.13

PROJECTION FROM THE LOCUS COERULEUS TO THE CEREBELLAR CAUDAL VERMIS: FLUORESCENT TRACING STUDY IN THE RABBIT

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The locus coeruleus (LC) located in the pontine tegmentum, is the major noradrenergic nucleus of the brain. It gives rise to fibers innervating extensive areas within the neuraxis, among other the cerebral cortex, basal forebrain, limbic system, thalamus, brainstem autonomic nuclei, spinal cord. Throughout these projections, LC as a crucial wakefulness-promoting nucleus, is involved in neuronal circuits controlling a number of physiological functions, e.g., regulation of arousal and autonomic activity. A few data concerning the LC-cerebellar projections indicate, that LC sends same fibers to the cerebellar vermis, and small collateral projection to both the cerebral cortex and cerebellum exist as well. The aim of present study was to identify LC neurons projecting to the caudal vermal lobule, i.e. the pyramis. Following fluorescent tracer injection into the pyramis, retrogradely labelled neurons, as parent for the LC – pyramis projection, were found in defined regions of LC. The projection is bilateral with ipsilateral predominance (82% ipsi-*versus* 18% contralateral). The LC noradrenergic connections may modulate response of the pyramis neurons and exert influence on activity of the postural muscles of upper trunk and the proximal forelimb muscles.

P4. Neuroscience methods

P4.1

THE SOURCES OF LOCAL FIELD POTENTIALS RECORDED ALONG THALAMO-CORTICAL PART OF SOMATOSENSORY PATHWAY IN A RAT

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Local field potentials recording is a tool well suited for the chronic monitoring of neuronal activity. However, due to the widespread propagation of electric field within a brain tissue a signal recorded in one place may possess a substantial contribution of synaptic currents from distant neuronal populations. In the rat vibrissae-barrel system the cortical representation of mystacial vibrissae is located closely above their somatosensory relays in thalamic nuclei. Since the order and dynamics of thalamic EP waves resemble those of cortical ones, it is crucial to determine to what extent these signals are generated locally or whether they reflect the electrotonic component from cortical sources and *vice versa*. Using two linear multielectrodes (100 µm inter-electrode distances) we recorded series of potentials evoked by deflections of a group of vibrissae, thus obtaining EP profile spanning the tissue from the cortical surface to the level below somatosensory thalamic nuclei. Kernel Current-Source Density analysis revealed that the subcortical EPs recorded in the external capsule and fimbria of the hippocampus comprised mostly of responses of cortical characteristics while those recorded within the thalamus mainly possessed components characteristic to the local, thalamic sources. Research supported by the polish National Science Centre grant N N401 533040.

P4.2

INTRODUCING THE METHOD OF MULTIELECTRODE ARRAYS IN THE ELECTROPHYSIOLOGICAL STUDIES OF THE POSTERIOR HYPOTHALAMUS *IN VITRO*

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Theta rhythm is the best synchronized EEG activity recorded from the mammalian brain. The generation of theta in the limbic structures depends on activation of the ascending brainstem-hippocampal synchronizing pathway. One of the main structures of this pathway is the posterior hypothalamic area (PHa) which most probably modulates hippocampal theta frequency. Multielectrode Arrays (MEA) are a well-known tool in both *in vivo* and *in vitro* electrophysiology. Our new *in vitro* multi-recording setup is constructed using a glass multielectrode matrix consisting of 256 electrodes fitted on 10 square mm. Each electrode is 40 µm high, cone-shaped and represents a single field recording channel. During experiments, brain slices are constantly perfused with prewarmed and oxygenated cerebrospinal fluid which prevents the tissue from dying and allows direct administration of chemicals. Our previous *in vitro* experiments show that the PHa is capable of generating local theta rhythm in both *in vitro* and *in vivo* conditions. The aim of the current study was to introduce a new method of *in vitro* multisite recordings from posterior hypothalamic slices. Application of MEA recording method in the studies of PHa theta rhythm is discussed. Supported by NCN grant 2011/01/B/N24/00373.

P4.3

NANOPARTICLES MEDIATED DELIVERY OF TIMP-1 ACROSS BBB AND ITS NEUROPROTECTIVE EFFECTS

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There is an increase in expression of Matrix Metalloproteinase-9 (MMP-9) during numerous pathologic conditions, including excitotoxicity and its inhibition is considered as a potential therapeutic target. Tissue Inhibitor of Matrix Metalloproteinase-1 (TIMP-1) is endogenous inhibitor of MMP-9 that can play an important role in neuroprotection. Here, we show that TIMP-1 and TIMP-1 loaded PLGA nanoparticles (NPs) have neuroprotective effects against Kainic Acid (KA) induced excitotoxicity in organotypic hippocampal slice cultures. Moreover, TIMP-1/TIMP-1 NPs decreases LDH release and further supporting its neuroprotective effect. For blood brain barrier (BBB) penetration the NPs were coated with polysorbate 80 (Ps80). We used rat brain capillary endothelial cell culture to study their uptake/binding, toxicity and BBB penetration. BBB penetration studies showed that in the group treated with TIMP-1 NPs coated with Ps80, 11.21% ± 1.35% of TIMP-1 was detected in lower compartment of endothelial cells. To summarize, TIMP-1 loaded NPs showed neuroprotective effects *in vitro* and they have BBB penetration properties.

P4.4

DELINEATION OF BRAIN STRUCTURES IN THE *MONODELPHIS DOMESTICA* OPOSSUM BRAIN

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The *Monodelphis* opossum became an important laboratory animal and is often used in biomedical research. However, data on the brain anatomy are scarce and there is no reliable brain anatomy reference. The aim of this study is to present neuroanatomical delineation of basic brain structures. Data which served for construction of the 3-dimensional atlas were magnetic resonance images (MRI) and stained brain sections. MRI was obtained 48 h after perfusion of the animal with 4% paraformaldehyde and gadotheridol contrast (ProHance 20:1 v:v). The second MRI was performed 30 days after perfusion of the same animal. Both MRIs were aquired using Bruker Biospin system with voxel reolution of 50 μm^3. For Nissl and myelin staining, coronal brain sections were cut in cryostat at 40 μm thickness. To minimize tissue deformation, sections were transferred from the cutting blade to slides using the Tape-Transfer System. Then brain sections stained either with Nissl or for myelin were imaged with a high resolution scanner and were transformed to three-dimensional form. By superimposing all three-dimensional data, several brain structures were delineated, e.g., the olfactory bulb, cerebral cortex, hippocampus, white matter and other. Supported by grant from the Polish Ministry of Regional Development POIG.02.03.00-00-003/09.

P4.5

IMAGING METHOD OF NEURONAL NUCLEI IN THE MOUSE BRAIN

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Little is known about the relationship between neuronal gene expression and the architecture of the neuronal cell nucleus. We wanted to study the cell nuclei in neurons of the mouse cerebral cortex, using confocal microscopic immunocytochemistry. We used a segmentation algorithm, based on continuous boundary tracing, able to reconstruct the nucleus surface and to separate adjacent nuclei (Walczak et al. 2013). The algorithm did not use a rigid threshold what made it robust against variations in image intensity and poor contrast. However, when we analyzed mouse, but not rat, neuronal nuclei there have occurred a considerable problem with an appropriate segmentation. This problem was related to the presence of the discrete chromocenters, which are much more prominent in the mouse that in other species. Therefore, in order to assure the proper segmentation, we used sections co-immunostained for the lamin protein. Our refined program is an efficient segmentation tool for crowded and overlapping objects in 3D space, regardless of the particular species. It allows us to study quantitatively the architecture of the neuronal nucleus using confocal-microscopic approach.

P4.6

RETINAL ORIGIN OF ELECTRICALLY EVOKED POTENTIALS IN EYE-EYE AND EYE-NECK MONTAGES

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Noninvasive current stimulation is a rapidly developing tool for rehabilitation of visual impairment. The therapeutic use of current stimulation requires solving many technical problems including optimal placement of stimulating electrodes (SE). In this study we asked the question about origin of electrically evoked potential (EEP) and it's dependence on the placement of the SE. In acute

experiments on rats under urethane anaesthesia, visually (VEP) and electrically evoked potentials were recorded using single- and multi-channel electrodes from 5 visual structures: retina (1 channel), lateral geniculate nucleus (8 channels), superior colliculus (7 channels) and visual cortex of both hemispheres (16 channels each). Recordings of EEPs were performed to electrical pulse current stimulation, delivered using two electrodes placed either on one eye-ball (eye-eye montage) or on the eye-ball and neck (eye-neck montage). To reveal the origin of EEPs in both electrode montages 5 µl of tetrotodoxine (TTX 0.5 mM), was injected into the eye to block retinal ganglion cells' activity and EEPs were recorded for both SE configurations. Lack of VEPs confirmed the successful block of ganglion cells' activity. We have observed full decay of EEPs after TTX injection independent on the SE configurations. These results indicate on the retinal origin of EEPs regardless of the reference electrode placement. Supported by ERA-NET Neuron project REVIS.

P4.7

GLOBAL ANALYSIS OF DRUG-REGULATED ALTERNATIVE EXPRESSION OF GENES IN MOUSE BRAIN

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Genomic response of neuronal cell to external stimuli includes expression of specific transcript isoforms, that use alternative transcription start sites and polyadenylation signals. We applied microarray profiling (Illumina Mouse WG-6) and next-generation sequencing (SOLID 5500xl) to screen for drug-induced activity-dependent transcriptional events in the C57BL/6J mouse striatum. We compared effects on gene expression induced by psychoactive drugs with diverse neuropharmacological mechanisms of action (antidepressants, antipsychotics, anxiolytics, psychostimulants and opiods). Using whole-genome approach we identified a pool of transcripts that are regulated by the psychotropic drugs in mouse striatum (317 transcripts). We found that drug-responsive transcripts are organized into three main co-regulated gene expression networks. Furthermore, using Bowtie read aligner and Cufflinks algorithm we identified specific gene isoforms responsive to drug treatment. 58% among the drug-regulated transcripts were defined as alternative transcription events. To search for transcriptional factors that control alternative gene transcription in the brain we developed seqinterpreter web-based tool (http://seqinterpreter.cremag.org). We found SRF, NPAS4 and GR as candidate regulatory factors. The complex program of regulation in gene transcription may further impact long-lasting alterations in brain function.

P4.8

AGE-RELATED DIFFERENCES IN DURATION COMPARISON REVEALED BY MMN

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The study offers a new approach to investigate age-related changes in duration discrimination in millisecond time domain. Forty healthy subjects: young (aged: 20–29 years) and elderly (aged: 61–71 years) were studied using Mismatch Negativity (MMN) paradigm. White-noise bursts of two different durations (50 ms and 10 ms) were presented binaurally in 2 oddball blocks. In one block (increment condition, IC), the repetitive sequence of 10 ms standards was interspersed by occasional 50 ms deviants. The order was reversed in the second block (decrement condition, DC). MMN was elicited in two age groups. The amplitudes were significantly higher in young than in elderly participants for both conditions, but higher in IC than in DC. Moreover, the IC resulted in significantly shorter latencies of MMN peak than the DC for two groups. These results suggest that the MMN is a good indicator for detection of changes in stimulus duration in some tens of milliseconds which corresponds to results of previous psychophysical studies. However, some subject-related factors (e.g., age, gender), as well as procedure-related ones (e.g. stimulus presentation condition) have to be taken into account while designing a reliable measurement in the future timing studies. Supported by the grant: INNOTECH-K1/IN1/30/159041/NCBR/12.

P4.9

HIGHLY REPLICABLE, FULLY AUTOMATED MEASURES OF PERSEVERATIVE BEHAVIORS IN INTELLICAGE SYSTEM

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Perseveration, defined as resistance to change in routine and repetitive behaviors, is one of the core symptoms of Autism Spectrum Disorders. It was proposed that an inability to break habits, experienced by autistic people, corresponds, in animal models, to impaired performance in the learning tasks that assess ability to change a response strategy to obtain reinforcement. However, the results of conventional behavioral tests can be confounded by anxiety related to handling and social isolation. In order to avoid such effects and to analyze phenotypes of subjects in an efficient manner, we developed a battery of automated tests aimed at appraising behavioral flexibility in mice. The tests were performed in the IntelliCage (IC), a computer-controlled system, which can be used

for long-term monitoring of group-housed animals. These tests allow for measuring of exploration patterns, pace and progress of appetitive and reversal learning. To standardize and evaluate the relevant IC tests, we compared valproate treated and control animals from two inbred strains of mice, C57BL/6 and BALB/c. We show that tested mice differ significantly in most of the examined parameters. The obtained results are highly replicable between tested cohorts of subjects, thereby allowing us to infer, that the reported battery of automated behavioral and cognitive tests is a valuable tool in verifying suitability of mouse models of ASD symptoms.

