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ABSTRACT BOOK

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SESSION 1

S1.1:

Muscarinic receptors are responsible for cholinergic modulation of corticothalamic transmission from layer 6 to posteromedial thalamic nucleus

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We have previously shown that synaptic transmission of layer 6 corticothalamic input to posteromedial nucleus of the thalamus is modulated by cholinergic agonist carbachol. Particularly, in rat brain slices carbachol depresses EPSP amplitudes recorded in PoM relay cells in response to stimulation of corticothalamic fibers projecting from layer 6 of primary somatosensory cortex but, at the same time, enhances their frequency facilitation. In this study we aimed to identify receptors responsible for this modulation. In presence of muscarinic receptor antagonist (scopolamine or atropine) both depression of corticothalamic EPSPs and increase of frequency facilitation were abolished. In contrast, high concentration of specific nicotinic agonist DMPP (dimethylphenylpiperazinium) neither depressed corticothalamic responses nor enhanced the frequency facilitation. Thus, cholinergic modulation of corticothalamic synapses in PoM is mediated by activation of muscarinic acetylcholine 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.

S1.2:

Influence of binocular deprivation on the gene and protein expression profiles in developing cat visual system

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Previous experiments in which we mapped the visually driven activity in the primary visual cortex (area 17) of kittens via detection of the expression level of the activity reporter gene zif268 revealed a clear age-dependent decrease in normal kittens, and significantly higher levels in binocularly deprived (BD) animals compared to age-matched controls. Based on these light-induced zif268 activation patterns, early onset BD seemed to halt primary visual cortex maturation.
Here we screened for proteomic changes in relation to area 17 maturation under normal visual stimulation (N) and affected by BD, applied for either two (2BD) or four (4BD) months from eye opening. Intracellular protein expression was judged by means of a functional proteomics approach (Two-Dimensional Difference Gel Electrophoresis and mass spectrometry) and Western analysis was applied for validation. Computational pathway analysis was carried out by means of Ingenuity Pathway Analysis (IPA).
We detected 25 proteins with a dysregulated expression as compared to age-matched controls in 2BD kittens and 10 proteins in 4BD kittens. The protein interaction analysis demonstrated that most proteins were associated with energy metabolism (9 proteins), mRNA

metabolism and transport (hnRNPL, hnRNPH), clathrin-mediated endocytosis (Hsc70, endophilin, alpha-synuclein), GABA release (Septin 5) and outgrowth of neurites (CRMP4). Expression of alpha-synuclein and Hsc70 was decreased in BD kittens, possibly pointing towards negative regulation of clathrin-mediated endocytosis. Septin 5, which inhibits exocytosis and is associated with GABA vesicles in synapses (Beites et al., 1999; Kinoshita et al., 2000), was upregulated in BD animals. Interestingly, in adult cat area 17, CRMP4, which is known to be present in all parvalbumin-positive neurons (a subset of inhibitory interneurons), was upregulated under BD whereas the level of another member of this family, CRMP2, which is expressed in pyramidal neurons, was not changed (Cnops et al., 2006). Western analysis further revealed that GAD65, the GABA synthesizing enzyme at the synapse, was downregulated in BD kittens, suggestive of negative regulation of inhibitory transmission, which normally should increase with age to open and subsequently close the critical period for ocular dominance plasticity (Huang et al., 1999). Altogether, these maturation control-related processes seem to demand increased metabolism as 7 out of 9 detected proteins that were involved in energy metabolism were upregulated in BD conditions.
Project co-financed by the European Union from the European Regional Development Fund within the frame of International PhD Projects Programme (MPD4-504).

S1.3:

Annexin A6 and phospholipase D1 in catecholamine exocytosis in PC12 cells:a plausible relationship?

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Neurons and endocrine cells synthesize and secrete catecholamines (adrenaline, noradrenaline and dopamine) in a regulated pathway. For more than 30 years, PC12 cells have been used as a model to monitor secretion of neurotransmitters. Recently, results obtained in our laboratory revealed that overexpression of annexin A6 (AnxA6) isoforms inhibits exocytosis of dopamine in this cell line. The aim of my work is to elucidate molecular mechanism of this inhibition and and to determine the role of AnxA6-1 and AnxA6-2 in exocytosis. By using confocal microscopy and Forster energy transfer technique, we observed interactions between AnxA6 and phospholipase D1 (PLD1). The later enzyme had been shown to be activated at exocytosis site on the plasma membrane. We also developed an in vitro method based on infrared spectroscopy which allows a direct measurement of PLD activity with its natural substrates. This enzymatic assay may serve as a tool for screening PLD inhibitors and to determine the effects of AnxA6 or other partners on PLD activity. To confirm AnxA6-PLD1 interaction, co-immunoprecipitation is used. By producing human recombinant PLD1, we would like to reconstitute its interaction with AnxA6 in vitro.

S1.4:

Post-traumatic epileptogenesis in APP/PS1 mouse model of Alzheimer`s disease

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Amyloidogenesis is a common pathology in traumatic brain injury (TBI) and Alzheimer`s disease (AD), both of which associate with an elevated risk of epilepsy. Moreover, TBI is a risk factor for AD, facilitating amyloidogenesis. To address the question whether increased amyloid-β load facilitates post-traumatic epileptogenesis we induced TBI in 13-15wk old APP/PS1 mice (n=14) and Wt littermates (n=17). 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 composite 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 (24h/7d, 2wk) performed at 6wk 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 (24h/7d, 2wk) starting at 14wk 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 in APP/PS1 injured mice results in more pronounced epileptogenesis and more severe motor and cognitive co-morbidities following TBI.

SESSION 2

S.2.1:

Endocannabinoid signaling in regulation of insulin secretion

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The pancreatic β cells exhibit remarkable abilities to change their function in response to altered tissue insulin demands. The mounting body of evidence suggests important role of lipid neuromodulators, endocannabinoids (eCBs) in regulation of insulin release. However, the molecular cascade linking agonist-induced cannabinoid receptor activation to insulin secretion remains unknown. Here, we aim to elucidate the role and mechanism of eCBs' action on

insulin secretion. We combine molecular pharmacology and genetic tools carring the experiments out in INS-1E β cell line and pancreatic islet isolated form wild type and cannabinoid receptor 1 (CB_1R) knockout mice. RT-PCR, western blot analysis, immunofluorescence and insulin secretion assay are used to investigate presence, role and mechanism coupling eCBs signaling to insulin release. Both β cells and pancreatic islets exhibit functional and autonomous eCBs signaling (receptors and enzymatic machinery regulating anandamide (AEA) and 2-arachidonoylglycerol (2-AG) bioavailability). We show that AEA and 2-AG potentiate insulin secretion. Observed eCBs' effect depends on CB_1R activation since it is absent in the pancreatic islets isolated from $CB_1R^{-/-}$ mice and impeded only when its antagonist (O-2050) or reverse agonist (AM251) are applied. CB_1R stimulation leads to activation of Akt and extracellular signal-regulated kinases 1/2 and further phosphorylation of focal adhesion kinase (FAK). CB_1R-mediated FAK activation induces the formation of focal adhesion plaques and stress fibers, facilitating the second-phase of insulin release. We show that inhibition of eCBs synthesis or FAK activity forecloses insulin release. The obtained results show FAK downstream from CB_1Rs mediates eCBs-induced insulin release by allowing cytoskeletal reorganization that is required for the exocytosis of secretory vesicles.

S2.2:

Neuroprotection from TIMP-1 and its Nanoparticles

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There is a marked increase in expression of Matrix Metalloproteinase-9 (MMP-9) during numerous pathologic conditions, including excitotoxicity. Therefore inhibition of MMPs is considered as a potential therapeutic target. Tissue Inhibitor of Matrix Metalloproteinase-1 (TIMP-1) is a 28 KDa 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. We also evaluated these NPs for their blood brain barrier (BBB) penetration. The NPs were coated with tween 80 and used rat brain capillary endothelial cell culture to study uptake/binding, toxicity and BBB. The results showed, the NPs without tween 80 coating have higher uptake/binding to endothelial cells as compared to tween 80 coated NPs. Lucifer yellow (LY) assay was used for toxicity studies, suggesting that NPs do not cause opening of BBB and hence they are non-toxic. For BBB penetration studies across cell monolayer, we used fluorescence spectrophotometric assay for dye loaded NPs and for TIMP-1 loaded NPs we used TIMP-1 ELISA. In the group treated with TIMP-1 NPs coated with tween 80, 11.21% ± 1.35% of TIMP-1 was detected in lower compartment of endothelial cells. To summarize, TIMP-1 loaded NPs coated with tween 80 are non-toxic to endothelial cells and they showed some sort of BBB penetration.

S2.3:

Synaptic machinery alterations and nucleotide receptors-related signaling in hippocampus of normal and dystrophic mice

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Duchenne muscular dystrophy (DMD) is a lethal neuromuscular disease caused by mutation of the dystrophin encoding gene. Although muscular disorder is the major life-threatening consequence of DMD, this disease is also one of a few well-known single gene defects, which result in cognitive impairment and mental retardation. In this study putative alterations of the P2X-dependent Ca^{2+} signaling in hippocampal neurons of dystrophic mice are the main focus. Knowing the cellular distribution of P2X7 receptors in the brain would be important not only for our understanding of this receptor function but may explain its involvement in DMD-associated mental retardation.
P2X7 receptor level has been found to be inversely dependent on the expression of $GABA_AR$ gene. “Inhibitory” interactions between $GABA_A$ and P2X native receptors has previously been described and we showed increased P2X7 expression level in dystrophic brains in our previous experiments. That is why we have examined $GABA_AR$ subunits distribution in hippocampi. The only change we have observed concerned decreased synaptic clustering of alpha1 and less inhibitory receptors within the synapse in dystrophic mutants, what may have functional implications and impair inhibitory modulations of pyramidal neurons.
Differences in the density of various inhibitory synapses within stratum pyramidale and stratum radiatum have also been observed. Moreover, immunostaining for proteins relevant to GABAergic synapses showed decreased VGAT, CB1 and NL2 in stratum pyramidale of mdx with more than expected puncta in stratum radiatum. This infers aberrant synapse formation and, collectively, may imply the cognitive impairment.

S2.4:

Deficit of arginine affects actin organization in neuronal tumor cells

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It was shown that arginylation of β-actin affects actin filament organization and in consequence cell migration [Kashina et al. (2006) Science 313, 192-196]. In this study, we have attempted for the first time to examine whether decreasing the level of arginylation by culturing glioma cells in arginine-deprived conditions affects their invasiveness. Noteworthy, while arginine is non-essential for normal cells it was shown to be essential for several cancer cell lines.
Human glioma U251MG cells were cultured up to 144 hours in three different conditions: (i) standard medium; (ii) medium lacking lysine and (iii) medium lacking arginine. At several time points, we have examined cell morphology (by means of scanning electron microscopy), cytoskeleton organization (by means of immunohistochemistry and evaluation of G- and F-actin content in cell fractions), adhesive complex formation (immunostaining for vinculin) as well as cell migration (by means of wound-healing test) and invasiveness (with the use of

Transwell system). We showed that arginine-deprivation but not lysine-deprivation significantly impaired cell growth and morphology (cells became elongated and their leading edges were significantly reduced), actin organization (less polymerized actin), adhesiveness (decreased amount of adhesive complexes), and significantly inhibited cell migration and invasiveness. The effects were seen during the course of the entire experiment.
We propose that arginine-deprivation, causing a decrease in actin-arginylation, may be considered as the novel strategy of anti-brain tumor metabolic therapy.

SESSION 3

S3.1:

Carnitine transporters in the blood-brain barrier

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L-carnitine (3-hydroxy-4-trimethylammoniobutyrate) (LC) in peripheral tissues participates in energy production. It's role is to transfer fatty acids from cytoplasm into the mitochondria. In the brain, which main source of energy is glucose, LC plays distinct functions. In recent years more attention is directed to LC and it's acylated derivatives as a neuroprotective agent. Carnitine is transported through the blood-brain barrier (BBB) by two transporters, sodium-dependent organic cation/carnitine transporter (Octn2), which transports LC with high-affinity and amino acid transporter $B^{0,+}$ ($ATB^{0,+}$) capable of transporting carnitine with low affinity. In our study we use co-culture model of rat glial cells and bovine brain endothelial cells grown on collagen coated microporous membrane. The present study focuses on localization, function and regulation by protein kinase C (PKC) of carnitine transporters in the BBB.

S3.2:

Glycogen synthase kinase-3beta affects size of dentate gyrus and species-typical behavioral tasks in transgenic and knockout mice

Ilona Kondratiuk

Glycogen synthase kinase-3 (GSK-3), a multifunctional serine-threonine kinase, is an important regulator in numerous signaling pathways and processes including adult brain neurogenesis. GSK-3 (mal)functioning was implicated in many diseases, in particular neurological and behavioral disorders. We investigated the impact of altered levels of the GSK-3β isoform on hippocampal size, number of doublecortin-positive cells, and hippocampal-dependent behaviors. Both GSK-3β transgenic mice (GSK-3β[S9A] mice) and GSK-3β neuron-specific knockout (GSK-3β(n-/-)) mice, showed reduced size of the dentate gyrus (DG) and were impaired in three hippocampal-dependent, species-typical behavioral tasks: digging, marble burying and nest building. We further demonstrate that the number of differentiating, doublecortin-positive new neurons is reduced in GSK-3β[S9A] mice, but not in GSK-3β(n-/-) mice. We conclude that GSK-3β activity must be critically controlled to allow wild type-like volume of the dentate gyrus and for normal execution of hippocampal-dependent, species-typical behavior.