P4.10

DIFFERENCES IN MISMATCH NEGATIVITY (MMN) RESPONSE TO PURE-TONE AND SPEECH SOUNDS IN NORMAL SUBJECTS: AN ADDITIONAL EXPLANATION

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The relation of automatic auditory discrimination, measured with MMN, with the type of stimuli has not been well established in the literature, despite its importance as an electrophysiological measure of central sound representation. In this study, MMN response was elicited by pure-tone and speech binaurally passive auditory oddball paradigm in a group of 8 normal young adult subjects at the same intensity level (75 dB SPL). The frequency difference in pure-tone oddball was 100 Hz (standard = 1 000 Hz; deviant = 1 100 Hz; same duration = 100 ms), in speech oddball (standard /ba/; deviant /pa/; same duration = 175 ms) the Portuguese phonemes are both plosive bi-labial in order to maintain a narrow frequency band. Differences were found across electrode location between speech and pure-tone stimuli. Larger MMN amplitude, duration and higher latency to speech were verified compared to pure-tone in Cz and Fz as well as significance differences in latency and amplitude between mastoids. Results suggest that speech may be processed differently than non-speech; also it may occur in a later stage due to overlapping processes since more neural resources are required to speech processing.

P4.11

SIMULATIONS OF LOCAL FIELD POTENTIALS AND CURRENT SOURCE DENSITY ANALYSIS IN SLICES WITH REALISTIC CONDUCTIVITY DISTRIBUTION

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To test methods of local field potential (LFP) analysis we need realistic ground truth data which demands plausible models of neural activity and of physical properties of the setup, tissue, and the electrodes. To interpret the recordings we often reconstruct the Current Source Density (CSD) from the LFP. In this work we study the effect of realistic conductivity profiles and the slice geometry on (1) computation of LFP generated by cell populations embedded in slice, as would be measured on multi-electrode array (MEA), and (2) current source density (CSD) reconstruction in the slice from such potentials. We show that the method of images approximates solution through finite elements well while being much more efficient computationally. Inclusion of slice properties with homogeneous and uniform conductivity in the slice noticeably modifies the observed activity (LFP) but inhomogeneity and anisotropy do not further change the profile and amplitude of the LFP. Supported with grants: IP2011 030971, N N303 542839, FP7-PEOPLE-2010-ITN 264872, POIG.02.03.00-00-018/08, POIG.02.03.00-00-003/09.

P5. Cognition and behavior

P5.1

THE ROLE OF INTERLEUKIN 6 IN BEHAVIOR OF AGED MICE

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Interleukin 6 (IL-6) is a cytokine playing an important pleiotropic role in the immune system. IL-6 is also involved in stress response, etiology of the age-related diseases and plays a role of mediator between the central nervous system and the immune system. To study effects of IL-6 on behavior during aging we examined aged (13 to 15 months) IL-6 deficient and wild type (WT) mice. Behavior was tested using the open field test, elevated plus maze test and registration of spontaneous activity in the individual home cages for 72 hours. These registrations showed that IL-6 deficient animals were less active than WT mice. The difference was more distinct during the dark phase. Interestingly, in the open field IL-6 deficient mice displayed higher locomotor activity than control WT mice and spent more time in the central part of the arena. In the elevated plus maze IL-6 deficient mice spent more time exploring open arms than WT mice. We conclude that IL-6 deficient aging animals show lower level of anxiety than WT control animals. After tests mice were perfused and brains were cut into 40 μm sections. Brain sections were immunohistochemically labeled for IL-6 and its receptor (IL-6R), also known as CD126. We found that cells immunopositive for both IL-6 and CD126 were present in the hippocampus and other brain structures. Supported by the National Science Center grant No 1577.

P5.2

PRECONTACT 50-kHz ULTRASONIC VOCALIZATIONS AND SEXUAL PERFORMANCE IN WAG/Rij AND SPRAQUE-DAWLEY MALE RATS: EFFECT OF NALTREXONE

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As we found previously, ultrasonic vocalizations in the 50-kHz band emitted before a female is introduced into a copulatory chamber (precontact vocalizations – PVs) is useful parameter describing the rewarding value of socio-sexual contact in male rats. In the present experiments, we have investigated the influence of opioid receptors on PVs in sexually experienced rats. The behavioral effects of an i.p. injection of the opioid receptors antagonist – naltrexone were analyzed in six month old sexually experienced Wag/Rij (rats with genetic absence epilepsy) and Sprague-Dawley male rats. We found higher number of PVs in WAG/Rij compared to Sprague-Dawley males. Naltrexone (3 mg/kg) significantly diminished number PVs. Spectral analysis of PVs ultrasonic vocalizations did not detect significant changes in frequency of calls after physiological saline and naltrexone treatment. Opioid receptors are involved in PVs probably *via* changes in rewarding value of socio-sexual contacts. Our results have shown that rewarding system of Wag/Rij rats with absent epilepsy is sensitive to socio-sexual reward and seems to be even more sensitive that in Sprague-Dawley males.

P5.3

CORTICAL ASYMMETRIES AND THE LATERALIZATION OF GESTURES AND LANGUAGE IN LEFT-HANDERS

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It has long been proposed that structural cortical asymmetries may underlie functional lateralization of the human brain. Given that interhemispheric differences in language processing are one of the most pronounced, most studies investigated whether this functional asymmetry has a structural correlate. Recently, it has been demonstrated that it is the insular cortex asymmetry (not the Broca's area or planum temporale) that is linked to the lateralization of verbal fluency in right-handers. Whether or not this effect can be seen in left-handers is unknown. Moreover, language and gesture representations are co-lateralized. Therefore, if common cortical asymmetries underlie both of these functions then the structure of the insular cortex could also determine them both. Finally, given that gestures are supported by a distributed praxis representation network involving parieto-frontal pathways, other asymmetries may also contribute to the lateralization of this function. Here, we demonstrate that despite the existence of significant asymmetries observed in the superior parietal lobes, only the inter-hemispheric differences in the insular cortex are related to gesture lateralization. Moreover, this study shows that even in left-handed individuals language representation is reflected in insular asymmetry. In sum, the structure of the insula may be paramount to cerebral specialization for both gestures and language.

P5.4

NEW METHOD OF INDUCING EXPERIMENTAL STRESS

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Existing procedures of inducing experimental stress in fMRI experiments are usually unrelated to the cognitive task which is the object of the study. Our experiment concerns the impact of experimental stress on well learned tactile discrimination task. Participants attended two weeks Braille training. The object of this training is not to learn people how to read braille, but to learn tactile discrimination of meaningful signs. Discrimination of two Braille signs is a simple task after the training. We tested the effect of experimental induced stress on this model of learning. For this purpose, we have developed a procedure for inducing experimental stress. This procedure consists of three parts by: two runs of tactile discrimination are divided by stress inducing procedure. In the stress inducing procedure participants are asked to discriminate between symmetrical and non-symmetrical signs and are exposed to negative feedback. Experimental stress was evaluated by a questionnaire and GSR, EMG and heart rate data were acquired during whole procedure. Results showed inducement of experimental stress reported by participants in a questionnaire, higher GSR values during experimental stress procedure and no impact of experimental stress on the performance in the second tactile discrimination task. The project was supported by The National Science Centre, grant number: 3608/B/H03/2011/40.

P5.5

SEX DIFFERENCES IN ANXIETY AND SOCIAL BEHAVIOR IN TEMPERAMENTALLY DIFFERENT RATS

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The experiment was designed to examine whether the relationship between anxiety level and social behavior depends on sex in animal model of individual differences (Roman RHA/RLA rats). Hyperemotional RLA and hypoemotional RHA males and females were subjected to a set of nonsocial (OF, EPM) and social tests. Females were tested during selected phase of ovary cycle – estrus or diestrus. Social settings ranged from low aversive – Social Affiliation/Recognition Test (SART / three chamber test) through mildly stressful social interactions (SI) to highly stressful Resident-Intruder paradigm (RI – males only). Between-line differences in emotional reactivity in nonsocial situations were observed both in males and females. The character of social interactions differs between RLA and RHA and between males and females. In SART RHA males explored more than RLA, without any preference toward the social stimulus. RLA males preferred to stay in the compartment containing unknown rat, although during SI and RI they avoided contacts. Females in SART exhibited no between lines differences and displayed significant preference toward social stimulus while during SI test differences between lines were observed. In all tests the influence of ovary cycle was insignificant. Our results suggest that the relation between anxiety level and social behavior is not linear and is gender specific.

P5.6

WIN 55,212-2 AND EXENDIN-4 ADMINISTERED TOGETHER REVERSE FOOD PREFERENCES IN RATS MAINTAINED ON A FREE-CHOICE, HIGH-SUGAR DIET

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Both the endocannabinoids and glucagon-like peptide-1 (GLP-1) are known to control intake of highly palatable food. We have investigated whether WIN 55,212-2 (a cannabinoid receptor 1 agonist) and exendin-4 (Ex-4, a GLP-1 agonist) may interact to change feeding behavior in rats maintained on a free-choice, high-sucrose diet. The rats were presented with both their regular and high-sucrose chow throughout the experiment. After 4 days, they were injected once daily for 3 days with either 1 mg/kg WIN 55,212-2, 3 μg/kg Ex-4 or both. Ex-4 and, unexpectedly, WIN 55,212-2 injected separately diminished the mean daily caloric intake. When both drugs were administered together, the daily caloric intake was further reduced, the consumption of high-sugar chow was almost completely inhibited but the intake of standard diet was increased and was significantly higher than that in either Ex-4-, WIN 55-212-2- or saline-injected rats. These changes were associated with a marked reduction in body weight in both WIN-55,212-2- and Ex-4+WIN-55,212-2-treated animals wherein the difference between these groups was not significant. In conclusion, subchronic treatment with WIN 55-212-2 resulted in the reduced total caloric intake and Ex-4 enhanced this effect. Moreover, combined administration of WIN 55-212-2 and Ex-4 reversed food preferences (i.e., decreased sweet chow and increased standard chow consumption).

P5.7

INFLUENCE OF CHRONIC FLUOXETINE TREATMENT ON APPETITIVE LEARNING IN MATRIX METALLOPROTEINASE-9 KNOCK-OUT MICE

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Matrix metalloproteinase-9 (MMP-9) is an extracellular endopeptidase which cleaves extracellular matrix proteins and plays a significant role in synaptic plasticity, learning and memory. Impairment of MMP-9 knock-out mice in appetitively motivated learning has been previously shown. In the present project we investigated whether chronic treatment with fluoxetine, antidepressant drug, which stimulates synaptic plasticity, would affect appetitive learning of MMP-9 knock-out mice. To this end, MMP-9 knock-out and wild type mice were treated with fluoxetine or vehicle for 35 days, and trained in sucrose-water discrimination task in the IntelliCage system. The IntelliCage system allows for long-term monitoring of the behavior of group-housed animals. For five days the mice had to discriminate between bottles (placed on two sides of the same corner of the cage) that contained either sweetened or plain water. The results suggest that chronic fluoxetine treatment improves appetitive learning of MMP-9 knock-out mice.

P5.8

COMPARATIVE ANALYSIS OF BRAIN CYTOARCHITECTURE OF LABROID FISHES WITH DIFFERENT DEGREE OF COGNITIVE ABILITIES

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Labroides dimidiatus (Labridae) is a model fish in research concerning cleaner fish mutualism. Recent studies shows that cleaners possess the ability to establish complicated relationships (Bshary and Würth 2001). This well-developed network is one of the most complex interspecies communication systems known in fishes and require complex cognitive abilities (Marshall et al. 2003). It makes the cleaners particularly interesting object of comparative analysis of morphology and cytoarchitecture of brain and brain centers in telencephalon, optic tectum and cerebellum, parts of the brain potentially involved in cognitive functions related to behaviors in complex social system (Demski and Beaver 2001). Similar conclu-

sion were drawn in primates, Carniforous and bats, where strong correlations between development of neocortex and size of social group (as measurement of the complexity of social behavior) were found (Dunbar 1992, Joffe and Dunbar 1997, Bshary and Würth 2001). According to the basic theoretical assumption, centers located in the telencephalon, optic tectum and cerebellum involved in skills associated with complex social behavior, learning and memory, differ qualitatively and quantitatively between cleaners and species with less complicated behavior.

P5.9

BEHAVIORAL CHARACTERIZATION OF CALCIUM BINDING PROTEINS (CaBPs) KNOCKOUTS WITH SPECIAL EMPHASIS ON AUTISTIC-LIKE TRAITS

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Calbindin D-28k (CB) and parvalbumin (PV) are cytosolic calcium-binding proteins expressed in many neurons without general preference for functionally and morphologically defined subpopulations. Deletion of CB and/or PV alters intracellular calcium signaling, and physiological properties of affected neurons. General knockouts for both proteins display a distinct and permanent motor impairment which is revealed only when adaptation of movement to novel environmental conditions is required. In order to determine whether the absence of CB and PV influences locomotor properties and behavior we compared mouse lacking CB, PV, or both with wild type controls. Animals from all groups were tested by means of an automated setup TruScan (USA) and following parameters were analyzed in an open task: locomotors activity, total number of movements, total movement time, total rest time, ambulatory distance traveled, total movement distance, time spent in defined part of arena, jumps, number of entries into the vertical plane, number of stereotypic movements, total time of stereotypic behavior and dark/light preference. Obtained results showed no obvious differences among tested groups of animals, however in future study more specific, autistic-like behaviors oriented tests will be applied.

P5.10

ULTRASONIC VOCALIZATION (USV) IN CALBINDIN D-28k MUTANT MICE

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Mice similarly to some other rodent species communicate with specialized sounds in the ultrasonic range called ultrasonic vocalizations (USV). Evaluation of this behavioral activity enables estimation of the social interactions in animal models of diseases involving psychiatric manifestations related to the social environment like autistic spectrum disorders (ASD). Because of the growing evidence for involvement of cerebellum in ethiology of ASD, we decided to change physiological properties of Purkinje cells and look for signs of alterations in USV activity of newborns. In our experiments we switched off expression of the major protein calcium buffer (calbindin D-28k) in these neurons by means of the Cre/loxP technology. It is known from previous reports (1), that the lack of calbindin D-28k in cerebellar cortex results in motor coordination deficits due to disturbed processing of calcium signaling in Purkinje cells. Because coordination of the vocal apparatus depends on the cerebellar input we were curious if altered Purkinje cells function results in altered USV calling.

P5.11

THE EFFECT OF NUMBER MAGNITUDE ON VOLUME OF ACTIVATION IN THE BRAIN – AN fMRI STUDY ON THE SPATIAL-NUMERICAL ASSOCIATION

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Brain representations of numbers are spatially represented on so-called mental number line. We investigated the activation patterns related to the number magnitude and its spatial position during fMRI scanning. Each trial started with a fixation point replaced by a cue stimulus – one of four digits (1, 2, 8 or 9) or non-digit symbols (#, %, & or §). The target stimulus consisted of a pair of such digits and/or symbols. Participants responded by indicating the position (left/right) of the cue digit/symbol within the target, which could be either congruent (e.g. 8 on the right) or incongruent (e.g. 9 on the left). The behavioural results showed faster reactions to low than high number magnitude and fMRI results revealed the asymmetry patterns dependent of the digit magnitude, irrespectively of stimuli congruency. In trials with low digit magnitude the right hemisphere as well as left cerebellum preponderance was observed, while in trials with high digit magnitude the opposite pattern was present. However, the analysis of the activation volume calculated in each condition and hemisphere, showed the main effect of digit magnitude, but no significance for congruency and hemisphere. These results suggest that number magnitude processing affects the reaction time and volume of brain activation.

P5.12

REPEATED RESTRAINT STRESS ALTERS GLUTAMATERGIC BUT NOT GABAERGIC TRANSMISSION WITHIN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS

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The hypothalamic paraventricular nucleus (PVN) plays a key role in the activation of the hypothalamic-pituitary-adrenal axis (HPA) in response to stressors. Wistar rats were subjected to restraint lasting 10 min and repeated twice daily for 3 days. Brain slices were prepared 24 h after the last restraint session and studied *ex vivo*. Whole-cell patch-clamp method was used to record spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs) from presumed parvocellular neurosecretory neurons in slices containing a part of the PVN. Repeated restraint stress resulted in an increase in the mean frequency of sEPSCs and in a decrease in the rise time and the decay time constant of sEPSCs. There were no changes in the mean amplitude of sEPSC. All measured parameters of sIPSCs remained unaltered. The relationship between the injected current and the spiking rate of parvocellular neurons was reduced. These data indicate that restraint stress, repeated for 3 days, selectively enhances the excitatory inputs to parvocellular neurons of the PVN, most likely *via* a combination of pre- and postsynaptic mechanisms. These changes are accompanied by a decrease in the intrinsic excitability of PVN neurons. Support: "DeMeTer" and statutory funds from the Institute of Pharmacology.

P5.13

BLOCKING MATRIX METALLOPROTEINASE-9 ACTIVITY IN THE CENTRAL AMYGDALA DECREASES c-Fos PROTEIN EXPRESSION FOLLOWING APPETITIVELY MOTIVATED TRAINING

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In addition to being widely investigated as a marker of neuronal activity, expression of c-Fos has also been shown to be closely linked with synaptic plasticity, learning and memory. Understanding c-Fos-dependent molecular underpinnings of the synaptic plasticity may be achieved by following its transcription-regulatory function, i.e. by identifying the genes it controls. MMP-9 (matrix metalloproteinase-9), an extracellular endopeptidase which cleaves extracellular matrix proteins and plays an important role in synaptic plasticity, learning and memory, have been documented to be c-Fos/AP-1 regulated at the transcriptional level, also in the activated neurons. We hypothesized that following the extracellular release of MMP-9 supply, c-Fos upregulation is necessary for MMP-9 replenishment. To test this hypothesis we injected PLGA nanoparticles releasing TIMP-1 (tissue inhibitor of matrix metalloproteinases-1, a specific inhibitor of MMP-9) to the central amygdala of mice. Then, the animals learned the appetitively motivated behavioral task in the IntelliCage system, which had been previously shown to specifically increase c-Fos expression in the central amygdala. We showed that blocking MMP-9 results in significantly decreased expression of c-Fos protein. This result is consistent with the hypothesis of the role of c-Fos in MMP-9 replenishment.

P5.14

TRANSCRIPTOME ANALYSIS OF FRONTAL CORTEX, HIPPOCAMPUS AND NUCLEUS ACCUMBENS IN ALCOHOL-PREFERRING AND NONPREFERRING RATS

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Alcoholism is a complex disease with hereditary influence. To elucidate genetic contribution, microarrays were used to probe for differences in gene expression in limbic system structures in strains of rats selected for several generations for alcohol preference: Warsaw High Preferring (WHP) strain and Warsaw Low Preferring (WLP) strain. Microarray analyses of medial prefrontal cortex (mPFC), hippocampus (HP) and nucleus accumbens (NAc) gene expression patterns revealed 237, 416 and 756 differentially expressed genes (DEGs) between the strains (FC>1.5; adj P<0.05). While the NAc showed a substantially larger number of DEGs, there was a considerable overlap in expression profiles between the studied brain areas: 104 common transcripts changed in the same direction in the mPFC, HP, and NAc. Several functional groups, including genes involved in the action of corticosteroids, prostaglandins, glutamate, or GABA activity were found to be significantly overrepresented and may play an important role in establishing a high level of voluntary alcohol drinking in our model. The results suggest candidate genes for alcohol preference quantitative trait loci (QTL) identification.

P5.15

HEMISPHERIC ASYMMETRIES FOR VISUAL PROCESSING OF TOOLS IN LEFT-HANDERS

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In typical right-handers, the processing of tool-related information is lateralized to the left hemisphere. Yet, the hemispheric dominance for tools in left-handers is still debated. Since visual half-field (VHF) paradigms provide a reliable measure of cerebral asymmetries, left vs. right hemisphere advantage for man-made object categorization was studied in 17 left-handers (9 women, mean age = 23 years) using a VHF test. The task was to decide whether one of the two bilaterally presented line drawings depicted a tool or non-tool. Given a higher incidence of atypical organization of functions in sinistrals, participants were divided into 2 groups, showing either right or left visual field advantage irrespective of the target objects. Nonetheless, significant effects of visual half-field were found exclusively for tool discrimination. Namely, subjects with the putative typical organization of functions (n=9) showed significantly faster response times for tools correctly categorized in the right visual field (i.e. processed in the left hemisphere) whereas those with atypically organized functions (n=8) responded faster to tools processed in the left visual field (right hemisphere). None of the groups showed any dominance for non-tools. The results indicate that even a simple visual processing of tools can vary significantly across left-handers. It remains to be seen if the observed patterns are linked to language dominance.

P5.16.

SEX DIFFERENCES IN FEAR EXTINCTION AND RENEWAL

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Posttraumatic stress disorder (PTSD) develops following exposure to a traumatic event, afflicting 7–12% of the population. Women are shown to be twice as likely as men to develop PTSD. Moreover, their susceptibility to PTSD following a trauma depending on the phase of menstrual cycle. Clarification of the biological mechanism underlying sex differences in the susceptibility to PTSD is necessary to design sex-specific therapies. We addressed this issue using an animal model of extinction and renewal of conditioned fear. We investigated the impact of estrus cycle phase on fear memory acquisition, extinction and recall. We hypothesized that hormonal status would influence memory formation and recall at all stages of the behavioral procedure. Therefore we employed a matrix design, carrying out fear conditioning, extinction and fear/extinction memory recall in either estrus or metaestrus. Males and gonadectomized have been trained at corresponding time intervals. While estrus cycle phase during fear conditioning did not affect the retrieval of fear memory, hormonal status during both extinction and following fear/extinction memory recall affected animals' freezing rates. Highest differences have been found in animals that have been tested in metaestrus and extinguished in estrus or metaestrus. Collectively, we claim that it is necessary to control the hormonal status of female animals used in experiments involving fear conditioning, extinction and renewal.

P5.17

STIMULATION OF THE BED NUCLEUS OF THE STRIA TERMINALIS AND THE MEDIAL SEPTAL NUCLEUS INFLUENCES NATURAL KILLER CELL CYTOTOXICITY OF RATS DIFFERING IN RESPONSIVENESS TO NOVELTY

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In our previous study we found that electrolytic lesion of the bed nucleus of the stria terminalis (BST) as well as the medial septal nucleus (MS) caused depression of the peripheral blood natural killer cell cytotoxicity (NKCC) in rats. In the present study we evaluated blood NKCC after 14 day electrical stimulation of the BST and the MS in conscious, freely behaving rats differing in responsiveness to novelty. Male Wistar rats divided into high (HR) and low (LR) responders to novelty, implanted with stimulating electrodes at the BST or at the MS, were subjected to 14 day electrical stimulation (constant current 0.1 ms duration cathodal pulses delivered at a frequency of 50 Hz during 30 min) of the BST and the MS. The chronic stimulation of the BST and the MS caused augmentation of blood NKCC in comparison to the sham operated group and to the baseline, which was more significant in HRs. A week after termination of the stimulation procedure NKCC returned to the baseline. The obtained results suggest that immunoenhancing effect on blood NK cell function is dependent on the behavioral outcome (intensive locomotor reaction) of the BST and the MS stimulation as well as on individual behavioral characteristics. This work was supported by a research grant NN303819040.

P5.18

FUNCTIONAL REORGANIZATION OF THE MESOLIMBIC SYSTEM AFTER ITS TERMINAL AREA DESTRUCTION INFLUENCES BLOOD LEUKOCYTES AND THEIR SUBSETS

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Contralateral nucleus accumbens shell (AcbS) lesions (Contra group) impaired (by about 20%) and ipsilateral AcbS lesions (Ipsi group) facilitated (by about 30%) motivational aspects of ventral tegmental area (VTA) stimulation-induced feeding or exploration which manifested as respective alterations in latency to reaction. Present work was aimed to examine how this motivational reorganization of AcbS-VTA circuitry affect on blood leukocytes and their subsets (morphological method). As compared to the respective sham animals, the chronic VTA stimulation and unilateral lesion of the AcbS caused a significant decrease in total leukocyte and lymphocyte numbers in Ipsi and Contra groups. Both groups showed also significant decreases in total leukocyte and lymphocyte numbers on the 2nd day after unilateral lesion of the AcbS. On the 14th VTA stimulation day following unilateral lesion of the AcbS total leukocyte and a large granular lymphocyte (LGL) number was higher in Ipsi group in relation to Contra group and in comparison with the respective sham group. Increased motivational drive associated with facilitation reactivity of the ipsilateral VTA to lesioning AcbS enhance total leukocyte number, especially LGL cells that are critical to the innate immune system.

P5.19

COGNITIVE CONTROL MODIFIED ON-THE-FLY IN THE ERIKSEN TASK

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In perceptual-motor tasks, the influence of flankers can be modified. Their influence can increase when they are more often congruent with the target and decrease when they are more often incongruent. The black horizontal string of shapes was presented on a white background. The string contained: two flanker arrows in the middle, the target arrow on the left (or right) and a square on the right (or left). In one half of trials the target was more often congruent (arrow pointing in the same direction as flankers) when it was presented on the left, in the second it was more often congruent when it appeared on the right. Our results showed that the reaction time for incongruent trials was shorter when the target was presented on the side with predominance of incongruent targets than for incongruent trials with target presentation on the side with predominance of congruent targets. Reactions were faster for congruent than for incongruent trials in both cases of predominance. Related results were observed in the modulation of N2PC amplitude. The influence of flankers can be modified by changing the proportion of congruent targets in the selected location.