S3.3:

Grafted embryonic motoneurons contribute to the functional improvement of denervated hindlimb muscles

Gál László

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In our study embryonic spinal cord tissue (E13-eGFP rat) was transplanted into the L4 segment of Sprague-Dawley rats after L4 ventral root avulsion and reimplantation. The aim of our experiments was to investigate the morphological and functional integration of the grafted motoneurons into the host circuits. Two experimental groups have been set up: in the control group the animals underwent ventral root avulsion and reimplantation, but did not receive embryonic graft, animals in the second group received an embryonic graft after avulsion and reimplantation. Six months later EMG was recorded from ankle flexors and extensors of the hindlimbs on the intact and operated sides. During locomotion the motoneuron activity was related to rhytmic locomotor limb movement. Two populations of motor unit action potentials (MUAPs) were distinguished in the grafted animals. The first population of MUAPs was recruited in a long period of time (firing below 50 Hz) in sitting position. The second group of MUAPs was detected during walking in a range between 50 and 200 Hz firing for short periods of time (<200 ms) with relatively higher amplitude. Retrograde labelling from the reinnervated muscles provided evidence that both the graft and host neurons contributed to the reinnervation of the denervated hindlimb muscles. Anterograde tracing with Phaseolus vulgaris revealed substantial reciprocal connections between the graft and the host spinal cord circuitry. These results have clearly shown that grafted embryonic motoneurons are able to integrate into the host circuitry.

S3.4:

Arc functional neighbourhood in the neuronal cell nucleus

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Arc protein is a versatile factor connecting memory formation and plasticity changes with 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 very 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.

SESSION 4

S4.1:

Novel strategy for the protection from oxidative stress in rodent mitochondria

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Mitochondria are considered one of the main sources of reactive oxygen species (ROS), which (when produced extensively) can evoke intracellular oxidative stress. Cells possess a number of both enzymatic and non-enzymatic defense systems to protect them from ROS. Here we propose a new strategy through which mitochondria alleviate the destructiveness of ROS through their withdrawal from the lesion-prone localization inside the mitochondrial matrix.

ROS production was measured in rodent mitochondria, isolated from various tissues (in ROS production promoting conditions) with the use of the AmplexRed/Resofurin fluorescent probe. Accumulation of oxidative stress was assessed by the inactivation of aconitase.

Release of ROS from mitochondria was significantly decreased by millimolar concentrations of GDP. At the same time intramitochondrial ROS accumulation, measured by inactivation of aconitase, increased.

Here, we suggest a novel function for UCPs other than their uncoupling activity, namely the removal of the superoxide anion radical from the mitochondrial matrix. Such a novel strategy along with the antioxidant defense system provides additional protection against ROS.

S4.2:

The role of transcription regulators Stat1 and Stat3 in microglia activation

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Uncontrolled and prolonged inflammation is associated with earlier onset and/or progression of virtually all neurological diseases including Alzheimer's and Parkinson's disease, brain trauma and stroke. Microglial cells accumulate in regions of degeneration and produce a wide variety of pro-inflammatory molecules. The Janus Kinase (JAK)-Signal transducer and activator of transcription (STAT) pathway converts the cytokine or TLR signals into gene expression programs that regulates immune and glial cell functions. Stat targets and molecular mechanisms underlying inflammatory activation of microglia are unknown. In primary

microglial cultures lipopolysacharide (LPS) induced stimulation leads to rapid activation of Stat1, 3, and 5. We mapped the genome-wide occupancy of active, phospho-Stats by hybridization of immunoprecipitation-enriched genomic DNA to promoter microarrays (3x720K RefSeq Promoter microarrays, NimbleGen). We found correlation of active Stat1 and 3 binding sites with changes in expression of many genes encoding cytokines/chemokines and transcription regulators. The most interesting hit, representing a newly identified Stat target, was jmjd3, encoding a JmjC family histone demethylase and transcription factor, which controls inflammatory gene expression in peripheral macrophages. Silencing of Stat1 and Stat3 blocked Jmjd3 and inflammatory gene expression in BV2 microglial cells, while overexpression of constitutively active Stat1 and Stat3 was sufficient to induce Jmjd3 and inflammation-related genes in the absence of LPS. Action of Jmjd3 did not depend on its histone demethylase activity, but was lost after interference with its transactivator domain. These data show that Stat1 and Stat3 are necessary and sufficient for initiation an appropriate inflammatory response.

S4.3:

New insights into interactions of hemin and calcium-regulated BK channel in rat brain mitochondria

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Heme is a prosthetic group that consists of an iron atom bound in the center of a porphyrin ring. It is an essential element of hemeproteins in all living organisms. Although heme is ubiquitous, its circulation is strictly controlled. Therefore it is believed that it may play as yet unknown regulatory functions.
The mitochondrial calcium-dependent BK channel (mitoBK$_{Ca}$) is one of the five known channels that contribute to potassium permeability of mitochondrial inner membrane. It is activated by calcium and voltage and inhibited by scorpion venom toxins such as charybdotoxin and iberiotoxin.
In the current study, we have checked the impact of the oxidized heme (hemin) on mitochondrial membrane potential and respiration rate of rat brain mitochondria. We have shown that hemin prevents the collapse of membrane potential that is normally caused by calcium-dependent BK channel openers (NS1619). A similar, though rather modest effect was observed in studies of oxygen consumption rate. We also report inhibitory effects of hemin on the reactive oxygen species-downregulating properties of NS1619.
Additionally, we have studied the single channel activity of mitoBK$_{Ca}$ by patch-clamp of mitoplasts isolated from a rat astrocyte cell line. Other results confirm the phenomenon of reversible inhibition of mitoBK$_{Ca}$ channel by hemin.
This might explain some of the cytotoxic effects of hemin observed in hemorrhagic stroke.

S4.4:

Dimerization of CacyBP/SIP as a "molecular switch" for ERK1/2 binding

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CacyBP/SIP (Calcyclin Binding Protein and Siah-1 Interacting Protein) has been shown to interact with several proteins. While the precise function of CacyBP/SIP remains unclear, it has been suggested that it might play a role in cytoskeleton organization during differentiation of neuroblastoma NB2a cells through interactions with tubulin and actin or through dephosphorylation of ERK1/2 [Schneider and Filipek, 2011].
In this work, we analyzed the CacyBP/SIP dimer organization and its potential impact on interaction with ERK1/2 kinase. Using biophysical methods such as size-exclusion chromatography (SEC) and multi-angle light scattering (MALS) we show that CacyBP/SIP forms remarkably stable dimers. We established the shape and size, as well as the 3D structure of CacyBP/SIP dimer at low resolution by small-angle X-ray scattering (SAXS). Computational modelling of the N-terminal domain of CacyBP/SIP suggests that it is responsible for dimerization. The involvement of this domain in dimer formation was confirmed by SEC-MALS. Mutations of key residues predicted to be engaged in formation of CacyBP/SIP dimers appear to alter the stability of the dimer as reflected in a dimer-monomer equilibrium. Interestingly, these residues are located within one of the two Kinase Interacting Motif (KIM) sequences identified in CacyBP/SIP, one in the N-terminal and the second one in the C-terminal domain. Interestingly, these KIM sequences are specific for ERK1/2.
Our studies suggest a "molecular switch" model in which CacyBP/SIP regulates ERK1/2 signalling, in the sense that dimerization will block the N-terminal KIM motif from interacting with ERK1/2.
This work was supported by the European Union through the International PhD Studies in Neurobiology Program (MPD4-502), NIH grant R01GM075156 to W.J. Chazin and by statutory funds from the Nencki Institute of Experimental Biology.

SESSION 5

S5.1:

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

S5.2:

Cat as animal model in studies of the visual system

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Cat is a predator with extremely good vision. This animal can react quickly to natural visual stimuli as mice or bats, or even spot of light shone on the wall. Cat's visual system is in many aspects similar to monkey or human visual system, thus this animal is a good model for studying visual system. In our laboratory we study visual responses of neurons in the superior colliculus (SC), the first structure after retina in the extrageniculate visual pathway. This structure is responsible for visually guided behavior and saccadic eye movement by which it takes part in basic processes of visual attention. SC is a laminar structure, located in the midbrain. Superficial layers of the SC contain neurons responding only to visual stimuli. Those neurons respond both to slow and to fast changes in the visual field. Some neurons respond to visual stimuli by sustained increase of firing rate, other produce oscillatory changes in spike generation. In our studies we are interested in role of such oscillations in processing of visual information and whether type of visual stimulation can influence oscillatory responses. Our results indicate positive correlation between velocity of presented stimulus and strength of oscillations in the SC. Moreover, our data indicate that oscillations improve processing of information by increase of reliability and temporal precision (calculated as standard deviation of first spike time in the response) and decrease of variability of neuronal responses. Finally we suggest that stimulus phase-locked oscillations enhance transfer of visual information in the visual system.

S5.3:

The role of STAT3 in population of cancer stem-like cells in human and murine melanoma cells

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Transcription factor STAT3 is a point of convergence for numerous oncogenic signaling pathways and through deregulation of gene expression may promote uncontrolled growth and survival of tumor cells. Recent evidence suggests an important role of STAT3 in cancer stem cells however its role in their biology is poorly understood. We found active STAT3 in both

primary and metastatic human melanoma cells and murine B16 melanoma cells. Metastatic human melanoma cells have increased levels of phosphorylated STAT3 in comparison to non-metastatic cells. Microarray analysis of transcriptome of melanoma cells depleted of STAT3 with siRNA treatment revealed a set of genes which are likely STAT3 targets. Chromatin immunoprecipitation followed by qPCR validated their dependence on STAT3 expression. The presence and potential role of active STAT3 in population of cancer stem like cells in melanoma was investigated. We have applied two protocols for enrichment of melanoma cell lines in cancer stem like cells: FACS sorting of rhodamine – subpopulation and sphere cultures. The expression of NANOG and OCT4 was determined with qPCR to confirm the stem-like phenotype. We set up sphere cultures from cell lines which have different requirement for culture conditions. Our preliminary results show the higher level of phosphorylated STAT3 in population of isolated melanoma stem like cells comparing to bulk cells. We also demonstrate a decrease in sphere forming ability of human and murine melanoma cells after STAT3 depletion with siRNA that suggests the involvement of this transcription factor in maintenance of cancer stem-like cells

S5.4:

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 um 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.

S5.5:

mTOR kinase role in dendritic arbor formation of neonatal born neurons

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Mammalian Target of Rapamycin (mTOR), play an important role in transmitting signals to cellular effectors during dendritogenesis. Dysregulation of mTOR activity is often connected to neurological disorders. Therefore, precise mTOR role in neuron development need to be established. For that we decided to standardize technique which allows in vivo visualization of neurons in olfactory bulb (OB). The olfactory bulb is one of two regions in the adult brain where new functional neurons are continuously incorporated into pre existing neuronal circuits. The OB is a destination for neuronal progenitors born in subventricular zone (SVZ), which migrate through the rostral migratory stream (RMS). Therefore, SVZ-RMS-OB is a unique system to study molecular mechanisms of neurogenesis, neuronal development and neuronal network reconstruction in vivo. While importance of mTOR has been previously demonstrated for dendritic arbor development of embryonic neurons, it remains unknown if exact same molecular mechanisms drive dendritic arbor development of neonatal born neurons. We have shown a high activity of mTOR kinase in OB, an area of adult-born neurons differentiation. Consequently, we have showed using in vitro cultured SVZ-derived, postnatally-born neuroprogenitors, forced to differentiate into neurons that mTOR activity is needed for proper development of their dendritic arbors. Moreover with use of mTOR inhibitor (Rapamycin) and in vivo electroporation we showed that decrease level of active mTOR leads to reduction in dendritic branching in olfactory bulb neurons. Both in vitro and in vivo studies suggest that mTOR is crucial for neuron development of neonatal born neurons. Currently, we are performing experiments, with use of in vivo electroporation to provide more direct evidence for a key role of mTOR for incorporation of neonatal born neurons into already functional circuits of OB.

S5.6:

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 μm3. 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.

S5.7:

New regulators of cell signaling among endocytic proteins

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Signaling via NF-κB transcription factors is of major importance in health and disease. Although numerous ligands, receptors and mediators of the cascade leading to NF-κB activation are well described, the knowledge of its basal regulation is largely limited. Similarly, little is known about the role of endocytosis and membrane trafficking in NF-κB signaling. The aim of this study was to identify endocytic proteins that regulate the basal NF-κB activity.
We performed a targeted RNAi-based screen in human cells using a luciferase reporter assay as readout. We found that the depletion of several endocytic proteins potently activates NF-κB-dependent transcriptional reporter under basal conditions, in the absence of cytokine stimulation. These effects were confirmed by quantitative PCR analysis which demonstrated increased expression of several NF-κB target genes upon knockdown of the selected genes. At the biochemical level, depletion of screen hits activates two branches of NF-κB signaling: canonical and non-canonical. Moreover, the two branches are activated independently of each other upon depletion of selected genes. Importantly, the role of the identified hits in NF-κB signaling seems to be evolutionarily conserved, as their knockdown in zebrafish embryos increases expression of NF-κB target genes.
Cumulatively, we identified novel negative regulators of basal NF-κB signaling, acting along with the IκB inhibitors. Inappropriate pathway activity may result in serious pathologies. Our data suggest a new mechanism to limit such activity under basal conditions.

S5.8:

Mitochondrial potassium channels in Dictyostelium discoideum

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Mitochondria are crucial not only in energy metabolism but also in regulation of cell senescence and apoptosis. The strict control of inner mitochondrial membrane permeability and selective ion transport is essential for mitochondria functioning. Potassium ions homeostasis is an important process for mitochondrial optimal functioning. Potassium channels such as ATP-regulated, large conductance calcium activated and voltage dependent channels were observed in inner mitochondrial membrane in various mammalian tissues. Recently, we have identified potassium channels in inner mitochondrial membrane of potato Solanum tuberosum and Acanthamoeba castellanii. Currently we characterize mitochondrial potassium channels from one of Dictyostelium species. It is commonly used as a model organism to study cell differentiation, metabolism and programmed cell death. Preliminary experiments are focused on biophysical and pharmacological characterization of mitochondrial ion channels. Purified inner mitochondrial membranes (submitochondrial particles) were reconstituted into planar lipid bilayer. To form model membranes asolectin from soybean mixture of phospholipids was used. We observed two types of potassium selective ion channels in submitochondrial particle samples: a large- and small-conductance channels. Experiments were performed both in gradient solution 50/150 mM KCl (cis-trans) and in symmetrical solution 150/150 mM KCl at voltages from -50 to 50 mV. Regulation of the channel activity by divalent cations such as Ca^{2+} and Mg^{2+} was explored. Additionally, interaction of the ATP with mitochondrial potassium channels was characterized. The knowledge on mitochondrial ion channels may contribute to understanding molecular mechanism of Dictyostelium discoideum functioning.

S5.9:

The interplay between the mitochondrial inner membrane formation MINOS complex and MIA pathway responsible for protein transport

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Mitochondria are essential organelles in the eukaryotic cell, which play a crucial role in energy metabolism and regulatory processes. The vast majority of the mitochondrial proteins are synthesized in cytosol and therefore must be imported into this organelle. All precursor proteins utilize the translocase of the outer mitochondrial membrane, TOM, as the main gate to enter mitochondria. After passing through the TOM complex the protein precursors that are directed to the mitochondrial intemembrane space use the specialized MIA (Mitochondrial Import and Assembly) pathway [1]. Mia40, the key component of this pathway, facilitates import and biogenesis of precursor proteins in a redox-dependent manner. Mia40 recognizes precursors emerging from the TOM complex and specifically transfers the disulfide bonds, thus enabling the proteins' transport, folding and assembly.

Our recent studies showed that the protein import activity of Mia40 is regulated by Fcj1 (Formation of Cristae Junctions 1) protein, a key component of the protein complex responsible for maintaining the proper morphology of mitochondria – MINOS (Mitochondrial INner membrane Organizing System) [2]. We investigate the mechanisms underlying the interplay between the process of inner membrane formation by MINOS complex and protein sorting facilitated by MIA.

[1] Chacinska A, Pfannschmidt S, Wiedemann N, (2004). Essential role of Mia40 in import and assembly of mitochondrial intermembrane space proteins. EMBO J 23, 3735-3746.
[2] von der Malsburg K, Müller JM, Bohnert M, et al. (2011) Dual Role of Mitofilin in Mitochondrial Membrane Organization and Protein Biogenesis. Developmental Cell 21, 694-707.

S5.10:

Search for new Antiviral compounds against human entroviruses using fragment screening methodology

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Picornaviridae are among the most diverse and oldest "known" viral families that include many important pathogens of humans and animals. They are small, icosahedral ss+RNA viruses, causing a variety of diseases. Vaccines are available for PV, HAV and FMDV, but no effective prophylaxis is implemented for other picornaviruses. So far, anti-viral research has focused on the capsid, whereas inhibitors targeting non-structural proteins (i.e. proteases, helicases, polymerases) have remained largely unaddressed.
We are developing the project focused on searching for novel antiviral compounds against human enteroviruses (HEV) via fragment screening methodology based on STD-NMR technique. The protein target is picornaviridae protease.
Validation and profiling of the most promising non-covalent hits were done using surface plasmon resonance (SPR) technology and proteolytic activity assay. Co-crystallization/soaking of the most potent compounds and their analogs with protein target are being carried out to obtain their 3D structures by X-ray crystallography. The data provided by NMR, SPR and crystallography techniques will identify the close contacts between a fragment hit and a protein. It can help to infer the requirements underlying the association and suggest novel non-covalent ligands by both fragment-growth and fragment-linking strategies. Consequently, new binders could be obtained that eventually will become leads for further development.

SESSION 6

S6.1:

The role of STAT3 and its transcriptional network in human melanoma cells

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Aberrant Stat3 promotes uncontrolled growth and survival through dysregulation of gene expression, including cyclin D1, c-Myc, Bcl-xL, Mcl-1 and survivin genes, and thereby contributes to oncogenesis. However, the mechanisms that associate STAT3 in cancer invasion are poorly understood.
STAT3 is one of the critical players in human cancer formation and genes regulated by it represent valid targets for novel anticancer therapy. The main aim of this study was to identify the genes targeted by inhibition of the STAT3 expression in melanoma WM239 and T1 cells and to study their roles in tumorigenesis in particularly metastasis.
Gene expression analysis of microarray data indicated 20 genes up or down-regulated following STAT3 silencing. Functional analysis of these differentially regulated genes revealed that STAT3 silencing significantly affected the expression of a small but interesting subset of genes involved in invasion (SERPINA3), antigen processing and presentation (CD74), chromatin remodeling (SMARCA2) and (TSPAN10).
SERPINA3 (serine protease inhibitor-3) is known to play a part in invasion, but its exact functional role remains elusive. PCR results confirmed a reduction in the level of SERPINA3 expression in STAT3-depleted cells. This was followed by a scratch assay that showed after silencing of STAT3 the WM239 and T1 cells have significantly reduced potential to migrate to cell free area when compared to the control cells. The matrigel invasion assay coupled with Laser Scanning Cytometry revealed that there is a reduction in the number of cells migrating through the matrigel after silencing STAT3 compared to cells treated with control siRNA.

S6.2:

Age-related differences in duration comparison revealed by MMN (poster)

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3) CBRU, University of Helsinki, Helsinki, Finland

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 yrs.) and elderly (aged: 61-71 yrs.) 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/1

S6.3:

Ultrastructural studies on CD14 distribution in LPS-stimulated macrophages

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Lipopolysaccharide (LPS) of Gram-negative bacteria induces pro-inflammatory responses of macrophages. LPS recognition requires its binding to CD14 protein on the macrophage surface which transfers LPS to a signaling complex of toll-like receptor 4 (TLR4). We undertook confocal and electron microscopy studies on CD14 distribution in macrophages. These included ultrastructural analysis of CD14 localization in the plane of sheets of the plasma membrane obtained by mechanical cleavage of J774 cells and subjected to immunogold labeling. We found that stimulation of cells with 100 ng/ml LPS induced transient clustering of CD14 in the plasma membrane reflected by a shift of CD14-attributed gold labels from singlets to aggregates of more than 10 particles. CD14 clusters colocalized with PI(4)P, a precursor of $PI(4,5)P_2$, a lipid controlling an assembly of TLR4 signaling complexes. In addition, numerous vesicles rich in CD14 fused with the plasma membrane during LPS action. In these vesicles, caveolin but not clathrin, was also found. Biochemical analysis indicated that CD14 was enriched in raft fractions of the plasma membrane at the onset of cell stimulation with LPS. The CD14-rich vesicles were devoid of MyD88, an adaptor protein crucial for TLR4 signaling. However, MyD88 colocalized with CD14 and $PI(4,5)P_2$ distributed outside the vesicles. We assume that the vesicles bring new pools of CD14 to the plasma membrane in LPS-stimulated cells and contribute to a moderate elevation of the amounts of surface CD14, as indicated by flow and laser scanning cytometry analysis. The MyD88-containing signaling complexes of TLR4 are formed outside of these structures.

S6.4:

Alterations in lipid metabolism by endogenously synthesized 2-AG leads to enhanced insulin action in skeletal muscle

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The endocannabinoid system (ECS) is a part of the neuromodulatory system and plays an important role in regulation of many physiological functions like pain perception and feeding behavior. Moreover ECS is also present in peripheral tissues where it has been shown to regulate energy metabolism, including lipogenesis in adipose tissue and liver and glucose uptake into skeletal muscle. One of the most abundant endocannabinoid present not only in nervous system but also in peripheral tissues is 2-Arachidonyloglicerol (2-AG). In a condition of obesity ECS becomes overactivated and might be involved in the pathogenesis of type 2

diabetes. It has been already shown that key components of the ECS, cannabinoid receptors (CB1R and CB2R) and enzymes that synthesize and degrade 2-AG, diacylglycerol lipase and monoacylglycerol lipase, are present in human and rodent skeletal muscles. However, it is still unknown what is the role of endogenously synthesized 2-AG in the modulation of insulin sensitivity in skeletal muscle. During our studies we showed that high fat diet increases the level of CB1R and the enzyme that synthesizes endocannabinoids. Using differentiated C2C12 cells, we showed that endogenously synthesized 2-AG increased insulin response. Yet, this effect was not mediated by CB1R signaling pathway. However, high level of endogenously synthesized 2-AG led to activation of peroxisome proliferator-activated receptors, decrease in free fatty acids content and changes in proteins involved in lipid metabolism. These results suggest that endogenously synthesized 2-AG might play an important role in enhancing insulin action by changing lipid metabolism in skeletal muscle.

S6.5:

TNFα affects mitochondrial metabolism and nitric oxide production in vascular endothelial cells

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Vascular endothelium is an active endocrine organ which produces and releases a wide range of diverse molecules that act inside the vessels as well as in a smooth muscle layer. Endothelium controls inflammation processes and plays an important role in maintaining a delicate balance between vasoconstriction and vasodilatation. Nitric oxide produced and released upon stimulation of endothelial cells serve as signalling molecule is of a crucial importance in proper endothelial functioning. Under various pathological conditions endothelium undergo proinflammatory stimulation. TNF-alpha is one of pivotal mediators of inflammatory response, hence, prosurvival pathway in endothelial cells probably due to activation of NF□B. It also stimulates proapoptotic events including an excessive mitochondrial ROS production. Here a mitochondrial response in human endothelial cells (EA.hy926) stimulated with TNF-alpha was investigated. TNF□ caused an inflammatory response (increased ROS generation and elevated ICAM protein level). These results paralleled with increased oxygen consumption, enhanced level of MnSOD and UCP2 protein content as well as slight increase in mitochondrial mass. Moreover, a rise of protein levels of selected respiratory chain complexes and transcriptional factors like TFAM, NRF1, PGC1□ which are involved in regulation of mitochondrial biogenesis was also observed. In addition, elevated level of NO (molecule associated with mitochondrial biogenesis) was found after TNF-alpha treatment. Thus, an observed stimulatory effect of TNF-alpha on mitochondrial metabolism most likely reflects increased amount of mitochondria rather than activation of biochemical processes per se. It could be a mechanism which is activated to prevent TNF-alpha induced cell death.

S6.6:

The novel role of Mia40 in biogenesis of membrane proteins in mitochondria

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Proper biogenesis of mitochondria is a crucial process for cell viability. The majority of mitochondrial proteins are synthesized on cytosolic ribosomes, therefore they have to be selectively transported to the final destination site in mitochondria. Mitochondria developed several machineries specialized in recognition and sorting of precursor proteins. The MIA (Mitochondrial Intermembrane Space Assembly) pathway is generally viewed to be dedicated to the redox-dependent import and biogenesis of intermembrane space (IMS) proteins. The Mia40 oxidoreductase, a central component of the pathway, is responsible for the maturation of the incoming precursor proteins. This process involves the transfer of disulfide bonds from Mia40 to the precursors proteins followed by their oxidative folding. Our results indicate that Mia40 is also involved in the biogenesis of the proteins localized in other mitochondrial compartments.We present the first evidence that the function of Mia40 is not restricted to the transport and oxidative folding of soluble IMS precursor proteins. We show that Mia40 is directly involved in the biogenesis of the inner membrane protein, Tim22 – an essential core component of the TIM22 translocase. This highly conserved membrane protein forms a disulfide-bonded intermediate with Mia40 upon import into isolated mitochondria. Interestingly, Mia40 recognizes and binds Tim22 precursor also via non-covalent interactions. We propose that Mia40 is not only responsible for disulfide-bond formation, but also serves as a chaperone that assists the Tim22 protein in its integration into the inner membrane of mitochondria.