P5.20

THE IMPACT OF MATRIX METALLOPROTEINASE 9 ON ALCOHOL-ADDICTION RELATED MOUSE BEHAVIOUR

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Alcohol addiction is a chronic, psychiatric disease defined by compulsive alcohol drinking and seeking. Long-term alcohol intake induces aberrant synaptic plasticity in the amygdala and striatum as well as enhanced c-Fos expression in the central nucleus of amygdala (CeAmy). Interestingly, human studies have implicated matrix metalloproteinase 9 (MMP-9), whose gene is regulated by c-Fos, in alcohol addiction. Notably, recently critical role of MMP-9 in reward-driven learning as well as synaptic plasticity has been revealed. In the present study we aimed at elucidating a role of MMP-9 in alcohol addiction in mice. First, we analyzed the effects of MMP-9 levels on the dendritic spine morphology in the CeAmy of C57BL/6 wild type (WT) and mice lacking MMP-9 (MMP-9 KO). Next, to verify the role of MMP-9 in alcohol addiction we subjected WT, MMP-9 KO and heterozygous mice to behavioral tests in the Intellicage system, previously shown to be suitable to investigated addictive behaviors. Dendritic spine analysis of the CeAmy revealed that MMP-9 KO mice have longer and thinner dendritic spines than WT mice. Preliminary data analysis from the Intellicages showed there were no differences between MMP-9-KO and WT mice during first hour and first day activity, as well as in neophobia. Currently we are investigating the pattern of development of the alcohol addiction, to evaluate the role of MMP-9 in aversive aspects of this behavior.

P5.21

COPING BEHAVIOR DURING THE DRIVING LICENSE COURSE PREPARING TO THE STATE EXAM DOES NOT CORRELATE WITH THE SALIVA CORTISOL AND BLOOD PRESSURE DURING THE COURSE

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We assesed the effectiveness of coping with stress during a 30 hours driving course by people with different levels of stress. Volunteers aged 18–30 took participated: 8 women and 7 men in the control group, and 9 women and 9 men preparing for the exam. Before the start of the course all participants took the Coping Inventory for Stressful Situations (CISS). Saliva samples to measure cortisol were collected from each of the participants: before the start of the course, before the 1st, 13th and 28th driving hour. Blood pressure was measured: before the course, during the 15th hour and the 30th hour of the course. Par-

ticipants in the control group had one saliva sample taken and their blood pressure was measured once. The results suggest that the hour of the course is related to the level of the cortisol – the highest level is achieved before the first hour of the course and then it decreases in time (ANOVA: $F_{3,64}$=14.9, P<0.001). Systolic pressure is related to the hour of the course ($F_{2,48}$=11.3, P<0.001) and it reaches its peak before the state exam. There is a similar relation in case of diastolic pressure ($F_{2,51}$=6.4, P=0.003). CISS test has shown that there are differences in copying with stress (between sexes as well as age-related).

P5.22

ALCOHOL DRINKING UNDER INTERMITTENT ACCESS IN GROUP-HOUSED C57BL/6J MICE

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Models of alcohol drinking in rodents are useful in determining factors underlying uncontrolled alcohol abuse. Here we describe a new model, which overcomes a limitation of previous approaches, the necessity of studying animals in isolation. Over the course of 4–5 weeks group-housed mice intermittently received free or instrumental access to 12% alcohol in the IntelliCage system. Animals developed stable alcohol preference which was similar across 6 cohorts tested (46.6 ± 12.8%). Compared to behaviors of single-housed mice, we found no escalation of drinking over time, and no difference in alcohol preference between males and females, but comparable levels of alcohol preference to those previously reported. Motivation to obtain alcohol or saccharin measured under a progressive ratio schedule was initially similar, but the breakpoint decreased over consecutive sessions in case of alcohol (from 19.8 to 15.9). Conversely, addition of 0.03% quinine had a smaller impact on alcohol than saccharine intake (72.2% vs. 97.2% decrease). We observed that sequences of animal entries to alcohol-containing corners diverged from random distribution. Thus, this model applied to group-housed mice induces stable levels of alcohol drinking and allows to measure its motivational aspects as well as explore relations between social structure and drinking behavior.

P5.23

OLD RHYTHM, NEW METHODOLOGY: DIFFERENTIAL TEMPORAL ORGANIZATION OF SLEEP-WAKE STATES IN THE LIGHT AND DARK PHASE

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Sleep-wake cycle, a dynamic process of alternating states of vigilance, is usually described by means of quantitative methods. In our EEG study performed on WAG/Rij rats, a validated, genetic animal model of absence epilepsy, correlation and cross-correlation functions over time were applied on 24 h data collected in 12:12 light-dark cycle in order to investigate temporal coupling between absence seizures and sleep-wake states, and between sleep-wake states themselves. We found significant light/dark-related differences in temporal organization: first, absence seizures showed bidirectional coupling with sleep-wake states in the dark phase only. Second, temporal relationships among states of vigilance followed phase-related alterations, which were the most prominent at the light onset. These results were confirmed in a non-epileptic control strain. Our approach suggests that different processes are governing sleep and wake in the light and dark period.

P5.24

DYNAMIC CHANGES IN MOUSE HIPPOCAMPAL GENE EXPRESSION DURING CHRONIC STRESS

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Transcriptome profiling of chronic social stress (CSS) effects on the brain revealed changes in expression of many genes responsible for alterations of structure and function of the central nervous system. However, there is still little information on dynamics of gene expression in the brain during chronic stress. The aim of the study was to investigate the time course of mouse hippocampal transcriptome response in CSS. Gene expression was assessed using Agilent microarrays. Number of genes showed specific expression patterns between CSS and control animals during subsequent time points, including genes involved in glutamate transport (Slc17a6), amyloid sequestration (Ttr) and neuroprotection (Igf2, Igfbp2). Study revealed dynamics of gene expression patterns and novel molecular effectors of behavioral effects of stress.

P5.25

LOCALIZATION OF RECENT AND REMOTE MEMORY TRACES FOR SUCCESSFUL AND IMPAIRED FEAR EXTINCTION

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Extinction of conditioned fear leads to formation of a new memory trace. There are, however, factors altering behaviors associated with such a memory trace, such as CS presentation outside the extinction context (promoting fear renewal) and re-emerging of fear with the passage of time after extinction (spontaneous recovery). The neuronal basis of these phenomena is poorly understood. The involvement of hippocampal-prefrontal cortical circuits was investigated only during initial processing of fear extinction memory. As has been shown before for fear conditioning, the mechanisms underlying matured memory may differ from those of recent memory. In our study we used c-Fos immunohistochemistry to generate a functional map of the neural circuits involved in contextual retrieval of recent and remote memories of extinguished fear. Presentation of the CS in the extinction context 24 h after extinction yielded low freezing and induced strong activation of infralimbic cortex (IL) and ventral hippocampus (vHIPP). Similar presentation after 28 days resulted in high freezing and much lower activity of IL and vHIPP. In contrast, presentation of the CS outside the extinction context after either 24 h or 28 days yielded high freezing and induced strong activation of prelimbic cortex. These results suggest remodelling of the fear extinction memory trace over time, as well as dissociable neuronal mechanisms underlying fear renewal and spontaneous recovery.

P5.26

NALTREXONE REDUCES ETHANOL CONSUMPTION AND NORMALIZES GHRELIN PERIPHERAL BLOOD LEVELS IN ALCOHOL DEPENDENT RATS

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Naltrexone is an opioid receptor antagonist used in the management of alcohol dependence. There is a hypothesis that ghrelin, hunger-stimulating peptide, could take part in the central effects of alcohol and also act as a peripheral marker of long-term ethanol consumption. The aim of this study was to assess the effect of naltrexone on ghrelin plasma level in the model of alcoholism. The study was performed using male Wistar alcohol preferring (PR) and nonpreferring (NP) rats. Naltrexone (0.1 mg/kg, i.p.), was administered to rats for 28 consecutive days. Peripheral blood was collected three times (preliminary, after preference period, and after naltrexone treatment) and the acetylated ghrelin and total ghrelin concentrations in plasma were measured using ELISA method. It was observed that chronic alcohol intake in PR animals led to decrease concentrations of both, active and total ghrelin in comparison to NP rats. After naltrexone administration the increase of active and total ghrelin levels were found in PR rats which corresponded with naltrexone lowering of alcohol consumption. Concluding, ghrelin could be of value as a possible indicator of antialcoholic activities of drugs shown by the lowering of alcohol intake.

P5.27

ULTRASOUND VOCALISATION (USV) ALTERATIONS IN PURKINJE CELL SPECIFIC TSC1 KNOCKOUT MICE

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Tuberous sclerosis (TSC) is a genetic disorder, characterized by the emergence of multi-system benign tumors. Symptoms related to central nervous system disruptions include seizures, developmental delay and a varying range of behavioral disorders. Behavioral disorders can be identified by analyzing various parameters, many of which can be observed during social interactions where different types of communication come into play. One of the more universal methods of communication in mammals is vocalization, which in mice is realized both in the audible and ultrasonic range. We recorded and analyzed mouse vocalizations during a mouse-pup isolation test, where newborn mice emit the largest amount of USV's. A range of sound parameters were analyzed including the rate of vocalization and numerous physical characteristics of sound, as well as single calls which were identified and classified.

P5.28

IMPACT OF TUBERIN (TSC2) GENE KNOCKOUT ON ULTRASONIC VOCALIZATIONS IN MOUSE PUPS

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Tuberin (TSC2) is one of the proteins involved in autism spectrum disorders' pathogenesis. Together with hamartin (TSC1), tuberin is responsible for protein synthesis, cellular growth and proliferation. Lack of these proteins causes tumors, hamartomas, observed in the central nervous system of affected patients. Additionally 25–60% of patients with tuberous sclerosis complex develop autism spectrum disorders. The highest concentration of tuberin has been noted in the cerebellum, particularly in the Purkinje cells. Purkinje cells may participate in the process of emission of ultrasounds (ultrasonic vocalization – USV), which can be analyzed as parameter of early stages of intercommunication development. Expression of tuberin in the cerebellar Purkinje cells was switched-of by means of the Cre/loxP transgenic technolgy. The study involved two groups

of mice: homozygous for the mutation TSC2Cre-/- and control group TSC2Cre+/+. All the mice were introduced into the study at the age of 2 days and the USV was recorded every two days until the age of 14 days. To record and analyze the ultrasounds we used the Ultrasound Recording Device and software by Avisoft Bioacustics. Preliminary data suggest subtle alterations of USV, but more experiment is needed to support first observations.

P6. Neurodegenerative diseases and disorders of the nervous system

P6.1

THE ROLE OF Cdk5/p35 AND cPLA2/LOX IN NEUROINFLAMMATION AND NEURODEGENERATION

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Cyclin dependent kinase 5 (Cdk5) is implicated in the pathomechanism of Alzheimer's disease (AD), as a kinase responsible for hyperphosphorylation of tau protein and aberrant metabolism of Amyloid β (Aβ) precursor protein. The previous data indicated the involvement of Cdk5 in regulation of cytosolic phospholipase A2 (cPLA2) gene, but its precise function is not fully understood. In our studies, we analyzed in animal AD model the role of Cdk5 in neuroinflammation and in regulation of cPLA2/lipoxygenase (LOX) pathway. Our data indicated an increase in gene expression for cPLA2, 5-LOX and 12/15-LOX in the hippocampus during the systemic inflammation. In parallel, we observed an increase in expression of the Cdk5 activating protein – Cdk5r1 (p35), suggesting the enhancement of Cdk5 activity and its possible role, as the regulatory factor. Using mouse AD model we demonstrated enhancement of 12-LOX expression and activity and cognitive impairment, which was prevented by 12-LOX inhibitor. Our results demonstrated the important role of inflammatory reaction in cognitive impairment. The relationship between Cdk5 and cPLA2/LOX during neuroinflammation may have a significant implication for the pathomechanism of AD, and presents Cdk5/p35 as the promising target for improvement of AD therapy. This study was funded by grant from The National Science Centre 2011/03/B/NZ3/04549.

P6.2

SPHINGOSINE KINASE AND GLYCOGEN SYNTHASE KINASE-3β IN MOLECULAR MECHANISM OF ALZHEIMER'S AMYLOID β TOXICITY

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Alterations of phosphorylation-dephosphorylation processes play a crucial role in the pathomechanism of Alzheimer's disease (AD). They affect signaling cascades and lead to hyperphosphorylation of tau protein. Glycogen synthase kinase 3β (Gsk-3β) is the main tau-kinase; however, little is known about the role of sphingolipid pathway in its regulation. Alteration of sphingolipid biostat may be an early event in etiopathology of AD. The question arises, if the sphingosine kinase (Sphk), a key enzyme in sphingolipid pathway, regulates Gsk-3β? We analyzed acute effects of exogenous amyloid β (Aβ) oligomers in PC12 cells, and prolonged exposition to endogenous Aβ in PC12 cells stably expressing human Swedish mutant APPsw gene. Our data indicated that in cells subjected to exogenous Aβ expression of Sphk1 and phosphorylation of Gsk-3β at Ser9 were enhanced, what could be considered as a component of protective mechanism. However, prolonged liberation of Aβ in APPsw cells evoked inhibition of Sphk1 expression, activation of Gsk-3β and death of significant population of cells. Consequently, an inhibitor of Sphk1 also reduced cell viability. Our data suggest the existence of specific relationship between Sphk1 and Gsk-3β and indicate their role in alteration of cell function and survival. The study was supported by MSHE Grant N401 587040.