S6.7:

Identification of endocytic proteins involved in IFN-α stimulated JAK-STAT signaling

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The role of endocytosis in signal transduction has been traditionally viewed as a mechanism for internalization and degradation of activated receptors in lysosomes. However, this model is changing with reports suggesting that endocytic proteins also participate in various aspects of cellular signaling and transcriptional regulation.
The aim of our study is to identify endocytic proteins involved in type-I interferon (IFN-α) stimulated JAK-STAT signaling. IFN-α is a cytokine with potent anti-viral and anti-proliferative activities. It is produced in response to pathogenic antigens. In humans, IFN-α uses the IFN-α receptor (IFNAR) complex, composed of IFNAR1 and IFNAR2 chains, for its signaling. It was shown that IFNAR1 receptor was internalized by clathrin- and dynamin-dependent endocytic pathway (Marchetti et al., 2006 Mol Biol Cell 17, 2896-2909). Therefore, it is reasonable to suspect that IFNAR might be exploiting a broader spectrum of endocytic machinery to modulate its signaling. However, so far, there is very little information to support this idea.
We performed small-scale RNAi-based screening to identify the endocytic proteins that perturb the JAK-STAT pathway. The methodology includes silencing of pre-selected individual endocytic genes. This was followed by analysis of transcriptional response using luciferase-

based reporter assays upon IFN-α stimulation. Genes that manifested more than two-fold change in the transcriptional activity upon silencing were considered as potential regulators of the JAK-STAT pathway. We have identified both positive and negative regulators of the pathway, including previously reported dynamin and clathrin. Subsequently, IFN-α target gene expression (such as OAS1, IFI44 and IFI6) was tested upon silencing of these potential regulators using real-time PCR assay. Searches of literature and online repositories are currently being performed to identify the interacting partners of these endocytic regulators. Expression pattern of the proteins interacting with the silenced endocytic regulators will be studied using western blots and co-immunoprecipitation assays. Results from these assays should elucidate the mechanisms by which the endocytic regulators alter the IFN-α signaling pathway.

S6.8:

Genetically encoded FRET-based biosensor for MMP-9 activity

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Here we developed a genetically encoded FRET-based biosensor to monitor the activity of matrix metalloproteinase 9 (MMP-9). MMP-9 is an extracellular acting endopeptidase implicated in both physiological and pathological processes. A genetically encoded FRET biosensor anchored in the cellular membrane provides an important advantage over currently employed probes. The sensor allows studying the proteolytic activity of MMP-9 with high spatiotemporal resolution at the exact region of MMP-9 action on the cell. Applicability of the sensor, both in vitro and in vivo in living cells, was demonstrated by ratiometric analysis of cleavage of the sensor by a purified auto-activating mutant of MMP-9.

S6.9:

Ultrastructural rearrangement of the neuronal cell nucleus in synaptic plasticity

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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 trancription events in neurons.
This work was supported by National Science Center grant GP4516 (Poland).

S6.10:

Structural studies of the protein machinery for DNA processing and translocation in bacterial conjugation

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Whatever the route used, horizontal gene transfer requires sophisticated multi-protein machinery to enable the long and charged DNA polymer to cross the cell envelope barriers. The best-studied system for cell-to-cell DNA translocation is bacterial conjugation, a major mechanism for genetic exchange in bacteria, which provides a route for the rapid acquisition of new genetic information and contributes to the spread of antibiotic resistance. Over the last decades research efforts in the field have resulted in the clarification of many aspects of this system and its machinery assembly.
The goal of our work is to explain, based on structural biology, the mechanism of action of some of the missing pieces of this phenomena. These factors include MobM relaxase, encoded on the Gram-positive streptococcal plasmid pMV158, as well as TrwC relaxase/helicase and TrwK protein (VirB4 homologue), the largest type IV secretion system component, both from the Gram-negative plasmid R388. Progress in the expression, purification, crystallization and structural characterization of these proteins is presented.

SESSION 7

S7.1:

Glycogen metabolism in cells with glycogen branching deficiency

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Glycogen branching enzyme (GBE1) deficiency leads to a genetic disease: glycogen storage disease type IV (GSD IV), affecting mostly the liver, skeletal muscles and the nervous system. The disease is a rare, genetic, autosomal recessive disorder. Its severity, age of onset and symptoms depend on the mutation the patient harbours. The GBE1 deficiency results is intracellular accumulation of poorly branched glycogen molecules, which have lower solubility and may lead to mechanical cell damage, slower glucose release from such glycogen, and in a consequence abnormal intracellular energy metabolism. It is not clear to which extent the cell malfunction is an effect of energy metabolism disruption or of mechanical damage to cells. In our study we work with primary human skin fibroblasts from patients with classical (childhood) and adult form of the disease. We have compared patient and control cells in

terms of GBE1 level, glycogen content, glycogen dynamics and branching. For the classical GSD IV model we find that the cells have a lower level of GBE1. The accumulation of glycogen is higher than in controls, even though glycogen synthase level remains unchanged. In stress induced conditions, this accumulation becomes even higher. In culture under starvation, patient cells are able to mobilize glycogen as much as the controls do.
Those results bring an interesting question – why do GBE1 deficient cells accumulate more glycogen? In further research on this model we will work on understanding that.

S7.2:

Infiltrating microglia show different gene expression profiles in low and high grade gliomas

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Microglia are myeloid cells residing in the central nervous system. These cells are supposed to participate in initiation of inflammatory and anti-tumor responses. In case of glioma microglia are attracted toward the tumor and accumulate in it. The number of infiltrating microglia positively correlates with tumor grade and invasiveness in in vitro and animal studies. Glioma cells secrete soluble factors which convert microglia and infiltrating macrophages into amoeboid cells with attenuated inflammatory responses and the switch to a pro-invasive phenotype. These alternatively activated microglia support tumor growth, invasion, angiogenesis and cause immunosuppression. To investigate the profiles of immune responses, we isolated human microglia from fresh, surgically removed low grade (WHO I, II) and high grade (WHO III, IV) glioma samples with the use of microbeads conjugated with $CD11b^+$. The expression of genes characteristic for classically activated brain macrophages: IRF7, NOS2, and IKBKB from the NFκB signaling pathway and for the alternative phenotype: ARG1, MT1-MMP, C-MYC was determined by qPCR. The results demonstrate distinct gene expression profiles in macrophages from benign and malignant glioma, and the expression of proinvasive phenotype genes in CD11b+ population from high grade gliomas.

S7.3:

Expression of Methyl-CpG-binding domain protein 3 (MBD3) in the rat model of temporal lobe epilepsy

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MBD3 is a member of the family of methyl-CpG binding domain containing proteins. In contrast to other MBD proteins it is not capable of binding to methylated DNA. It affects target gene activity as a subunit participating in formation of NuRD complex which is involved in transcriptional repression. MBD3 can act as transcriptional repressor. The aim of this study

was to characterize the MBD3 protein expression in the animal model of temporal lobe epilepsy induced by status epilepticus evoked by electrical stimulation of the amygdala.
Western blot analysis on proteins isolated from the dentate gyrus and extrahippocampal temporal tissue was performed. Three bands representing MBD3 were detected. Densitometric analysis revealed a decrease in MBD3 protein expression in the extrahippocampal temporal lobe 14 days after status epilepticus. Only decrease in intensity of the two upper bands reached statistical significance (0.75±0.32 fold of control, p=0.02, n=12). No difference between groups was observed in the dentate gyrus.
For immunohistochemical studies brains of control and stimulated animals were perfused and fluorescent immunostaining was performed. Sections of the brain were labeled with anti-MBD3 antibody and antibody directed against neuronal marker NeuN. The number of neurons and MBD3-positive cells as well as intensity of MBD3 fluorescence were measured.
Alterations in the expression of MBD3 protein in the temporal lobe during epileptogenesis may lead to changes in transcription of genes crucial for epilepsy development.

S7.4:

Identification of serum response factor (SRF) –dependent genes in the kainic acid model of aberrant synaptic plasticity

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Epilepsy is a chronic neurological disorder characterized by recurrent unprovoked seizures. Aberrant synaptic plasticity is known to play a pivotal role in epilepsy, yet the molecular mechanism underlying this pathology remains still unknown. SRF is a transcription factor that plays a prominent role in various programs of gene expression in the brain, including neuronal plasticity. Moreover, SRF protein accumulation and phosphorylation as well as increased binding of SRF to DNA was observed after pilocarpine and kainic acid (KA)-induced seizures, what suggests that SRF activation can be engaged in plasticity changes associated with the development of epilepsy. Recently, we have shown that SRF can regulate expression of matrix metalloproteinase 9 (MMP-9), an endopeptidase involved in physiological and pathological neuronal plasticity, including epileptogenesis. The aim of the current study was to identify new target genes regulated by SRF in the KA model of aberrant synaptic plasticity. Conditional, inducible, forebrain specific SRF knockout mice were used. 6 hours after KA-induced seizures dentate gyrus of the mouse hippocampus was dissected and RNA was isolated and analyzed by microarrays. 431 genes induced after seizures and significantly downregulated in SRF KOs were identified (FDR <1%, fold induction after KA > 1.5). Among those genes we distinguished and verified some potential plasticity related genes, including: aggrecan (Acan), that is a component of perineuronal nets – substructures of the neural extracellular matrix, and lipocalin 2 (Lcn2, NGAL), that is a small, secreted, protein that recently has been suggested to play a role in regulation of dendritic spines morphology.

S7.5:

Lipid metabolism in obestity-induced and endurance training-induced left ventricular hypertrophy

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Both physiological and pathological cardiac hypertrophy cause changes in lipid metabolism in the heart, although little is known about the underlying molecular changes. Therefore, the main goal of this study was to determine how cardiac hypertrophy that is caused by obesity (high fat diet (HF) fed rats) or endurance training influences lipid metabolism in the myocardium. Induced by training physiological hypertrophy was accompanied by an increased expression of lipogenic genes, decreased protein levels of fatty acid (FA) elongases, and the activation of sterol regulatory element-binding protein 1c and Akt signaling. Additionally, FA oxidation pathways regulated by AMP-activated protein kinase (AMPK) and peroxisome proliferator activated receptor α (PPARα) were upregulated in trained hearts. Cardiac lipid content was not changed by physiological stimulation, underlining balanced lipid utilization in the trained heart. On the other hand, pathological hypertrophy induced by HF diet did not affect the oxidative pathways regulated by AMPK and PPARα. Interestingly, pathological hypertrophy leads to cardiac triglyceride (TG) accumulation accompanied by the increased expression of TG-synthesis genes (DGAT and GPAT) in compare to chow-fed group. A possible explanation for this phenomenon is a decrease in lipolysis, as evidenced by the decreased content of adipose triglyceride lipase (ATGL) activator CGI-58 which attenuate TG contents. Obtained results show that cardiac lipid metabolism is differentially regulated in response to physiological and pathological hypertrophic stimuli and suggest that activation of lipogenesis might be involved in the regulatory mechanisms of heart adaptation to stress.
Support: NCBR LIDER/19/2/L-2/10/NCBiR/2011

S7.6

Does cholesterol accumulation in NPC fibroblasts affect mitochondrial metabolism?

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Niemann-Pick type C is one of autosomal recessive diseases called lysosomal storage disorders. NPC occurrence is estimated at approximately 1:150000 of healthy born children. NPC is caused by mutation in NPC1 gene, encoding protein involved in endosomal transport which results in cholesterol and glycolipids accumulation in the late endosomal/lysosomal compartment of the cell. NPC is characterized by wide spectrum of symptoms, including organ enlargement and progressive neurodegeneration leading to atonia, ataxia, bipolar disorders and dementia, and eventually death.
In this work we analyzed morphology and dysfunction of mitochondrial network in NPC cells. As an experimental model we used fibroblasts from healthy volunteers and NPC cell lines with defective cholesterol transport. These cell lines were tested for the level of NPC1 protein and the amount of cholesterol and lysosomes. We used several methods including measurement

of a mitochondria membrane potential reactive oxygen species production, and calcium homeostasis in NPC and control cell lines.
The NPC phenotype of our cell lines was characterized by the decreased level of NPC1 protein, increased cholesterol accumulation and higher amount of lysosomes. There was no significant differences in mitochondrial morphology. However, we observed significantly higher mitochondrial mass and an increase in mitochondrial potential of NPC cell lines in comparison to control lines.
Functional and structural differences between NPC and control cells suggest complex molecular mechanism of NPC disease development and sustenance.
This work was supported by the National Science Center grant NN401642740 and by statutory funds from the Nencki Institute of Experimental Biology

S7.7:

Lithium chloride protects neurons from toxicity of cytosolic PrP

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Prion protein (PrP) is mostly extracellular glycoprotein anchored in plasma membrane, constitutively expressed in the nervous system. In misfolded state this protein is prone to form aggregates, which are found in diseases called transmissible spongiform encephalopathies (TSE). In some of these diseases PrP abnormally localizes in cytosol (cytoPrP) interacting with proteins, which are not its physiological partners. In previous studies we have demonstrated that PrP is causing tubulin oligomerization, inhibits microtubule formation and disassembles microtubular cytoskeleton of epithelial cells. We have also showed that microtubule associated proteins (MAPs) that regulate microtubule stability can prevent above-mentioned effect of PrP. According to our study at least two proteins of this group: Tau and MAP2 where able to prevent deleterious effect of PrP but not in phosphorylated state. Interestingly, hyperphosphorylated Tau has been frequently detected in TSE. Extending the application of the above one should be able to modulate effect of cytoPrP on microtubular cytoskeleton by influencing level of phosphorylation of MAPs. In our study we used synthetic peptide encompassing first 30 amino acids of PrP (PrP1-30) as a model of cellular prion protein. The peptide consists of N-terminal signal sequence responsible for penetration of the molecule through cell membrane and major tubulin-binding site (23-30). To reduce level of phosphorylation of MAPs we chose inhibitors of GSK-3 a kinase known to be responsible for modification of these proteins. Specific inhibitor of the kinase - CT98014 and less selective LiCl were employed. By means of confocal microscopy we demonstrated disassembly of microtubular cytoskeleton and loss of neurites of primary neurons exposed to PrP1-30. In contrast, the cells treated with PrP1-30 in the presence of either LiCl or CT98014 did not differ from control cells. In cytotoxicity tests (MTT, LDH) we confirmed protection of neurons from toxic effect of PrP1-30 by both inhibitors of GSK-3. Our observations may help in understanding the molecular mechanism of neurotoxicity of cytoPrP.