P6.3

DYSFUNCTION OF STORE-OPERATED CALCIUM ENTRY AS AN EARLY EVENT IN THE PATHOGENESIS OF NEURODEGENERATIVE DISEASES

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Store-operated calcium entry (SOCE) is a mechanism that regulates calcium influx from the extracellular space which affects calcium signalling in the cell and has been implicated with neuronal cell death. We hypothesized that SOCE might be altered at the early stages of Alzheimer's (AD) and Huntington's (HD) disease. We used PC12 cells with an inducible expression of mutated full-length huntingtin as a cellular model of Huntington's disease. Calcium measurements were performed by single cell imaging with the Fura-2. We found SOCE parameters were changed as a result of the expression of mutant huntingtin. We next investigated if these differences were caused by changes in the mRNA expression of genes involved in SOCE. Similar analyses are currently being conducted using mouse models of HD (YAC128) and AD (APP V717I) using custom-made TaqMan Low Density Arrays containing probes for genes involved in calcium homeostasis and signalling. Our preliminary results suggest that the expression of mutant proteins such

as huntingtin or amyloid precursor protein affect the expression of selected components of calcium homeostasis and signalling pathways.

P6.4

IS SMN IMMUNOEXPRESSION IN HYPERTROPHIED NISSL GRANULATIONS IN AMYOTROPHIC LATERAL SCLEROSIS A MORPHOLOGICAL EQUIVALENT OF REINNERVATION?

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Reinnervation observed in early stage of amyotrophic lateral sclerosis (ALS) is a compensatory mechanism for motoneuron loss. Since survival motor neuron (SMN) protein could be involved not only in neuroprotection but also in the transport of mRNAs in motoneuron axons, we examined its immunoexpression in anterior horn motoneurons of ALS patients with reinnervation in EMG. SMN immunolabel was observed in neuron cytoplasm and neurities but it was particularly intense in enlarged Nissl granules. This finding may mirror increased synthesis of the protein in rough endoplasmic reticulum being not only an attempt of motoneuron to selfprotection but also necessary for nerve sprouting. Study supported by the Ministry of Sciences and Higher Education grant NN 401 014 640.

P6.5

THE ROLE OF GLYCOGEN SYNTHASE KINASE-3 BETA IN ALPHA-SYNUCLEIN-EVOKED TAU PHOSPHORYLATION

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Hyperphosphorylation of tau is involved in the pathomechanism of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Recent studies suggested the significance of alpha-synuclein (ASN) in tau phosphorylation, however, the molecular mechanism responsible for ASN-mediated tau modification remains to be elucidated. In this study, we investigated the role of extracellular ASN in tau phosphorylation in PC12 dopaminergic cells and the involvement of glycogen synthase kinase-3 beta (GSK-3β) and cyclin-dependent kinase 5 (CDK5) in ASN-induced tau modification and cell death. We found that exogenously added ASN (10 μM) stimulates the phosphorylation of tau at Ser396 in PC12 cells. A specific GSK-3β inhibitor (SB-216763) prevented ASN-evoked tau hyperphosphorylation without effect of CDK5 inhibitors. Furthermore, we found that ASN enhanced of GSK-3β protein level and activity. Cell viability determined by MTT assay and Hoechst 33258 staining showed that ASN induced PC12 cell death that presented typical apoptotic morphology. SB-216763 prevented apoptotic cell death evoked by ASN. Concluding, extracellular ASN is involved in GSK-3β-dependent tau modulation and its proapoptotic effect might be mediated at least in part by GSK-3β-catalysed tau phosphorylation and cytoskeleton destabilisation. Supported by a grant from The National Science Centre 2012/05/B/NZ3/02047.

P6.6

BIOELECTRICAL BRAIN ACTIVITY AND ATTENTIONAL FUNCTIONS IN TEENAGERS WITH ADHD AND HEALTHY CONTROLS

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Attention deficit hyperactivity disorder (ADHD) is a common behavioral diagnosis based on the presence of developmentally inappropriate levels of inattentiveness, overactivity and impulsivity. The prevalence for ADHD among children is estimated at about 3–10%, affecting boys 5 times more often than girls. The aim of the study was to investigate the patterns of attentional functions and brain activity measured with electroencephalography (EEG) in a clinical group aged 11–16 compared with healthy, age- and sex-matched controls. We focused on efficiency of alerting, orienting and executive networks assessed using Posner's Attention Network Test (ANT) paradigm. Further, the EEG recordings were collected while the participants performed the ANT test. The obtained results, including reaction time (RT) values, Event Related Potential (P300) and time-frequency analyses, are discussed within the context of existing theories of ADHD-related deficits. The project was supported by The National Science Centre, grant number: 2011/01/D/NZ4/04958.

P6.7

POSSIBLE ROLE OF MICROVESSEL-LOCATED P2X7R IN BBB FUNCTION DURING THE COURSE OF EAE

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Multiple sclerosis is a serious problem of medicine and one of the most frequent reasons of disabilities of young adults. EAE is a commonly used rodent model of MS. In physiological conditions the blood-brain barrier (BBB) maintains CNS homeostasis and prevents uncontrolled inflow of immune cells from the blood circuit. During the development of EAE, damaged BBB fails to protect CNS from autoreactive immune cells. In this study we try to investigate a possible role of P2X7R in pathological changes of BBB during EAE. Analyzing BBB tightness we observed decreased expression of Claudin5 (protein component of tight-junctions) in the isolated microvessels' fraction using western blot technique. These data were confirmed by immunofluorescence staining of brain sections against Claudin5. To assess functional state of BBB we carried out immunohistochemical staining against albumin and we observed its extravasation in early phase of EAE. All these data suggest dysfunction of BBB at the early stage of the disease. In parallel we observed overexpression of P2X7R in microvessels' fraction and noticed correlation between its expression and increased BBB permeability using antagonist of P2X7R. Supported by grant nr: 2012/05/N/NZ4/02191.

P6.8

EFFECTS OF LACTOCOCCUS LACTIS PRODUCING MYELIN PEPTIDES ON HISTOPATHOLOGICAL CHANGES IN SPINAL CORD OF RATS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

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Experimental Allergic Encephalomyelitis (EAE) is the animal model of Multiple Sclerosis (MS), human autoimmunological disease that causes neurodegeneration. The autoimmune base of the disease leads to treatment searching in immunological mechanisms. Earlier we proposed the application of pig spinal cord hydrolysate as a mean to induce oral tolerance. The positive effects observed in EAE rats, stimulated us to develop bacteria that may express active peptide related to myelin fragment. For our experiments we used mixture of Lactococcus lactis producing fragments of three main myelin peptides, MBP (MBP 85-97), PLP (PLP 139-151) and MOG (MOG 35-55). We fed female Lewis rats with spectrum of bacteria doses, from 101 to 108 cells/ rat daily, for twenty days, from day −10 to 9. At the day 0 we evoked EAE in rats. Based on the results obtained from clinical symptoms, we selected two doses, 103 and 106 bacteria cells/rat for investigation of histopathological changes in spinal cord of animals. We observed slighter inflammatory cells infiltration in spinal cord in EAE rats fed with both doses of bacteria in comparison to non-fed ones. Supported by N302 009 32/1139 grant.

P6.9

TREATMENT WITH OMEGA-3 POLYUNSATURATED FATTY ACID DOCOSAHEXAENOIC ACID REDUCES THE UPREGULATION OF CHONDROITIN SULPHATE PROTEOGLYCANS FOLLOWING SPINAL CORD INJURY

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Spinal cord injury (SCI) results in devastating consequences due to the inability of the central nervous system to regenerate. However, plasticity is thought to contribute to functional recovery. Here, we examined the effects of treatment with the omega-3 fatty acid docosahexaenoic acid (DHA) on changes in the expression of chondroitin sulphate proteoglycans (CSPGs) in the extracellular matrix and in perineuronal nets (PNNs), since it is thought that CSPGs are inhibitory to regenerating axons and that PNNs restrict synaptic plasticity. Hemisection was performed at thoracic level 12 in adult rats. Rats received intravenous injections of 500 nmol/kg DHA or saline 30 min post-injury. Spinal cords were dissected out 14 and 56 days later and sections were stained for PNNs, the CSPGs NG2 and neurocan, GFAP, and serotonin (5-HT). DHA treatment resulted in reduced lesion size, a smaller increase in neurocan, NG2, and GFAP expression at the scar border, and reduced neurocan immunoreactivity in PNNs 1 mm rostral and 3.5 and 4 mm caudal to the lesion. The decrease in 5-HT terminals contacting neurons seen caudal to the injury was not affected by DHA treatment. DHA may facilitate functional recovery after SCI by reducing the levels of CSPGs at the scar border, favouring axonal regeneration, and by decreasing neurocan expression within PNNs, which might promote synaptic plasticity.

P6.10

SOLID PITUITARY ADENOMA IN EKER RATS SUBJECTED TO KETOGENIC DIET

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Tuberous sclerosis (TS) is a genetic disease causing non-malignant tumors growth in the brain (e.g. pituitary adenoma) and in other organs. Ketogenic diet is already used in TS patients in treatment of epilepsy. However the mechanism of its influence on tumor growth is still not clear. The Eker rat is a useful model of TS: it has a spontaneous germ line mutation of the TSC2 gene what predisposed them

to multiple tumors. In Eker rats, pituitary adenomas are common, occurring in 58% of adults (more than 18-months-old). Methods: Forty six 8-month-old Eker rats (males and females) were used. Twenty six (experimental group) have been maintained on high fat, low carbohydrate ketogenic diet for 6 months, while 20 (control group) received a standard rodent diet. At the age of 14 months rats were sacrificed. Anteroposterior, vertical, and transverse diameters of the found pituitary adenomas were measured. Size of tumors was calculated by using the formula for volume of the ellipsoid. Results: 8% animals from experimental group and 20% from control group have developed pituitary adenomas. Mean tumor volume in experimental group was 143 mm^2 vs. 217 mm^2 in control animals. Conclusion: Eker rats fed with ketogenic diet develops solid pituitary adenoma in 14 months of age. Incidence of these pituitary tumors in Eker rats fed with ketogenic diet (8%) was lower when compared with rats from control group (20%).

P6.11

3' UNTRANSLATED REGION POLYMORPHISMS OF MATRIX METALLOPROTEINASE 9 AND THEIR ROLE IN SCHIZOPHRENIA: THE ROLE IN THE LOCAL TRANSLATION

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Recent studies have implicated MMP-9 in schizophrenia, in particular, Domenici et al reported highly elevated plasma levels of MMP-9 in schizophrenic patients and Rybakowski et al. demonstrated an association of MMP-9 5'UTR polymorphism -1562C/T with schizophrenia. Furthermore, Dziembowska et al. have shown that MMP-9 is locally translated in neurons in response to synaptic stimulation. Since 3'UTR plays essential role in mRNA transport to the dendrites and in its local translation, MMP-9 3'UTR polymorphisms may affect synaptic availability of the enzyme. In order to verify if SNPs affect local translation of MMP-9 or its mRNA transport we have made two types of vectors with human MMP-9 containing two 3'UTR variants as well as the inactive form of MMP-9 under human synapsin I promoter. The gene constructs enable MMP-9 protein visualization by its fusion to Venus fluorescent protein and additionally contain myristoylation sequence, which is responsible for the cell membrane docking. In result, locally translated MMP-9 at the synapse could be observed. Currently, we investigate if the polymorphism influences efficiency of MMP-9 mRNA transport and local translation under basal conditions or after stimulation. To enable MMP-9 mRNA tracking in the dendrites we will use MS2 system on living neurons under basal conditions and after stimulation of the two studied 3'UTR variants.

P6.12.

TRANSGENIC MICE WITH DYSREGULATED CA^{2+} HOMEOSTASIS IN NEURONS AS A MODEL OF AGE-INDUCED NEURODEGENERATION

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Altered Ca^{2+} homeostasis has recently emerged as one of the early events responsible for Alzheimer's disease (AD). Disturbances in Ca^{2+} signaling are found before any obvious extracellular Aβ pathology in patients with sporadic AD and it has been shown frequently, that Ca^{2+} dysfunction augments Aβ formation and Tau hyperphosphorylation. It is suggested, that brain ageing is a result of a subtle, but long-lasting dysregulation of Ca^{2+} homeostasis in neurons, which may explain that age is the major risk factor in AD. Our group showed that the intracellular Ca^{2+} level in resting neurons can be modulated by overexpression of STIM proteins. These proteins sense calcium level in ER and are involved in the Store Operated Calcium Entry (SOCE). The objective of our project is to understand how elevated basal Ca^{2+} level in neurons contributes to neurodegeneration. To achieve this goal constructs for STIM proteins and ORAI1 Ca^{2+} channels were created and procedures of transgenesis were completed to generate transgenic mice. The animals are now being tested for the transgenes. We will next analyze Ca^{2+} homeostasis in neurons of the transgenic mice. If they have expected phenotype, other properties will be monitored including behavior and susceptibility to neurodegeneration.

P6.13

THE INTERACTION BETWEEN PAROXETINE AND SOME CYTOKINES IN THE ANIMAL MODEL OF DEPRESSION

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Knowledge about the role of cytokines in the action antidepressant drugs is still insufficient. The aim of the this study was to evaluate the effect of paroxetine (PAR) on the levels of tumor necrosis factor alpha (TNFα), and interleukin-10 (IL-10) in the blood of rats in an experimental model of depression. Male Wistar rats were subjected to chronic mild stress (CMS) for 6 weeks. Following the development of anhedonia, the stressed and control rats (non-stressed animals) were treated with PAR (12.5 mg/kg b.w., p.o. 1× daily) for

three weeks. On the last day of the experiment, an acute lipopolysaccharide (LPS, 100 μg/kg b.w., i.p.) was injected to PAR or vehicle-treated rats and TNFα and IL-10 levels were assayed using ELISA methods. In stressed animals the level of TNFα was found to be significantly higher compared to non-stressed animals. PAR resulted in significant decrease in TNFα level and simultaneously the increase of IL-10 in the stressed animals. In conclusion, PAR induced normalisation of the TNFα level increased by stress and LPS and elevation of IL-10 level in animal model of depression what confirms the hypothesis that level of cytokines can be modulated by antidepressant drugs.

P6.14

PREVENTION OF EPIDURAL FIBROSIS BY A CHITOSAN AND/OR ALGINATE GEL IN A RAT LAMINECTOMY MODEL

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Epidural fibrosis is a frequent complication of lumbar disc surgery, however its influence on incidence of failed back surgeries remains controversial. A bilateral laminectomy (L2–L4) with associated disc injury was performed in 32 adult male Wistar rats. In three experimental groups (n=8 each), spinal cord on laminectomy site was covered with thin layer of gel: alginate, chitosan, or mixture of both. Control group (n=8) was left without treatment. After 4 or 8 weeks, rats (n=4 for each group) were sacrificed and spinal lumbar segments with surrounding muscles were removed and prepared for histologic analysis. Transverse and longitudinal sections were subjected to Masson-Goldner Trichrome staining and examined for severity of epidural fibrosis. Epidural scarring of variable density was found in all laminectomy sites. All experimental groups showed less epidural fibrosis, dural adhesion, fibroblast density, foreign body reaction, and nerve root retraction as compared to the control group. However, no significant differences were found between experimental groups. We suggest that both chitosan and alginate gels form effective barriers for collagen penetration preventing spinal cord from epidural fibrosis following disc injury.

P6.15

POST-TRAUMATIC EPILEPTOGENESIS IN APP/PS1 MOUSE MODEL OF ALZHEIMER`S DISEASE

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To address the question whether increased amyloid-β load facilitates post-traumatic epileptogenesis we induced traumatic brain injury (TBI) in 13–15 week old APP/PS1 mice and Wt littermates. Gene expression profiling of perilesional cortex, ipsilateral thalamus and hippocampus was performed using Affymetrix microarray system. APP/PS1 injured mice showed motor deficits compared to APP/PS1 controls (P<0.01) and Wt injured mice (P<0.01) in Neuroscore. Latency to find the platform in Morris water-maze was longer in APP/PS1 injured mice than in Wt injured group (P<0.05). Probe trial showed impaired spatial memory in APP/PS1 injured mice compared to APP/PS1 controls (P<0.05). Video-EEG monitoring (24 h/7 days, 2 week) performed at 6 week post-TBI revealed spontaneous seizures in 86% of APP/PS1 injured mice and 36% of APP/PS1 controls (P<0.05). None of Wt controls and 7% of Wt injured mice displayed spontaneous seizures (P<0.01 compared to APP/PS1 injured mice). Video-EEG monitoring (24 h/7 days, 2 week) starting at 14 week post-TBI showed spontaneous seizures in 50% of APP/PS1 injured mice and 13% of APP/PS1 controls (P>0.05). Neither Wt injured mice nor Wt controls had spontaneous seizures. Microarray data analysis revealed changes in transcriptome between groups. Enhanced amyloidogenesis results in more pronounced epileptogenesis and more severe motor and cognitive co-morbidities following TBI.

P6.16

DECREASE OF RGCS NUMBER IN GLAUCOMA MODEL VS. OPTIC NERVE TRANSACTION IN RATS

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Introduction: Transection of the optic nerve and glaucoma causes both structural and functional damage to retinal ganglion cells with subsequent vision defect or loss. This study was undertaken to compare the lost of RGCs after optic nerve transection and glaucoma model. Materials and methods: Wistar rats were divided into two groups. The first group underwent bilateral stereotactic injection of fluorescent tracer – Fluorogold (FG) into the superior colliculus to label RGCs. After one week the right optic nerve was transected. Left eye without optic nerve axotomy was established as control. In the second group intraocular pressure (right eye) was elevated by injection of polystyrene microbeads into anterior chamber (Bead model) and measured by Icare TonoLab. RGCs were labeled by FG before euthanasia. Fourteenth days following optic nerve transection and intraocular pressure elevation the total number of FG-positive RGCs

was counted in seven radial sections through the optic disk. Results and conclusions: After axotomy the number of surviving cells was reduced to 20.2 % (from 2249.5 ± 127.2 – in control group to 454.7 ± 96.5 – in group after axotomy), in glaucoma model to 79.9% (from 2249.5 ± 127.2 – in control group to 1798.3 ± 118.96 – in glaucoma model).

P6.17

LESION OF THE AMYGDALA NUCLEI IN RATS DETERMINES BEHAVIORS DIAGNOSTIC FOR AUTISM SPECTRUM DISORDERS

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Autism spectrum disorders (ASD) are a group of pervasive developmental disorder. All of them have been developing from birth, accompanying human through whole life and have strong influence on social functioning, communication as well as cognitive abilities. Neurobiological research (e.g. neuroimaging) indicate that patients with ASD demonstrate impairments of amygdala structure and functioning. It is worth to compare if previous results in human may be observed in animals. The aim of this study was display that lesion of amygdala nuclei may indicate behaviors diagnostic for ASD. For that purpose on a group of male Wistar rats (n=40) was conducted an electrolytic lesion of basolateral (BLA) or centromedial (CeA) amygdala. Animals were divided into groups separately for BLA and CeA: control (without operation), sham (operation, without lesion) and lesioned. After convalescence rats were observed in different behavioral tests which measured social functioning (social interactions), anxiety (elevated plus maze), spatial memory (water maze) and communication (smell preference). Obtain results suggest that amygdala lesion decreased social functioning or anxiety (BLA and CeA), communication (BLA), motor activity (CeA). In spite of this spatial memory increased (BLA). On the base of behavioral results it is likely that lesion of amygdala nuclei may be perceive animal model for further studies. (support: NN303 819040).

P6.18

YAC128 MOUSE MODEL-DERIVED iPSCS SHOW MARKERS OF HUNTINGTON DISEASE

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Huntington disease (HD) is an incurable brain disorder caused by expansion of CAG repeats in a HTT gene resulting in toxic huntingtin with long polyglutamine tract. In HD, neurons die in cerebral cortex and striatum and therefore a treatment option is a cell therapy using cells generated from induced pluripotent stem cells (iPSC) from patients. We have established a model of such therapy comprising iPSCs lines from the adult dermal fibroblasts of YAC128 HD mouse model. The cells were reprogrammed using transposable and excisable piggyBac vector expressing OSKML transcription factors. These iPSC cells show pluripotency both in *in vitro* (Tuj-positive neurons and beating cardiomiocytes) and *in vivo* (teratoma formation) differentiation assays, thus being suitable for experimental cell therapy. In addition, our YAC128/iPSC show alterations of Wnt/β-catenin and MAPK signaling pathways probably resulting from expression of human mutant huntingtin. Thus, cells suitable for cell therapy would need silencing of the mutant huntingtin. Therefore we have generated a series of therapeutic constructs based on piggyBac transposon expressing anti-huntingtin siRNAs in sh-miR backbone. We show that the construct when integrated into iPSC genome efficiently silences mutant huntingtin expression. Our platform is a useful model for investigating cell therapy outcomes in the HD mouse model.

P6.19

FIRST HUMANIZED ATAXIN-3 KNOCK-IN MOUSE MODEL PRESENTS MOLECULAR AND HISTOPATHOLOGICAL PHENOTYPES OF SPINOCEREBELLAR ATAXIA TYPE 3

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Spinocerebellar ataxia type 3 (SCA3) is a human neurodegenerative disorder caused by the expansion of CAG repeats in the coding region of the ataxin-3 gene. We generated the first humanized SCA3 knock-in mouse model by introducing human cDNA for ataxin-3 with 91 CAG repeats into the mouse ataxin-3 locus. The resulting animals express human mutant ataxin-3 protein in multiple brain structures and non-neuronal tissues. Like in human patients, the humanized allele shows both somatic and intergenerational CAG instability. The intergenerational instability is significantly associated with the gender of parent. Offspring inherits expanded CAG repeats in paternal transmissions and contracted CAG repeats in maternal transmissions. Moreover, mice show early upregulation of Serpina3n gene expression in the brain as early as at 7 weeks of age. This upregulation is also present in astrocytes isolated from neonatal animals, which suggest that mutant ataxin-3 has a more direct influence on a Serpina3n expression. The knock-in animals also demonstrate histopathological hallmarks of SCA3, including the damage of Purkinje cells in the cerebellum and the presence of intranuclear ataxin-3 inclusions.

P7. Synaptic plasticity and neurotransmission

P7.1

COOPERATIVE INVOLVEMENT OF SEROTONERGIC SIGNALLING AND MMP-9 IN SYNAPTIC PLASTICITY

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The brain plasticity is a re-organization of the neuronal and synaptic networks that allows for changes in response to incoming environmental stimuli. Pathological forms of neuronal plasticity underlie the multiple neuropsychiatric disorders like depression. Clinical observations on the efficacy of antidepressants targeting serotonergic system strongly suggest that serotonin and its receptors play a pivotal role in modulation of pathological plasticity. It is known that matrix metalloproteinase-9 is one of the most important biomarker in depression and polymorphism in this protein affect bipolar disorder. We have recently shown that MMP-9, having an established role in synaptic plasticity, influences dendritic morphology in a similar way to that obtained after the 5-HT7 receptor stimulation, e.g. it induces formation of long, thin dendritic spines. It is also known that stimulation of 5-HT7 receptor leads to activation of small Rho GTPase – Cdc42 in fibroblast cell line and in neurons. In this work we investigate whether MMP-9 substrate represents a novel downstream effector of 5-HT7 receptor. Our results indicate that stimulation of the 5-HT7 receptor increases MMP-9 activity toward its synaptic substrates and results in activation of small Rho GTPases.

P7.2

CONTRASTING EFFECTS OF REPEATED RESTRAINT STRESS ON LONG-TERM POTENTIATION IN THE RAT HIPPOCAMPUS AND FRONTAL CORTEX

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Wistar rats were subjected to restraint lasting 10 min and repeated twice daily for 3, 7 and 14 days. Brain slices were prepared 24 h after the last restraint session and studied *ex vivo*. In slices of the frontal cortex field potentials (FPs) were evoked by electrical stimulation of underlying sites in the cortical layer V and recorded in layer II/III. In the hippocampal slices field excitatory postsynaptic potentials were evoked by stimulation of Schaffer collaterals and recorded in the stratum radiatum of the CA1 area. In cortical preparations significant differences between experimental and control groups were evident already after 3 days of restraint stress and consisted of an increase in the maximum FP amplitude (3.0 mV vs. 2.2 mV, respectively) as well as a reduced long-term potentiation (LTP; 112 % vs. 139 % of baseline, respectively). While in the frontal cortex there were no differences after 7 or 14 days of restraint, in the hippocampus a significant effect of restraint stress, namely a reduction of LTP (from 146 % to 120 % of baseline) was observed only after 14 days of restraint. These results indicate that repeated restraint stress differentially modulates synaptic transmission and plasticity in the two brain areas studied. Support: "DeMeTer" and statutory funds from the Institute of Pharmacology.

P7.3

MUTATION IN F64 POSITION OF GABAAR α SUBUNIT AFFECTS THE RECEPTOR GATING

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GABAA receptor (GABAAR) is a pentamer, formed by 2α, 2β and γ subunit. GABA binding site is localized at the interface between α and β subunits. Our aim was to characterize how mutation localized at the binding pocket (α1F64) influences agonist binding and conformational transitions between bound receptor states (gating). We used patch-clamp technique with ultrafast perfusion system and HEK 293 cells expressing native or mutated GABAARs. All mutations (α1F64C/L/A) right-shifted the dose-dependent curve and accelerated current deactivation, indicating impairment of binding. Reduction of fast desensitization, which in the case of α1F64C was complete, indicates changes in gating. Moreover, the mutation decreased the maximum open channel probability, a key feature of receptor gating. Experiments performed with different agonists confirmed mutation-induced changes in the channel's opening/closing transitions (gating). Quantitative analysis based on model simulations indicated that this mutation mostly affected the channel state which precedes opening and is interpreted as a macromolecule destabilization ("priming" or "flipping") following agonist binding, whereas desensitization or efficacy are affected to a smaller extent. Our data thus suggest that mutation of α1F64 residue affects the "transition wave" from binding sites to the channel gate.