S7.8:

Adipose-derived Wnts modulate beta cell adaptation during progression of type 2 diabetes

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Wnt pathway plays important role in pancreatic beta cell survival, proliferation and insulin secretion. That is why we checked whether adipose-derived Wnts contribute to pancreatic beta cell adaptation towards systemic insulin resistance and how Wnts expression profile changes during progression of type 2 diabetes.
The experiments were performed either on INS-1E beta cell line or on isolated pancreatic islets treated with fat cell conditioned medium from insulin-resistant or control insulin-sensitive 3T3-L1 adipocytes. The experiments were also carried out on rat models of insulin resistance induced by high-fat diet. Our study showed that Wnt signaling was significantly upregulated in both, INS-1E cells and pancreatic islets incubated with conditioned medium from insulin-resistant adipocytes. These changes were accompanied by enhanced insulin secretion and increased proliferation of pancreatic beta cells. Furthermore, in vivo in insulin-resistant pre-diabetic rats the level of Wnt4 (Wnt inhibitor) was decreased whereas the level of Wnt3a (Wnt activator) was augmented in both white adipose tissue and blood plasma. Aforementioned changes were associated with activation of Wnt signaling, increased insulin secretion and beta cell proliferation in pancreatic islets isolated from insulin resistant rats. However in diabetic rats the level of adipose-derived Wnt activator was reduced, compared to pre-diabetic group, and this phenomenon was accompanied by the lack of activation of Wnt signaling in pancreatic islets and regression of beta cell adaptation.
Obtained results suggest that Wnt signaling is an important component of the crosstalk between adipose tissue and pancreas in maintaining beta cell adaptation towards systemic insulin resistance.

S7.9:

Grainyhead-like 1 (GRHL1) transcription factor in signaling pathways and in development of skin cancers

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The Grainyhead-like 1 (GRHL1) protein belongs to a family of transcription factors, which is involved, among others, in epidermal barrier formation and maintenance.
Here we present evidence for skin tumor suppressive properties of GRHL1. Grhl1(-/-) mice upon exposure to carcinogens developed more squamous cell carcinomas, with an earlier onset, than wild type littermates. To explain the observed phenotype we examined the properties of Grhl1(-/-) keratinocytes.
Previous work demonstrated that GRHL1 directly regulates the expression of desmoglein 1 gene and Grhl1(-/-) mice have defects in epidermal architecture. Our histological and molecular analysis of epidermis of the transgenic animals demonstrated that it exhibits hallmarks of mild chronic skin inflammation.

To elucidate mechanisms by which the deletion of Grhl1 leads to described phenotype we measured the levels of expression of genes potentially regulated by GRHL1, such as PTEN. In a related report it has been shown that a close homologue of GRHL1 - GRHL3 - directly regulates the expression of PTEN, and Grhl3-deficient mice develop squamous cell carcinoma induced by PTEN-dependent activation of PI3K/AKT/mTOR signaling. In contrast, our results demonstrated lack of changes in expression of PTEN upon Grhl1 deletion. This suggests that mechanism of increased tumor susceptibility in Grhl1(-/-) mice is different from Grhl3(-/-) animals. It is likely to involve EGFR/MAPK signaling - pro-proliferative signaling pathway which, when over-activated in keratinocytes, is sufficient to induce inflammatory response in the skin. The analysis of these pathways in Grhl1(-/-) mice skin is in progress, and the latest results will be presented at the Conference.

S7.10:

p38 MAPK– a master regulator of cellular faith

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p38MAPK pathway is an important regulator of cellular responses to many extracellular stimuli like genotoxic and environmental stress, interleukins and growth factors. This family of protein kinases consists of four members - p38α, p38β, p38δ, and p38γ, where p38α is ubiquitously expressed at high levels in most cell types. The extracellular stimulus usually leads to activation of p38 MAPKs via cascade of phosphorylation events and results in its activation. There are three MAP2Ks that are known to activate p38 MAPKs – MKK3, MMK6 and MKK4, which in turn, are activated by phosphorylation on two conserved residues catalyzed by ten MAP3Ks. Due to involvement of p38α MAPK in multiple functions, like cell proliferation, differentiation, survival, migration, inflammation and tissue homeostasis, this kinase has multiple downstream targets. There are more than 96 proteins which have been described as direct targets of p38α, among which are many transcriptional factors, kinases, cell cycle regulators, caspases and others, involved in particular functions like cytoskeleton remodeling, protein degradation or membrane trafficking.
In order to investigate effects directly caused by p38 MAPK, we generated an inducible system in osteosarcoma U2OS cell line with constitutively active MKK6EE. Upon activation of p38, cells undergo multiple changes, including cell cycle arrest, ROS production and in the later time point - cell death and senescence. This is being accompanied with morphological changes in cell shape, mitochondrial and nuclear morphology. Following changes underlie multiple targets of p38MAPK.

SESSION 8

S8.1:

Identification of microenviromental factors that control OPC differentiation during CNS remyelination

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Demyelination is a pathological process that occurs in the central nervous system. Natural, endogenous response to demyelination is remyelination, a regenerative process driven by the progenitor cells - the precursors of oligodendrocytes (OPC). In the previous studies we have shown that OPCs are multipotent and can differentiate into oligodendrocytes on a classic pathway of differentiation and to Schwann cells and astrocytes on an alternative pathway. We also observed that Schwann cells derived from OPCs in the CNS occupied almost exclusively tissue around blood vessels in astrocyte deficient areas. Therefore we postulated occurrence of specific niche creating microenviroment that modulates fate of OPC and favor their differentiation into Schwann cells. During our studies on demyelination/remyelination we have also noticed massive regeneration of blood vessels in the area of remyelination. We hypothesize that mutual interactions and the outcome of these events create in a vascular niche specific microenvironment instrumental for differentiation of oligodendrocyte precursors. Using laser capture microdissection technique we separated the vascular niche from the rest of the lesion area and investigated changes in global gene expression using the cDNA microarrays. Based on comparative bioinformatic analysis and verification of the results by RT-PCR we selected candidate genes which significantly discriminate two niches. Candidate gene selection revealed genes involved in cellular differentiation as well as genes involved in angiogenesis and neovascularization. The aim of present study was to identify the factors and their downstream effectors that significantly discriminate vascular and nonvascular niche and determine the fate of oligodendrocyte precursor cells.

S8.2:

GRHL genes in human non-melanoma skin cancers

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Grainyhead-like proteins (GRHL) constitute a family of transcription factors that were conserved in the course of evolution of multicellular organisms. In mammals there are three GRHL genes present on different chromosomes, which are expressed in a spatio-temporally-specific fashion. The GRHL factors are critical for development and homeostasis of the surface epithelium. Many of their target genes (like E-cadherin, desmoglein 1, PTEN, hTERT, PCNA) were previously implicated in carcinogenesis. Based on literature data and our preliminary results we hypothesized that GRHL genes' or GRHL proteins' dysfunction is involved in epidermal carcinogenesis. The aim of our research is to investigate whether various types of human skin cancers are accompanied by changes in the expression levels of GRHL genes and to establish the causes of these changes. We collected NMSC samples as

well as control healthy tissue from 35 Polish patients. In these samples we observed significantly correlated downregulation of both GRHL1 and GRHL3. To explain changes in GRHLs expression levels we decided to search for: specific point mutations, loss of heterozygosity and copy number variation, changes of methylation profile in regulatory sequences, miRNAs specifically regulating GRHLs expression. Global changes in transcriptomes of different NMSCs with different GRHLs expression are also studied. To detect and identify GRHL gene disruptions in skin cancers, we use: Targeted Deep Sequencing, DNA-methylation analysis, Human Gene Expression Microarrays, Lentiviral-based systems with miRNAs. Our findings will provide new molecular insights into the links between the GRHL genes and epidermal neoplasia in the human context.

S8.3:

FAP251 and FAP61 proteins are required for cilia motility

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Cilia are highly evolutionary conserved microtubule based protrusions which are present in nearly all eukaryotic cells except higher plants and fungi. There are two types of cilia– motile and immotile. Immotile, so-called primary cilia, are found on the surface of various cells in multi-cellular organisms and perform important sensory functions. Motile cilia, despite sensory functions, enable cell motility, movement of mucus in respiratory system, or flow of the cerebrospinal fluid. Defects in cilia assemble or function cause a wide range of human diseases called ciliopathies.
Cilia are build of several hundreds of proteins. The function of the majority of these proteins remain unknown.
Here we present data concerning two ciliary proteins, FAP251 and FAP61. Both proteins localized in cilia. The domain analysis of FAP251 protein indicated that deletion of WD-40 domain but not C-terminal end prevents ciliary localization. Overexpression of FAP251p had no effect on cilia while prolonged expression of FAP61p resulted in formation of short cilia and reduced ability to regenerate cilia. Disruption of either FAP251 or FAP61 gene in Tetrahymena cell caused decrease in cells swimming rate and reduced rate of formation of food vacuoles (phagocytosis). Rescue experiment with construct enabling expression of GFP or myc tagged FAP251 fusion protein confirmed that observed phenotype is a sole effect of the protein depletion. The length and number of cilia in FAP251 and FAP61 knock-out cells is as in wild type cells however the ultrastructure analysis indicated defects in the assembled radial spokes, macrocomplexes known to be indispensable for proper cilia beating.

S8.4:

Investigating the role of reactive oxygen species and ATM pathway in vascular smooth muscle cells senescence

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Senescence is a mechanism characterized by an irreversible growth arrest and certain alter functions of the cells. It is related to accumulation of unrepairable DNA double strand breaks (DSBs) which activate DNA damage response pathway (DDR). DSBs can be caused by DNA damaging agents and reactive oxygen species (ROS) which level could be increased during senescence. They can be produced either by mitochondria or professional ROS producers such as NADPH oxidases (Nox). Since senescent vascular smooth muscle cells (VSMCs) are known to taking part in pathogenesis of cardiovascular diseases, we took under investigation activation of DDR pathway and ROS level in these cells. To this end we cultured human VSMCs (hVSMCs) till they terminally stopped proliferation (replicative senescence, RS) or treated early passage cells with DNA damaging agent – doxorubicin to induce stress-induced premature senescence (SIPS). Activation of the DDR pathway and a secretory phenotype was observed during senescence. To elucidate the role of this pathway in this process, we silenced ATM kinase using siRNA and analyzed the SIPS markers in hVSMC treated with doxorubicin. Downregulation of ATM leads to a decreased level of some of senescence markers confirming the role of DDR pathway in senescence of hVSMCs.
Both RS and SIPS of hVSMCs was correlated with increased ROS production. Interestingly we were able to decrease the level of ROS using DPI – a Nox family inhibitor in proliferating as well as in senescent cells. It suggests that NADPH oxidases are important ROS producers in both proliferating and growth arrested cells.

S8.5:

Kinesin-14 Ncd: mechanism of force generation and EB1-dependent localization at microtubule plus end

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Ncd is a kinesin-14 from Drosophila melanogaster. It moves towards the microtubule (MT) minus-end. Its C-terminal motor domain and N-terminal tail domain contain MT-binding sites. Therefore Ncd is able to bind two MTs simultaneously and slide or crosslink them during mitotic spindle formation. To perform this role correctly the motor has to be localized near the MT plus ends. Ncd achieves this localization by the interaction with EB1 – a protein which tracks the growing MT plus ends.
The detailed mechanism of EB1-dependent tip-tracking of Ncd is still not fully understood. It is not known where the complex is formed and whether Ncd binds to EB1 only or interaction with a site composed of EB1 and the MT surface is necessary. Our aim was to elucidate these issues.
We deleted the MT-binding sites in Ncd tail. Then we reconstituted MT dynamics in vitro and observed the localization of Ncd tail fused to GFP in the presence of EB1. Although the truncated motor did not interact with microtubules, it was found that it was able to track the plus ends of MTs in the presence of EB1. It suggests that the most plausible mechanism is the

binding of Ncd to EB1 which was already associated with the MT end. However, the question remains how Ncd transits from EB1-mediated MT-binding to direct MT-binding which is necessary for MT sliding.