P7.4

EFFECTS OF NEONATAL MATERNAL SEPARATION ON LTP SATURATION AND LTD SATURATION IN LATERAL AMYGDALA OF THE RAT

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The maternal separation (MS) procedure represents a paradigm to study disturbances in brain function that might occur in response to stressful events during postnatal development. However, mechanisms by which early life stress affects synaptic plasticity in the amygdala are poorly understood. This study was aimed at finding the effects of repeated MS on saturation of LTP and LTD in the cortical (CoI) and the thalamic input (ThI) to the lateral amygdala (LA) of rats. Wistar dams with their offspring were housed under 12:12 L/D conditions with food and water available *ad libitum*. On each postnatal day (PND) 1–21 rats were subjected to MS (3 h/day). For *ex vivo* electrophysiological experiments rats between PND 35 and PND 55 were used. The animals were anesthetized and brain slices containing LA (450 μm) were cut. Field potentials (FPs) were evoked by the stimulation of CoI or ThI to LA. LTP was induced using theta-burst stimulation (TBS) and LTD was induced using low frequency stimulation (LFS). Both protocols were repeated every 45 minutes until maximum level of LTP or LTD was reached. In CoI we observed impairment of maximum LTP and enhancement of maximum LTD in slices prepared from MS subjected rats, however in ThI, both maximum LTP and maximum LTD was impaired when compared to control, animal facility reared (AFR) rats.

P7.5

DYSTROGLYCAN INFLUENCES THE MORPHOLOGY AND FUNCTION OF HIPPOCAMPAL NEURONS *IN VITRO*

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Dystroglycan (DG) is a cell adhesion receptor composed of α- and β-subunits that form a transmembrane link between the extracellular matrix and the intracellular actin cytoskeleton. Loss of DG function is implicated in muscular dystrophies and the aetiology of epithelial cancers. We have previously reported that β-DG is a target for matrix metalloproteinase-9 (MMP-9), an extracellularly operating enzyme, known to be pivotal for synaptic plasticity, learning and memory. This may suggest an important role of β-DG cleavage by MMP-9 in neuronal activity. Although it has been demonstrated that deletion of DG in neurons blunted hippocampal long-term potentiation (LTP), detailed knowledge concerning mechanisms of action of DG in neuronal cells is still lacking. To study the role of DG in neuronal structure and function we used the lentiviral vector (LV) to deliver shRNA, specifically silencing DG in cultured hippocampal neurons. We found that knockdown of DG simplifies dendritic arbor morphology as well as decreases the total length of dendrites. To determine whether DG deletion influences the dendritic spine shape and motility we performed life imaging of MMP-9-treated cultures. We observed differences in spine remodeling between control and LV-infected neurons. Our results suggest that DG is required for proper neuronal maturation and dendritic spine plasticity.

P7.6

LACK OF MATRIX METALLOPROTEINASE 9 (MMP-9) AFFECTS LTP IN THE BASAL AND CENTRAL NUCLEI BUT NOT IN THE LATERAL NUCLEUS OF THE AMYGDALA

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Matrix metalloproteinase-9 (MMP-9) is an extracellularly operating endopeptidase, which cleaves extracellular matrix proteins and plays an important role in synaptic plasticity, learning and memory. It is expressed in neurons in many different brain structures, including the hipocampus, prefrontal cortex and amygdala. MMP-9 is involved in maintenance of long-term potentiation (LTP) in the hipocampus and prefrontal cortex. On the other hand, its role in synaptic plasticity in the amygdala is much less known. It has been shown that the MMP-9 knock-out (MMP-9 KO) mice are impaired in amygdala-dependent appetitively motivated learning. The amygdaloid complex consists of several cytoarchitectonically and functionally distinguishable nuclei. To investigate MMP-9-dependent synaptic plasticity in different amygdalar nuclei, we studied MMP-9 role in LTP evoked in the central (CE), basal (BA) and lateral (LA) nuclei of the amygdala. In our *in vitro* extracellular recordings we used slices from MMP-9 KO and control mice. LTP in the BA-CE and LA-BA pathways was induced at the same level in the MMP-9 KO and control slices but it was disrupted several minutes after induction. In contrast, LTP in the external capsule-LA pathway was not disturbed in MMP-9 KO. These data suggests that MMP-9 is involved in stabilization but not in induction of LTP only in particular nuclei of the amygdala.

P7.7

GSK3 REGULATES Arc PROTEIN EXPRESSION IN PRIMARY NEURONAL CULTURE – POSSIBLE APPLICATIONS FOR SYNAPTIC PLASTICITY

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GSK3 alpha/beta (Glycogen Synthase Kinases alpha/beta) are serine/threonine kinases ubiquitously expressed in the nervous system. GSK3's appear to be critical for LTD formation and overactivation of GSK3 inhibits LTP expression. Here we report

that GSK3 regulate expression of Arc/Arg3.1 protein (Activity Regulated Cytoskeleton Associated Protein/Activity Regulated Gene 3.1), involved in diverse forms of synaptic plasticity, including LTP, LTD and homeostatic plasticity. We hypothesize that Arc could be one of the putative GSK3 effectors in neurons. The combination of low dose NMDA and GSK3 inhibitors up-regulated Arc expression at the protein but not at the mRNA level. Recombinant Arc protein is phosphorylated *in vitro* by GSK3 beta. Currently, we are characterizing the mechanism of GSK3-dependent Arc degradation. We also observed that the co-treatment of neurons with GSK3 inhibitors and NMDA induced alterations in the dendritic spine morphology. We are employing shRNA technology to determine if Arc contributes to the observed alterations in dendritic spines morphology and what is the role of GSK3-dependent Arc degradation in different forms of synaptic plasticity. Supported by 7FP EU grant 223276 "NeuroGSK3" and NCN 05397 grant from National Center for Science.

P7.8

CIRCADIAN SYNAPTIC PLASTICITY IN THE MOUSE BARREL CORTEX

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Mice show circadian rhythms in behaviour and metabolic processes but also in neuronal plasticity associated with the sensory input to the whisker representations in the somatosensory (barrel) cortex. We analyzed daily structural changes in the barrel cortex of the C57/BL mice in a light/dark (LD 12:12) regime and in constant darkness (DD). Using serial EM sections of the barrel cortex of mice sacrificed during their active or rest period, we observed up-regulation of the density of excitatory synapses located on single-synapse spines during the rest period in LD 12:12 and an increase in the density of inhibitory synapses located on double-synapse spines during the active period in LD 12:12 and DD. In conclusion, the mouse barrel cortex shows daily changes in the density of synapses and dendritic spines similar to the daily pattern of locomotor activity of the animals. Moreover, the excitatory and inhibitory synapses are differently regulated during the day/night cycle. This example of plasticity seems to be regulated by both, the circadian clock and light. Supported by a grant to MJ (2011/01/D/NZ3/00207).

P7.9

THE INVOLVEMENT OF TOR PROTEIN KINASE AND PI3K CLASS 1 IN THE REGULATION OF CIRCADIAN NEUROPLASTICITY IN THE VISUAL SYSTEM OF *DROSOPHILA MELANOGASTER*

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In the visual system of *D. melanogaster* numerous circadian rhythms have been described. The most pronounced rhythmic changes have been detected in L2 monopolar cells located in the first neuropil of the optic lobe (the lamina). The L2's dendritic tree perimeter oscillates in a circadian manner and is the largest at the beginning of the day. The aim of our study was to understand molecular mechanisms controlling cyclic L2 dendritic tree changes. We have examined a possible involvement of TOR and PI3K class 1 proteins in this process. First the expression of tor and pi3k class 1 genes was investigated in the fly's brain at different times of the day in 12 h of light and 12 h of darkness (LD12:12) or constant darkness (DD) by means of Real-Time PCR. Next the analysis of circadian changes in the perimeter of GFP labeled L2 dendritic trees was examined in control and in flies with down-regulated expression of tor or pi3k class 1 genes. The obtained results showed that the expression of tor gene changes during the 24 h cycle and is the highest when the L2 cell dendritic trees are largest. The silencing of tor gene expression resulted in the disruption of circadian changes in L2 interneurons. It indicates that TOR signaling pathway is involved in the mechanism of circadian neuroplasticity in the visual system of *D. melanogaster*.

P7.10

THE EFFECT OF CARBENOXOLONE ON CARBACHOL--INDUCED POSTERIOR HYPOTHALAMIC THETA *IN VITRO*

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Current evidence strongly suggests that gap junctions (GJs) communication underlies the mechanisms of oscillation and synchrony in the central nervous system. In our previous work we have documented that GJs are highly involved in the generation of theta rhythm in both *in vivo* and *in vitro* hippocampal formation. Specifically, the blockage of gap junction by application of carbenoxolone (CBX) abolished hippocampal theta field potential and this effect was found to be hardly reversible. In our recent studies we have showed that posterior hypothalamic area (PHa), well known as an extrinsic modulator of hippocampal theta frequency, is also capable

of independent theta rhythm generation. The aim of the present study was to investigate the effect of gap junctions blockage on cholinergically-induced posterior hypothalamic theta *in vitro*. Two experimental procedures were applied. (1) PHa slices were preincubated in 100 μM CBX and then theta rhythm was eleicited by 50 μM carbachol (CCH); (2) PHa theta activity was induced by perfusion of the slices with 50 μM CCH, and then the slices were perfused with 50 μM CCH + 100 μM CBX. In both experimental conditions the blockage of gap junctions with carbenoxolone failed to abolish the cholinergic theta rhythm in the posterior hypothalamic area. The mechanisms underlying the generation of PHa theta activity are discussed. Supported by NCN grant 2011/01/B/N24/00373.

P7.11

ACTIVITY-DEPENDENT REARRANGEMENT OF THE NEURONAL CELL NUCLEUS

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It is now firmly established that long-lasting synaptic plasticity involves dramatic changes in gene expression occurring under the influence of specific signaling pathways and transcription factors. Numerous studies have shown that DNA and histone epigenetic modifications play key roles in neuronal plasticity. Recent studies in non-neuronal cells, indicated the existence of epigenetic mechanism of yet another class, related to the nuclei structural remodeling and very poorly understood in neurons. Therefore, we decided to study the ultrastructure of the cell nuclei in the hippocampal dentate gyrus granule neurons upon seizures induced by kainic acid, an analog of glutamate. Under these conditions the granular neurons instead of degradation, undergo an intensive plasticity phenomena. We found that seizures led to rapid and dramatic enlargement and striking reorganization of internal component-structures of interchromatin granule clusters (IGCs) in granular cell's nucleus. Moreover, unlike IGCs of control animals, the reorganized IGCs contained activated RNA polymerase II CTD phosphoepitopes. These observations may suggest involvement of IGC in activity-dependent transcription events in neurons.

P7.12

REGULATION OF DENDRITOGENESIS BY ZBP1 DEPENDS ON ITS PHOSPHORYLATION AT Ser181

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Zipcode Binding Protein 1 (ZBP1) is one of proteins involved in local translation, a mechanism present in various cell types. In neurons, processes coordinated by ZBP1 are indispensable for proper axonal growth cone and spine formation, as well as dendritic arborization. Recently we also showed that ZBP1-dependent dendritic transport of b-actin mRNA and its local translation is needed for dendritic tree arborization. We also proved that phosphorylation of ZBP1 by Src kinase is important for this process. Now we demonstrate that ZBP1 is effectively phosphorylated *in vitro* by mTOR kinase. We took advantage of recently published information regarding potential mTOR-dependent phosphorylation sites in ZBP1, i.e. Ser181 (Dai et al. 2011), and examined role of this phosphorylation in (1) dendritic arborization and (2) cellular distribution of ZBP1. To address these questions, we constructed non-phosphorable (S181A) and phosphomimicking (S181E) mutants of ZBP1 fused to GFP. We observed that S181E but not S181A reversed morphological deficits caused by ZBP1 knockdown. Another observation was that distribution along the dendrites of non-phosphorable mutant was more even that distribution of wild type ZBP1, which is denser at the dendritic branching points. Thus we concluded that Ser181 phosphorylation is involved in ZBP1 functions during dendritic growth. This research has been supported by National Science Center (NCN) grant 2011/01/N/NZ3/05405.