S8.6:

The effect of exogenous AnxA2 on expansion and mineralization of human osteosarcoma (OS) cells in vitro

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Osteosarcoma (OS) is an aggressive bone cancer affecting teenagers. Annexin A2 (AnxA2), protein involved in early mineralization of osteoblasts, is proposed to have an impact on OS progression. We have shown that AnxA2 and its binding partner S100A10 are selectively translocated into matrix vesicles as well as secreted into the extracellular milieu by OS cells undergoing mineralization. To elucidate the role of secreted AnxA2 in modulation of OS cells phenotype in vitro, we analyzed the mineralization and cancerogenic potential of human OS cells (osteoblast-like Saos-2 cell line and osteolytic, highly metastatic 143B cell line) grown in the presence of human recombinant AnxA2 in the standard media. As a control, OS cells were treated either with AnxA6 or bovine serum albumin. Stimulation with ascorbic acid and β-glycerophosphate served to testify mineralization ability of OS cells in vitro. Then, each experimental group was tested for mineralization markers (like TNAP or Runx-2) and cancerogenic potential (by assessment of migration, invasion or adhesion of cells). The uptake of FITC-conjugated AnxA2 by OS cells was confirmed by confocal microscopy. Our finding supports the hypothesis that the induction of mineralization process might lead to limitation in OS cells expansion. This research was supported by grant 2012/05/N/NZ3/00330 to A.C. from the Polish National Science Centre and Polish-Portugal Executive Program for years 2011–2012 (project 760).

S8.7:

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. Rybakowski et al. demonstrated an association of MMP-9 5'UTR polymorphism -1562C/T with schizophrenia. 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 various 3'UTR variants as well

as the inactive form of MMP-9 under human synapsin promoter. First construct enables MMP-9 protein visualization by its fusion to Venus fluorescent protein and additionally contains myristoylation sequence which forces membrane docking. This will allow to observe locally translated MMP-9 at the synapse. 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.

S8.8:

Alterations in microRNA level in the dentate gyrus in epileptic rats

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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 LNA 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.

S.8.9

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.

S8.10:

Functional Neuroanatomy of Language

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Phonological processing refers to identification and discrimination of phonemes in fluent speech and underlies auditory comprehension. It reflects an individual's capacity to differentiate words, syllables, rhymes in verbal utterances. Despite a lot of studies, the neuroanatomy of phonological processing is still not clearly defined in existing literature.
The aim of our study was to identify brain regions involved in phonological processing in vision, audition and independently of the modality in healthy volunteers, using fMRI method.
Forty four subjects (28 female, 16 male, $\bar{x}$ ± SD 47.3 years ± 18.6) participated in the block design study, performed in 3T MRI scanner. Participants performed two visual and two auditory tasks during the scanning. For each modality both experimental and control tasks were applied. The visual tasks required rime detection (experimental task) and stimulus detection (control task). The auditory tasks required identification of words that started with a given letter (experimental task) and detection of a rising/ falling tone (control task). Two contrasts were considered: visual task or auditory task. Next, the conjunction analysis was used to map the common region activated in both visual and auditory experimental tasks, thus, to find the region involved in phonological processing, independently of the sensory modality.
Results revealed that phonological processing in visual modality activates temporal and frontal regions, mainly in the left hemisphere. Phonological processing in auditory modality activates more bilateral language network, while phonological processing independently of the modality activates the network of the left hemispheric structures which play a central role in language comprehension.
Acknowledgements: Supported by 507/1/N-DFG/2009/0

S8.11:

L1 overexpression in rats with complete spinal cord transection influences retraction of CST axons and alters expression of neuronal plasticity engaged molecules

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To evaluate L1-CAM potential after spinal cord transection in rats, we injected adeno-associated viral vector encoding L1 (AAV5-L1) into first lumbar spinal segment, immediately after transection at Th10/Th11. It led to chronic (5 weeks) increase of L1 mRNA and protein below the lesion. At first we analyzed its impact on transected corticospinal tract (CST), anterogradely traced with fluorescent dye DiI. AAV5-L1 injected rats showed reduced retraction/increased outgrowth of CST in 2 mm segment from the lesion border as compared to rats receiving AAV5-EGFP. To answer the question whether L1 molecule, overproduced below the lesion, may be shed to produce soluble L1 forms, reaching CST, we analyzed the expression of metalloprotease ADAM10 responsible for L1 membrane shedding; no significant changes of ADAM10 in Th4-L6 segments were found between AAV5-L1 and AAV5-EGFP groups. We evaluated also the potential of spinal neurons to respond to L1 by increasing transcripts of synaptophysin, adenylate cyclase1 (Adcy1) and growth-associated protein 43 and found L1 significant effect on them below, with upregulation of Adcy1 also above the lesion. Taken together L1 overexpression below the lesion promotes plasticity both below and above the site of transection.
Support: Polish-German S007/P-N/2007/01 & NCN GP2241 grants.

S8.12

p53-independent pathways in the DNA-damage induced senescence of cancer cells

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Cancer cells upon DNA damage undergo so named stress-induced senescence (SIPS). In DNA damage-induced senescence the key protein is p53, which is activated by ATM kinase. In turn p53 transactivates CDKN1 encoding for cdk's inhibitor p21. Recently we have shown that not only p53 wild type (WT) but also p53 knock-out (KO) HCT116 cells undergo SIPS upon curcumin treatment, even though curcumin does not cause direct DNA damage (Mosieniak et al., 2012). Nonetheless, senescence was dependent on p21 expression. Now, we were interested whether other proteins than p53, namely NF-κB, which acts downstream of ATM, can activate CDKN1 and induce senescence in cells treated with DNA damaging agent. To this end we treated p53 WT and p53 KO HCT116 with Topo2 inhibitor (doxorubicin) for 7 days. We showed that both p53 WT and p53 KO cells treated with doxorubicin displayed hallmarks of senescence, such as increased size and granularity, β-galactosidase activity and cell cycle arrest. Both p53 WT and p53 KO cells exhibited senescence-associated secretory phenotype – they produced VEGF and IL-8. Interestingly, p53 KO cells produced even more IL-8 than p53 WT cells. Western blot analysis of the total and phosphorylated proteins of the NF-κB signaling pathway, such as IκBα, IKKα, IKKβ, and p65, unexpectedly showed no activation of NF-κB. It seems that NF-κB is not involved in DNA damage-induced senescence of HCT116 cells. The signalling pathways which could be activated after treatment with DNA damaging agents and lead to SIPS are under elucidation.

POSTER SESSION

P1:

Phosducin-like protein 2 (Phlp2p), a potential regulator of ciliogenesis in Tetrahymena

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Recent studies have implicated the phosducin-like protein-2 (PHLP2) in the regulation of CCT, a chaperonin whose activity is essential for folding of tubulin and actin. However, the precise molecular function of PHLP2 is still unclear. Our latest studies suggest that the activity of Phlp2p is essential for cilia assembly and function. To better understand the mechanisms of Phlp2p function we investigated the localization of Phlp2p in Tetrahymena cells. Both, GFP or HA-tagged Phlp2 proteins localized in cilia, basal bodies and cytosol. However, while GFP-Phlp2p was distributed evenly along entire cilia length, Phlp2p-HA was observed in cilia as patches and resembled the localization of the components of the intraflagellar transport. Overexpression of GFP-Phlp2p caused a dominant-negative effect. GFP-Phlp2p expressing cells had fewer cilia and showed decreased rate of proliferation, motility and phagocytosis, as compared to wild type or Phlp2p-HA overproducing cells. Moreover, after deciliation GFP-PhLP2 cells were not able to regenerate cilia. Interestingly, the negative effect of GFP-Phlp2p overexpression on ciliogenesis was abolished by simultaneous overexpression of Phlp2p-HA. Thus, elevated level of non-functional GFP-Phlp2p inhibits ciliogenesis presumably by displacing endogenous Phlp2p or by titrating out key components for cilia assembly. High level of functional HA-tagged protein can “rescue” dominant negative effect by changing the ratio between functional (HA-tagged) and non-functional (GFP-tagged) forms of Phlp2 proteins.

P2:

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.

P3:

Lyn kinase differently regulates MyD88- and TRIF-dependent signaling pathways of TLR4 activated by LPS

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Toll-like receptor 4 (TLR4) is localized in the plasma membrane of macrophages and is activated by lipopolysaccharide (LPS) of Gram-negative bacteria. Activated TLR4 binds MyD88/TIRAP adaptor proteins triggering production of pro-inflammatory cytokines. After internalization, endosomal TLR4 binds TRIF/TRAM adaptors and induces synthesis of type I interferons and chemokines. We examined an involvement of raft-residing Lyn kinase in the two signaling pathways of TLR4. We found that LPS induced dose- and time-dependent activation of Lyn, as indicated by phosphorylation of tyrosine residue 396 of the catalytic domain of Lyn and simultaneous dephosphorylation of inhibitory tyrosine residue 507. Integrity of rafts was crucial for the activity of Lyn. To interfere with the activity of Lyn, we obtained a series
of mutated forms of this kinase fused with GFP which were next overexpressed in RAW264 macrophage-like cells. The transfection efficiency reached 40% of cell population. An expression of K275R kinase-dead Lyn increased TRIF-dependent production of chemokine RANTES by 60-75% in cells stimulated with 1-1000 ng/ml LPS. On the other hand, this mutant form of Lyn only moderately up-regulate MyD88-dependent production of TNFα and MIP-2,
as established at the protein and mRNA levels. Similar effects on RANTES production were exerted by R155A and W98A Lyn kinase mutated in its SH2 and SH3 domains, respectively. Taken together our data suggest that Lyn kinase phosphorylates an unknown adaptor protein and binds to it via SH2 and SH3 domains. This protein complex can serve as a strong negative regulator of TRIF-dependent signaling pathway of TLR4.

P4:

PML nuclear bodies upon neuronal stimulation

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PML protein is a crucial element of characteristic structures involved in creating dynamic architecture of the cell nucleus – PML bodies. However, the PML function at the systemic level is far from understanding. It is widely believed that besides its important role in the prenatal

neurogenesis, PML is not expressed in the adult brain. However, our studies reveal that PML protein does exist in the adult mouse brain, and forms nuclear body-like structures in the neuronal nuclei. Our anatomical study indicates that the most pronounced expression of PML occurs in cerebral- and cerebellar cortices. Such a pattern suggests the involvement of PML in higher levels of information processing in the brain. Furthermore, a brief seizure evoked by pentylenetetrazole upregulates PML, and evokes a series of changes in the morphology of the nuclear PML aggregates in the cerebral cortical neurons, culminating in their accumulation and dispersion throughout the nucleus, as observed by high-resolution fluorescence microscopy followed by three-dimensional quantification. Quite unexpectedly, an electron-microscopic immunogold analysis revealed that PML aggregates don't form morphologically recognizable objects, e.g. the classic nuclear bodies with fibrous capsule. Nevertheless, these studies demonstrated that seizure causes PML aggregates to tightly associate with decondensed chromatin fibrils. This observation suggests that PML function in neurons might be associated with activity-dependent gene expression. The changes in PML expression seem not to be seizure-specific, as they are evoked also by immobilization stress. Taking into consideration our studies, and the literature data, we foresee that PML has an important function(s) in the brain.

P5:

Voiced-unvoiced contrast discrimination across the life span

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Voice-Onset-Time (VOT) is one of the crucial feature in auditory language comprehension. The difference between voiced and unvoiced Polish stop-consonants may be described in terms of the VOT. It is the time interval between the release of stop closure of articulators and the onset of vocal folds vibration, related to articulation of the vowel. The aim of the experiment was to investigate discrimination of voiced and unvoiced contrast across the life span from 5 to 69 years of age.
We studied 83 subjects (49 male and 44 female) aged from 5 to 69 years classified into 3 age groups: children, adults and elderly people. All participants were Polish native speakers, had normal level of intelligence, normal hearing and no neurological or psychiatric disorders.
Subjects were exposed with 16 pseudowords (/Domek/ or /Tomek/) which differ in the VOT value (from -90 ms to +50 ms). They were asked to indicate the recognized word on the response cards. One card displayed the picture /Domek/, the other one /Tomek/.
We observed no differences between children, adults and elderly people in voiced and unvoiced contrast discrimination at all applied VOT values. The main conclusion is that the discrimination of voiced – unvoiced contrast is a stable feature across the life span from 5 to 69 years.
Supported by: INNOTECH-K1/IN1/30/159041/NCBR/12

P6:

The novel PERK-eIF2α prosurvival signaling in CML cells promotes protective autophagy and resistance to imatinib - induced cell death

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Chronic Myeloid Leukaemia is common hematological malignance caused by translocation between chromosomes 9 and 22 resulting in creation of fusion gene and then fusion protein BCR-ABL. Constitutively active kinase BCR-ABL activates many prosurvival pathways. Imatinib is a first line therapy in CML treatment which is a highly specific inhibitor of BCR-ABL activity. Around 70% patients treated with Imatinib responds and gives long time remission. Unfortunately there is a group of patients developing resistance. Recently we discovered that PERK-eIF2α signaling pathway is a novel prosurvival mechanism existing in CML cells. Activation of PERK-eIF2α correlates with occurrence and progression of CML as well as resistance to Imatinib. Because it was shown that PERK-eIF2α can promote autophagy in solid tumors, the aim of my work was to investigate whether PERK-eIF2α phosphorylation pathway promotes induction of autophagy in response to imatinib, as a part of the resistance phenotype. My preliminary results suggest that active state of the PERK-eIF2α signaling pathway promotes autophagy in CML cells. Failure of the PERK-eIF2α reduces autophagy in response to imatinib. Reduction of autophagy in CML cells by inhibition of the PERK-eIF2α signaling pathway can be a possibility to suppress resistance to imatinib.

P7:

Stearoyl-CoA desaturase activity is required for membrane translocation of protein kinase C-theta induced by lipid overload in skeletal muscle

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Skeletal muscle insulin resistance (IR) as linked to type 2 diabetes and obesity is among the most common metabolic defects, affecting over 5% of the population in western countries. One of the key risk factor that contributes to obesity-associated diseases such as type 2 diabetes is insulin resistance. Previous results have shown, that protein kinase C (PKC) activation induced by diacylglycerols (DAGs) is one of the sequels of the dysregulation of intramuscular lipid metabolism and is thought to play an important role in the development of insulin resistance (IR). We tested the hypothesis that DAGs with different acyl chains have different biological effects and that DAG species enriched in monounsaturated fatty acids (MUFA) act as better activators of PKC. The experiments were performed in vivo on the skeletal muscles of rats fed high-fat (HF), high-tristearin (TS) or high-triolein (TO) diets. To define the importance of endogenously synthesized MUFA on DAG-induced PKCθ activation, we performed experiments on stearoyl-CoA desaturase 1 knockout mice (SCD-1 KO) and mice with muscle-specific overexpression of SCD-1 (SCD-1 mTg) as well. The results show that the content of total DAGs and the levels of saturated DAG species is affected in both insulin-resistant (HF and TO) and highly insulin-sensitive (TS) groups. An increase in MUFA-

containing DAGs levels was most constantly related to increase in PKCθ membrane translocation and IR. In the muscles of MUFA-deficient SCD-1 KO mice, the DAG content and the induction of PKCθ translocation by the HF diet were significantly different from muscles overexpress SCD-1. Collectively, our data indicate that DAGs composed of 16:1 and/or 18:1, rather than the levels of total or saturated DAGs, are related to PKCθ membrane translocation. Moreover, our results show that the availability of dietary MUFA and/or the activity of SCD-1 plays an important role in muscle DAG accumulation.

P8:

Regulation of cholinergic innervation of motoneurons by different methods of activation of the spinal network: locomotor exercise or electrical stimulation of proprioceptive fibers in the tibial nerve; the role of neurotrophins

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Spinal cord (SC) injury leads to a reduction of the number of synaptic terminals on rat motoneurons (Mns) as indicated by a decreased expression of synaptophysin around neurons in the motor nuclei of spinal animals. Recovery processes after SC injury, supported by locomotor training of rats, resulted in up-regulation of synaptophysin interpreted as an enrichment of synaptic input to motoneurons. We asked whether cholinergic innervation of α-motoneurons participates in this reorganization. Cholinergic terminals were detected using anti-vesicular acetylcholine transporter (VAChT) antibody in the soleus (Sol) and tibialis anterior (TA) Mns prelabeled with injected intramuscularly dyes. Spinalization caused a selective decrease (by over 50%) of the number of VAChT-positive boutons apposing perikarya of the Sol but not TA Mns. Locomotor training, partly reduced the deficit in cholinergic innervation of Sol Mns but also increased the number of VAChT positive boutons in TA Mns above the control level. We then asked whether direct electrical stimulation of low-threshold proprioceptive afferents in the tibial nerve which, as we found, upregulated neurotrophin-3 (NT-3) expression in the SC, could counteract the selective deficit of cholinergic innervation of Sol Mns. The tibial nerve was stimulated unilaterally for 7-days with high-frequency bursts of pulses eliciting the H-reflex. Stimulation caused an increase of NT-3 by over 80% in L3-6 spinal segments (evaluated with ELISA). Our recent immunohistochemical studies, evaluating the effect of stimulation on synaptic changes on motoneurons innervating the ankle extensor muscles, particularly vulnerable to the spinal injury, are in progress.
Supported by BIOIMAGINE, MSE grant NN401048033.

P9:

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.

P10:

Sgt1 as a component of chaperone complexes - the role of Sgt1A and Sgt1B isoforms

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The Sgt1 protein (suppressor of G2 allele of Skp1) was identified in yeast, but later it was found also in plant and mammalian cells (Kitgawa et al., 1999; Spiechowicz and Filipek, 2005). In most of the studied organisms, including mouse and rat, there is only one Sgt1 isoform but in human two isoforms, named Sgt1A and Sgt1B, are present (Niikura and Kitagawa, 2003). Silencing of both Sgt1 isoforms in human causes mitotic delay and in consequence cell death, which is in agreement with the results showing the involvement of Sgt1 in kinetochore assembly. Interestingly, Sgt1 plays a role in kinetochore complex together with Hsp90 (Steensgaard et al., 2004; Davies and Kaplan, 2010).
In this work we search for the function of particular Sgt1 isoforms in human cells. In order to selectively silence the Sgt1A or Sgt1B isoform, specific siRNA molecules were designed. The most effective siRNAs caused 55%, 50% and 84% decrease in the level of Sgt1A, Sgt1B or both isoforms (used as control) in HEp-2 cells, respectively. We also looked for proteins associated with the Sgt1-Hsp90 chaperone complex and found that CacyBP/SIP might be a component of this complex. We showed that CacyBP/SIP co-precipitates with Sgt1 in the presence of phosphatase inhibitors suggesting the role of Sgt1 phosphorylation in this interaction. From the other site, co-precipitation of CacyBP/SIP with Sgt1 depends on the presence of radicicol, which point out the role of Hsp90 in CacyBP/SIP-Sgt1 interaction. Our data suggest that Sgt1 might play a role in kinetochore complex and cell cycle progression via interaction with both Hsp90 and CacyBP/SIP.

P11:

Cytostatic dose of curcumin induces senescence of vascular smooth muscle cells

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Curcumin, a natural polyphenol, has documented anti-inflammatory and anti-oxidant properties. A plethora of studies revealed its potential in therapy of many diseases with inflammatory origins – cancer and cardiovascular disease. Curcumin is believed to be safe even in high doses (8-12 mg/day). Its plasma concentrations do not exceed 2 μM. It was shown that curcumin can induce cell death as well as senescence of cancer cells.
The aim of this study was to investigate if curcumin can induce senescence in normal cells. We found that vascular smooth muscle cells (VSMCs) are relatively sensitive to curcumin since 2,5 μM curcumin inhibits proliferation, 5 μM is cytostatic and concentrations higher than 10 μM induce cell death. To induce cellular senescence we chose 5 μM curcumin. VSMCs treated with this concentration display several markers of senescence such as increased number of cells arrested in the G2/M phase of the cell cycle, elevated cellular granularity, increased activity of senescence-associated-β-galactosidase, senescence associated secretory phenotype. Additionally, an increased level of the marker specific for VSMCs senescence – AGTR1 (receptor for angiotensin II), was observed. In order to find the mechanism of senescence after curcumin treatment we analyzed the DNA damage response pathway (DDR). Western blot analysis revealed transient activation of the components of the DDR pathway such as p53 and p21, however we observed a decreased number of DNA double strand breaks.
Our results suggest that VSMCs are very sensitive to curcumin and cytostatic doses can induce senescence but the mechanism is not yet elucidated.

P12:

The influence of S100A6 on epidermal differentiation

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S100A6 is a calcium binding protein which expression is mostly confined to fibroblasts and epithelial cells. Although its gene is located on chromosome 1q21 in a gene cluster known as Epidermal Differentiation Complex (EDC) [1], little is known about its role in epidermal differentiation.
First, we examined S100A6 expression during calcium induced differentiation of human primary and spontaneously immortalized keratinocytes (HaCaT cells) and observed a significant decrease in S100A6 mRNA level in differentiated cells. Then we developed HaCaT cells with either diminished or elevated S100A6 level in order to investigate its influence on epidermal differentiation. Western Blot analysis revealed that HaCaT cells overexpressing S100A6 had higher keratin 14 content (marker of undifferentiated cells) which did not decrease during differentiation, higher keratin 10 level (the early differentiation marker) and did not express loricrin (the late differentiation marker). Furthermore, HaCaT cells with elevated S100A6 level proliferated more rapidly and adhered more efficiently to fibronectin – the constituent of the basement membrane. These data suggest that HaCaT cells overexpressing

S100A6 show the hallmarks of undifferentiated cells even when cultured in conditions promoting differentiation.
On the other hand, HaCaT cells with diminished S100A6 level showed a significant decrease in keratin 14 and increase in loricrin level. Additionally these cells had high p63 (a transcription factor essential for keratinocyte differentiation) expression even before triggering the differentiation process. These data suggest that their phenotype is more differentiated than that of control HaCaT cells.
Thus, S100A6 seems to be an important factor regulating epidermal differentiation.

P13:

Nuclear translocation of myosin VI upon stimulation of neurosecretory PC12 cells: a possible role of MVI in gene expression

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Myosin VI (MVI) is a unique, actin-based motor protein that moves along actin filaments in the opposite direction of all other myosins. It is implicated in processes associated with the actin-cytoskeleton such as for example: endocytosis, cell migration, cytokinesis and possibly in secretion. Our previous studies demonstrated that in neurosecretory PC12 cells MVI localized both in the cytoplasm and nucleus. Moreover, we showed that nuclear localization of MVI was even more pronounced upon stimulation with 59 mM KCl, especially after 5 min of treatment. Stimulation-dependent MVI translocation was accompanied by its co-localization with several nuclear proteins involved in transcription and nascent transcript maturation, namely active form of RNA polymerase II, transcription factor Sp1, PML bodies, SC35-containing nuclear speckles and hnRNP U. Also, MVI colocalized with transcriptionally active sites as measured by BrUTP incorporation. Our mass spec analyses of the eluate fraction of MVI-based pull-down assay identified numerous nuclear proteins, including hnRNP U and several proteins involved in transcription and post-transcriptional processes. Interestingly, we also observed that after stimulation total amount of MVI in the cell was increased, with the highest increase after 5 min. This was accompanied by increase of PML, SC35, eiF2α (eukaryotic initiation factor), and SF2/ASF (Serine/arginine-rich splicing factor 1) levels. Additionally, in PC12 cells with MVI knockdown a significant increase of hnRNP U and PSF (polypyrimidine tract-binding protein-associated splicing factor) levels was found.
These data indicate the existence of functional interactions between MVI and transcription machinery.

P14:

New ligands of S100A6 (calcyclin) in Wharton's jelly

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Wharton's jelly (WJ), a connective tissue in the umbilical cord, is composed of a very low number of cells (myofibroblasts mainly) which produce high amounts of extracellular matrix including proteoglycans. The S100A6 protein binds Ca^{2+} and belongs to the S100 protein family. Interestingly, this protein is characteristic for tissues which are specific for pregnancy such as deciduas or Wharton's jelly. Because S100A6 is present in WJ we searched for its potential ligands in this tissue.

By applying affinity chromatography on the S100A6 resin and mass spectrometry analysis we found that some proteins from the extracellular matrix of WJ could bind to S100A6 in a Ca^{2+}-dependent manner. Among them are extracellular proteins such as lumican and PRELP (proteins belonging to the family of small leucine-rich repeat proteoglycans) and an intracellular protein, cofilin1. Immunoprecipitation of S100A6 and Western blot analysis with anti-S100A6, anti-lumican or anti-PRELP antibodies confirmed the interaction between S100A6 and these proteoglycans. Moreover, immunohistochemical staining of umbilical cord paraffin slices revealed that S100A6 co-localizes with these proteins. Further studies, using ELISA and cross-linking experiments, showed direct interaction of S100A6 with lumican and with cofilin1.

The Ca^{2+}-dependent interaction between the S100A6 protein and cofilin1, lumican or PRELP and co-localization of S100A6 with these proteins in the extracellular matrix of WJ suggest the involvement of S100A6 in extracellular signaling pathways.

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P15:

The influence of different transcription factors on the regulation of CacyBP/SIP gene expression

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CacyBP/SIP was discovered as a calcyclin (S100A6) binding protein (1) and later as the Siah-1 interacting protein (2). At present, other targets of CacyBP/SIP are known. Among them are Skp1, tubulin, actin, tropomyosin and ERK1/2 kinase (3). As to the interaction with ERK1/2, it has been revealed that CacyBP/SIP dephosphorylates this kinase (4) and, in consequence, decreases the activity of Elk-1 transcription factor which is involved in the regulation of cell proliferation and differentiation pathways.

In this work we examined the regulation of CacyBP/SIP gene expression. For that we analyzed its promoter sequence (1,6 kb downstream from the Transcription Start Site, TSS) using the MatInspector program. We found the binding sites, among others, for transcription factors such as NFAT (Nuclear Factor of Activated T cells) and CREB (cAMP response element-binding), which may link CacyBP/SIP expression with the activity of other kinases and phosphatases, a binding site for E2F transcription factors that promote proliferation or cell

death and a binding site for DREAM (downstream regulatory element antagonist modulator) which might transmit the effect of intracellular calcium concentration on CacyBP/SIP gene expression.