P7.13

THE INFLUENCE OF *TOXOPLASMA GONDII* INVASION ON NEUROTRANSMISSION IN MICE

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The parasitic protozoan *Toxoplasma gondii* is capable of altering intermediate host natural defensive behavior, which is believed to facilitate the parasite's transmission in the environment. Despite extensive research on the subject, the exact mechanism behind the host manipulation remains obscure. However, key neurotransmitter levels are listed among possible contributing factors. Thus, the study was aimed at evaluating the monoaminergic activity in specified brain regions of *T. gondii* infected mice of both genders. The obtained results show that parasite invasion influences all tested monoamine systems and the observed changes depend on gender and time after infection. The parasite-induced neurotransmission alterations were mostly pronounced during acute toxoplasmosis and they involved a decrease in noradrenergic system activity in females and its slight increase in some brain areas of males as well as a rise in serotonin and dopamine systems activity in males. These findings may contribute to a better understanding of *T. gondii* involvement in the host behavior control and in the occurrence of certain CNS disorders in humans. The study was supported by the Polish Ministry of Science and Higher Education (grant N N302 636340).

P7.14

MMP-3 AND MMP-9 DIVERGENTLY REGULATE LTP IN HIPPOCAMPAL MOSSY FIBER-CA3 AND CA3-CA1 PROJECTIONS

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Matrix metalloproteinases (MMPs) comprise a family of proteolytic enzymes that modify membrane and extracellular matrix proteins. Broad-spectrum MMP inhibitors were shown to impair LTP consolidation in two hippocampal projections: mossy fiber (MF)-CA3 and CA3-CA1 which deeply differ in LTP induction as well as in expression sites and induction mechanisms. The aim of this study was to address the specific roles of MMP-3 and gelatinases in LTP in these projections. Using field potentials recording in acute mice brain slices we have shown that specific MMP-3 inhibitor (NNGH) disrupts LTP late phase in CA3-CA1 pathway (NNGH: 119 ± 10% of baseline two hours after induction, n=9; CTR: 177 ± 29%, n=8; P=0.01) but does not affect LTP in MF-CA3 (NNGH: 186 ± 27%, n=7; CTR: 172 ± 11%; n=7, P=0.34). Another MMP-3 inhibitor UK356618 gave similar results. Interestingly, knock-out mice without functional MMP-9 show impaired long term plasticity in MF-CA3 pathway (KO: 115 ± 17%, n=8; CTR 181 ± 13%, n=13; P<0.01) and a weak if any change in LTP in CA3-CA1 projection (KO: 139 ± 8%, n=7; CTR: 159 ± 13%, n=8; P=0.22). These results suggest that the role of particular MMPs in LTP expression is not universal in considered projections. Moreover, we provide the first evidence that MMP-3 and MMP-9 proteases differentially modify LTP consolidation in the MF-CA3 and CA3-CA1 pathways. Support: NCN grant N N401541540.

P7.15

THE ROLE OF MATRIX METALLOPROTEASE SUBTYPES IN EPSP-TO-SPIKE (E-S) PLASTICITY IN CA3 ASSOCIATIONAL NETWORK

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Learning and memory formation are often linked to long-term synaptic plasticity but some components of memory storage are coded by nonsynaptic changes, i.e. neuronal excitability. Matrix metalloproteases (MMPs) play a crucial role in long-term synaptic plasticity, but to what extent they affect other neuronal functions remains poorly understood. Here we studied the impact of MMP-3 and MMP-2/9 specific inhibitors on evoked EPSPs and population spikes (PS) in CA3 hippocampal autoassociative network in rat P30–P60 brain slices. We found that MMPs inhibition reduced long-term E-S coupling and spiking coherence evoked with stimulation of associational/commisural synapses alone (4×100 Hz) or paired in bursts with mossy-fibers. Moreover, broad spectrum MMPs inhibitor did not occlude with E-S plasticity recorded in the presence of GABAARs or L-type calcium channels blockers but significantly reduced LTP of NMDAR-mediated EPSPs. Finally, MMPs inhibition determined the saturation level of E-S coupling depending on synaptic activity pattern. In conclusion, our data provide a novel link between MMPs activity (particularly MMP-3), postsynaptic depolarization and neural excitability. By regulating E-S plasticity and by limiting the number of neurons firing, MMPs could influence information processing in CA3 associational network. Supported by MNiSW grant "Iuventus Plus" IP2010_047870 and partially by 3/Pbmn and N N401541540 grant.

P8. Glia

P8.1

CYTOTOXIC EFFECTS OF ZINC ON CHOLINERGIC AND ASTROGLIAL CELLS

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Zinc excess in the synaptic cleft may be one of pathologic signals triggering chronic neurodegenerative events. The aim of this work was to find relationships between Zn accumulation and integrity of cholinergic and astroglial cells. Exposition of cAMP/RA-differentiated (DC) and nondifferentiated cells (NC) cholinergic SN56 neuroblastoma and astroglial C6 cells to Zn yielded its concentration dependent accumulation. It caused inhibition of pyruvate dehydrogenase, aconitase and ketoglutarate dehydrogenase activities. Zn accumulation caused concentration-dependent death of both neuronal and astroglial cells. After 24 h exposition of SN56 cells to 0.15 mM Zn their death rates were equal to 35 and 50% for NC and DC at cation levels equal to 4.0 and 5.5 nmol/mg protein, respectively. In the same conditions, the death rates of astroglial NC and DC were close to 1–2% only, at intracellular Zn levels of 1.6 and 2.1 nmol/mg protein, respectively. Higher about 0.25 mM Zn levels were required to evoke death rates of astroglial cells, similar to those seen in neuronal cells. In such conditions Zn levels in astroglia were about 6.4 and 27.0 nmol/mg protein, respectively. Such differential sensitivity of astroglial and neuronal cholinergic NC and DC to Zn may be due to respective differences in densities of ZnT1 transporters in their plasma membranes. Supported by M.S&H.E. project IP2010035370 and GUMed fund ST57.

P8.2

THE ROLE OF ADHESION PROTEIN CD44 IN THE SHAPE CHANGES OF ASTROCYTES

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CD44 is a widely distributed type I transmembrane glycoprotein and functions as the major hyaluronan receptor on most cell types. CD44 through interaction with actin cytoskeleton affects the transmission of signals from the outside to the inside of the cell in many tissues and organs. Primary cultures of astrocyte are diverse in their morphology and many factors can influence on it. *In vivo* astrocyte also are able to change their shape in response to various stimuli. The appearance of reactive astrocytes *in vivo* with thicker and longer processes and increased cellular content of glial fibrillary acidic protein (GFAP) has been observed in the CNS after various types of injury caused by physical, chemical, and pathological trauma. Furthermore, it has been showed that CD44 expression increases after brain injury. In our study we investigated the influence of knock down of CD44 by specific shRNA and CD44 overexpression on the astrocytes shape changes. Our results indicate that knock down of CD44 in astrocytes results in more regular and flat shape. In contrast the overexpression of CD44 promotes more irregular, radial-like shape of astrocyte. Our data support the hypothesis that CD44 plays role in morphological changes of astrocyte and give the opportunity to investigate its role in pathological processes such as brain injury.

P8.3

MODIFIED PROTOCOL FOR CULTURING PREDEGENERATED ADULT RAT SCHWANN CELLS

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The purpose of this experiment was to optimize the methodology of culturing predegenerated Schwann cells. Right sciatic nerves of adult rats (n=3) were cut and left for 7 days. Then, 1-mm fragments of predegenerated (P) and intact (C) nerves were separately planted in 12-well culture plates precoated with laminin or fibronectin. Medium for culturing of endothelial cells EBM-2 (endothelial cell culture medium) was compared with DMEM (Dulbecco's Modified Eagle's Medium). Additionally, culture media were supplemented with factors supporting SCs growth: bovine pituitary extract (5 µg/ml), heregulin (40 ng/ml), and insulin (2.5 ng/ml). After 7 or 14 days, plates were subjected to analysis. Cell culture purity was determined under the fluorescent microscope by estimating the percentage of GFAP, N-Cadherin and NGFR p75-positive cells, and intensity of cell growth - by counting the number of cell islets migrating from nerve explants. Percentage of cells confirmed as Schwann cells was 94–97. Number of islets was significantly higher in both time-frames: (1) in plates precoated with fibronectin in both groups; (2) in P than in C groups. Thus, nerve predegeneration, application of EBM-2 as culture medium and fibronectin as coating appeared a good method for obtaining cultured Schwann cells to be used in different experimental models in rats.

P8.4

ACTIVITY-DEPENDENT MOTILITY OF PERISYNAPTIC ASTROCYTIC PROCESSES: LINKS TO FUNCTIONAL SYNAPTIC PLASTICITY

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Astrocytes are generally accepted as important players in synaptic function and development. On the other hand astrocytes *in vivo* have a very complex 3D structure, which is shaped by their numerous and highly ramified thin peripheral processes. Morphology of perisynaptic astrocytic processes (PAP) is subject to constant remodeling, for instance, long-lasting PAP retraction in hypothalamus is known to alter synaptic transmission and deficiency in PAP movement can prevent dendritic spine formation and synaptic maturation. The PAP motility is likely to be actin-based since they are known to contain actin and actin-related proteins. We use actin-binding deficient Profilin-1 (abdProf-1) as a genetically-encodable tool to selectively suppress activity-dependent morphological plasticity of astrocytes in combination with membrane targeted form of GFP (LckGFP) to trace PAPs precisely. This approach combined with astrocyte-specific viral gene delivery allows us to learn how suppressed morphological response of astrocytes can affect synaptic function in the mouse brain.

P8.5

EFFECT OF NOVEL PENTABROMO-BENZYLISOTHIOUREAS ON T98G HUMAN GLIOBLASTOMA CELL LINES AND SEGA-DERIVED CULTURES

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Gliomas are the most common primary brain tumours characterized by infiltrative cell growth. The specific novelty inhibitors of constitutively active serine/threonine kinase (CK2), i.e. isothiourea derivatives induce apoptosis and affect proliferation in some human cancer cells. We examined the cytotoxic and proapoptotic effect of selected modified isothiourea derivatives – pentabromobenzylisothioureas (ZKKs) against T98G adult human glioblastoma cell lines and cultures derived from rare, low-grade pediatric brain tumour of a mixed glioneuronal lineage (subependymal giant cell astrocytoma – SEGA), and normal human cultured astrocytes. ZKK-3 and ZKK-2 appeared to be the most effective compounds that induce apoptosis and exhibit strong anti-proliferative effect on neoplastic astroglial cells determined by flow cytometry analysis and Multisizer3 Beckman Coulter. Treatment of T98G cell line and SEGA-derived cell cultures with 50 μM ZKK-3 and ZKK-2 for 48 h resulted in apoptosis and inhibition of cell proliferation up to 60% and 50% respectively. These results might suggest the potential anti-tumour effect of selected ZKKs related with glioma-derived neoplasms.

P8.6

THIAMINE PYROPHOSPHATE DEFICIENCY CAUSES ENERGY DISTURBANCES IN ASTROGLIAL C6 AND MICROGLIAL N9 CELLS

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Inhibition of pyruvate (PDHC) and ketoglutarate (KDHC) dehydrogenase complexes induced by thiamine pyrophosphate deficits is known to cause disturbances of cholinergic transmission in the brain, yielding clinical symptoms of cognitive and motor deficits. However, particular alterations in distribution of acetyl-CoA, in the glial cells of thiamine pyrophosphate-deficient brain remain unknown. Therefore, the aim of our work was to find out how amprolium-induced thiamine pyrophosphate deficits (TD) affect distribution of acetyl-CA in the compartments of glial cells. As an experimental model we used astroglial C6 and microglial N9 cell line cultured in low thiamine medium. In such conditions microglial N9 cells displayed significantly greater loss of viability than the C6 ones. In both groups of the cells the activity of the key enzymes of energy/acetyl-CoA metabolism such as: PDHC, KDHC, aconitase was inhibited by amprolium-induced thiamine deficits. It explains why acetyl-CoA levels in the mitochondrial compartment were decreased in the cells. Supported by the Ministry of Research and Higher Education projects: IP 2011 046071, 01-0100/08 and St 57.

P8.7

IN SEARCH OF SIGNALS THAT TRIGGER GPR17 EXPRESSION IN MICROGLIA CELLS

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After insults microglia cells act by migrating to the site of injury, phagocyting cell debris and secreting inflammatory mediators, among which are cytokines, chemokines, cysteinyl leukotrienes (cysLTs) and purinergic molecules. The recently discovered GPR17 is structurally related to P2Y purinergic and cysLT receptors. Little is known about its regulation in microglia except that, in animal models, GPR17 was found in these cells exclusively after ischemic injury. The aim of this study was to identify *in vitro* signals that can trigger GPR17 expression in reactive microglia *in vivo*. Real-time PCR showed that in primary rat microglia cells, a low level of GPR17 can be increased by conditioned medium from oxygen and glucose deprived neurons or by prolonged deprivation of growth factors, but not by typical "danger signals" like ATP or cysLT. Among other known activators of microglia cells, lipopolysaccharide caused a decrease of GPR17 expression, but GPR17 receptor agonists and zymosan (a stimulator of phagocytosis) led to its increase in a time-dependent manner. It is still not known how long, after induction, GPR17 receptors remain in the membrane and what function they may play. However preliminary results showed that GPR17 expression may participate in the acquisition of a detrimental or a beneficial phenotype of microglia.