P16:

Role of heat shock protein 72 in regulation of insulin sensitivity in skeletal muscle

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Insulin resistance, a condition in which cells fail to respond normally to insulin, is associated with many related health complications, including type 2 diabetes and heart disease. It is known that heat treatment improves glucose tolerance and prevents lipid-induced skeletal muscle insulin resistance. It was observed that expression of heat shock protein 72 (Hsp72), cytoprotective chaperone protein, in skeletal muscle in humans is positively correlated with insulin sensitivity and inversely correlated with the percentage of body fat, the mechanism of which is practically unknown. Therefore, the aim of the study was to investigate the molecular mechanisms involved in Hsp72-associated regulation of insulin sensitivity in skeletal muscle. Our study showed that C2C12 myotubes with overexpression of Hsp72 are characterized by a better insulin response. Moreover overexpression of Hsp72 decreases both palmitic acid (16:0) and C2 ceramide induced insulin resistance in C2C12 cells. Additionally, activity of 5'AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) was increased in C2C12 cells with overexpression of Hsp72 both under normal conditions and after 16:0 treatment. Level of fatty acid synthase (FAS) protein was increased in C2C12 myotubes with overexpression of Hsp72 after 16:0 treatment. This study showed that overexpression of Hsp72 increases insulin sensitivity in C2C12 cells via mechanism that involves changes in expression and activity of proteins controlling lipid metabolism.

P17:

Efficient isolation of rare stem and precursor cell populations responding to the central nervous system traumatic injury by use of fluorescence activated cell sorting

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The central nervous system is populated by a number of stem and progenitor cell populations which, when responding to the tissue injury, might have potential to support regenerative processes in damaged tissue. The aim of present study was to develop a strategy for highly effective and pure sorting of these unique populations. We used fluorescence activated cell sorting, which appears to be the ideal tool for efficient isolation of such populations. We have chosen mouse model of stab wound injury of cerebral cortex, of which 3 or 6 days after injury we were obtaining material for further analysis. Intact and injured brain cortex was dissociated and the cell suspension was cleared of myelin followed by the lineage-positive cells depletion. Rare populations of $Sca1^{+}lin^{-}CD45^{-}$ (so called very small embryonic-like stem cells) as well as

$Sca1^{+}lin^{-}CD45^{+}$ hematopoetic stem cells and oligodendrocyte precursor cells were sorted with FACS Aria I according to the specific cell markers. Using immunohistochemical staining and gene expression analysis we confirmed an identity of isolated populations.

Proposed strategy of effective isolation of rare stem and precursor cell populations may be a useful tool for further functional studying their biology and differentiation potential in vitro and in vivo.

P18:

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, for example: presence or the absence of neurons in culture or reagents which can increase intracellular cAMP levels. 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 flatted 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.

P19:

Tangled in the signal network: how calcium and adhesion modulates Rho-dependent signaling?

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Cell migration is one of the crucial attributes of a living organism. It is responsible for embryogenesis, regeneration, immune defense, as well as such undesirable phenomena, as the spreading of cancer cells. Two major conditions must be fulfilled for cell migration to occur: polarization and adhesion. These processes are spatially and temporally regulated by

signaling pathways related to RhoA and Rac1 proteins – the key regulators of actin cytoskeleton dynamics: contractility and polymerization, respectively.
We indicated the mutual compensation of these two signaling pathways in glioma C6 cells by blocking each of them. Under both experimental conditions stimulation of P2Y2 receptors with UTP resulted with cells recovery to control morphology and motility. We examined the differences between cell migration parameters (average velocity, walk persistence, directionality) and adhesion areas in control cells, those with blocked RhoA/ROCK or Rac/PAK pathway (by Y-27632 and NCS inhibitor respectively) and in cells in calcium-free medium. We showed that NCS and calcium-free environment prevent cell recovery from ROCK inhibition after UTP stimulation. Under these experimental conditions interaction of αvβ5 integrins with $P2Y_2$ receptors is decreased, as microscopy and biochemical studies showed, inhibiting cofilin phosphorylation via Rac1/PAK signaling pathway and changing the migration parameters. The dependence between examined signaling pathways and the pattern of glioma C6 cells migration is discussed.

P20:

Towards a computational model of learning and social interactions of mice in IntelliCage

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It was showed that co-housing APP.V717I transgenic mice (a model of Alzheimer's Disease) with wild type companions can boost transgenic mice's ability to find the location of a reward (sweetened water provided in one of the cage's cornes; pure water provided in the others) [1]. It was also shown that the ability is correlated with the circadian rhythm.
In an attempt to find a specific mechanisms of learning, decision making and social interactions we employed a collection of computational models of behaviour, based on the Rescola-Wagner learning rule.
We discovered that model with decisions based purely on the learned rewards, with the probabilities of actions given by the softmax distribution fits the trend of the learning curve well (for both strains housed separatedly), but it does not capture the circadian oscillations.
The learning curve of a model assuming minimal interactions between animals (blocking a corner by a mouse already occupying it) starts to exhibit circadian oscillations arround the trend.

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P21:

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.

P22:

Conformational variation of nematode thymidylate synthase monitored by structure-based approaches

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Parasitic worms, including numerous nematodes are common infectious agents. One of them is Trichinella spiralis, known to cause trichinellosis. No effective chemotherapeutic treatment has been proposed so far against the muscle stage of this disease.
Thymidylate synthase (TS) catalyzing $N^{5,10}$-methylenetetrahydrofolate-dependent methylation of dUMP, is a target in chemotherapy. High specific activity of this enzyme is present not only in T. spiralis adult forms, but also in developmentally arrested muscle larvae, the latter devoid of DNA synthesis, thus not expected to show such activity. Similar activity has been found in Caenorhabditis elegans developmentally arrested dauer larvae, a developmental step corresponding to T. spiralis muscle larvae. TS present in these developmental stages is probably catalytically irrelevant but may play a regulatory role, as the enzyme shows certain non-catalytic activities, including capacity to bind mRNA and to inhibit translation. Therefore possibility of selective interference with nematode TS activities could provide both therapeutic method and means to study physiological meaning of high expression of TS in cells of developmentally arrested forms of nematodes.
C. elegans TS structural crystallographic data were used as an input for computer simulations. Molecular dynamics and essential dynamics methods were applied, to learn more on possible

conformations of the enzyme homodimer with ligands bound either in both active sites or in one of them. The results showed clear differences among conformations and protein motions in the studied systems, which will allow to identify differences in spatial organization of nematodial and mammalian TS, exposing potential sites of selective binding and enabling virtual selection of ligands capable of such binding to the enzyme.

P23:

Thermal unfolding of Large Mechanosensitive Channel (MscL)

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Mechanosensitive (MS) channels play an important role in a variety of physiological processes including: touch, hearing, balance and circulation. The MscL channel is homopentameric membrane protein protecting bacteria from hypoosmotic stress. Each subunit has two transmembrane domains M1 and M2. M1 domains associate near the five-fold symmetry axis to form narrow constriction and M2 helices are more peripheral. Under extreme hypooosmotic conditions MscL opens to form a large nonselective pore that protects the cell from lysis by releasing osmolytes. In spite the fact that MscL is one of the best-studied MS proteins, its molecular mechanism of transition from the open to the closed state is still poorly understood.
To better understand the mechanism of MscL gating, we conducted calcein efflux experiments. First we reconstituted purified MscL protein into liposomes containing high concentration of fluorophore calcein. Calcein exhibits concentration dependent self quenching. Liposomes containing high concentration of calcein have no fluorescence. Open channels release calcein from liposomes , decreasing its concentration and resulting in the increase of fluorescence. Using such fluorescence measurements we can monitor number of open channels. To check maximum fluorescence we use detergent Triton-X 100, which destabilizes liposomes releasing all calcein. We tested 40 chemical compounds using this method. Our results suggest that some of them can be MscL channel activators.

P24:

αCaMKII-autophosphorylation protects dendritic spines from chronic alcohol drinking effects

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Alcohol causes numerous changes in the nervous system, among which dendritic spine morphology alterations have just begun to be studied. αCaMKII-autophosphorylation has been shown to regulate spine morphology in vitro. We provide evidence that after chronic alcohol exposure αCaMKII-autophosphorylation-deficient mice (T286A mutants) – in contrast to wild-type controls - develop structural changes in dendritic spines in hippocampus that correlate with addiction-like behavior. We also found that T286A mice are less prone to extreme

behavior; they show neither very high motivation for alcohol nor drastic avoidance. Thus our data suggest that αCaMKII-autophosphorylation is involved in the development of alcohol addiction-related behaviors as well as homeostatic control of dendritic morphology.

P25:

Cardiac lipid metabolism – the role of stearoyl-CoA desaturase-4

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An increasing body of evidence shows that metabolic disturbances contribute to cardiac myocyte dysfunction, independent of associated coronary artery disease. In inherited disorders accumulation of unmetabolized lipids in cardiac myocytes is associated with ventricular systolic dysfunction. In obesity, increased myocardial oxygen consumption together with its decreased efficiency may contribute to diastolic and systolic dysfunction and heart failure. Recent studies on genetically obese animal models showed that disturbances in the regulation of intracellular lipid metabolism in cardiomiocytes result in “fatty heart” and cardiomyopathy. The aim of the presented study was to investigate the role of steraoyl-CoA desaturase 4, the enzyme that plays important role in lipid metabolism, in the cardiac fatty acid utilization. SCD 4 is expressed exclusively in the heart and catalyzes crucial step in synthesis of monounsaturated fatty acids. The obtained results showed that SCD4 overexpression significantly increases lipid droplet accumulation and triglyceride (TAG) and free fatty acid (FFA) contents in HL-1 cardiomyocytes. On the other hand, decrease in TAG and FFA contents were associated with reduced SCD4 expression. SCD4 plays also an important role in the regulation of FA □-oxidation as evidenced by modification of the expression of important proteins involved in this process, i.e. PPARα and AMPK. In conclusion, the presented results suggest that SCD4 downregulation leads to a significant decrease in the accumulation of lipids in the heart that possibly can reduce the level of apoptosis of cardiomyocytes and significantly improve left ventricular function. However, more research is needed to identify exact role of SCD4 in cardiac metabolism regulation.

P26:

Mitochondrial dynamics in primary fibroblasts derived from patients with Alzheimer’s and Parkinson’s diseases

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Defects in mitochondrial function have been implicated in many neurodegenerative diseases. Continuous fusion and fission of mitochondria are required to maintain their proper functions. The fusion is needed for maintaining a correct respiratory and metabolic activity of mitochondria, the fission, in a consequence, gives an opportunity to remove dysfunctional mitochondrial fragments from healthy mitochondrial network and is important for their transport

in the cell and proper segregation during the cell division. In order to understand the role of mitochondrial dynamics in cellular signaling in neurodegenerative diseases we have studied human primary cultures of skin fibroblasts derived from patients with sporadic Alzheimer's and Parkinson's disease (AD and PD). Mitochondrial dynamics is controlled by a number of proteins. We have investigated the level and the modifications of these proteins in AD and PD fibroblasts. We have found different level of Drp1 protein and changes in the level of phosphorylated Drp1 in AD and PD cells in comparison to controls. The shape and organization of mitochondrial network within AD and PD cells are also slightly modified.

P27:

Tumor-derived integrin ligands are responsible for pro-invasive polarization of glioma-associated microglia and macrophages

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Numerous clinical and experimental studies show that microenvironment plays important role in growth and metastasis of many tumors including malignant gliomas. Both microglia (brain resident macrophages) and bone marrow-derived macrophages infiltrate glioma, but instead of initiating the anti-tumor response, these cells support tumor progression by inducing extracellular matrix remodeling, angiogenesis and immunosuppression. The identity of glioma-derived molecules responsible for polarization of tumor-associated brain macrophages into pro-invasive cells are still unknown. We identified osteopontin and lactadherin as candidate molecules involved in pro-invasive polarization of brain macrophages. These proteins are αvβ3/αvβ5 integrin ligands and are able to interact with receptors present on microglia and macrophages. Both proteins are highly expressed in glioma cells, but not in non-transformed astrocytes. We generated clones of rat C6 glioma cells stably expressing shRNAs specific to lactadherin and osteopontin, and studied tumor progression after implantation of those cells into the brain of Wistar rats. As we did not detect differences in proliferation and viability of control cells and gene silenced clones in vitro, therefore we concluded that autocrine production of lactadherin and osteopontin has the negligible effect on tumor cell growth. Knockdown of either of proteins resulted in significant reduction of tumor volumes. Immunostaining for Iba1 demonstrated similar numbers of infiltrating microglia/macrophages but the reduced number of amoeboid, arginase 1 expressing cells, particularly in osteopontin-depleted tumors. Knockdown of either of proteins affected the blood vessels density lactadherin or osteopontin-depleted tumors. Our results suggest that glioma-derived osteopontin and lactadherin are crucial for polarization of glioma infiltrating microglia/macrophages into the pro-invasive phenotype and their targeting could be a new therapeutic strategy.

P28:

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.

P29:

Effect of CacyBP/SIP phosphatase on CREB activity in neuroblastoma NB2a cells

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The CacyBP/SIP protein was discovered as a calcyclin (S100A6) binding protein (1) and later as a Siah-1 interacting protein (2). CacyBP/SIP is present in different cells and tissues with the highest level in brain, where it was suggested to play a role in cytoskeleton reorganization (3,4). Recently, it has been shown that CacyBP/SIP has phosphatase activity towards ERK1/2 kinase and, in consequence, inhibits the activity of the Elk-1 transcription factor (5).
In this work we examined the influence of CacyBP/SIP on transcription factors engaged in different aspects of neuronal activity such as SRF, CREB, AP-1, STAT-3, NFκB and NFAT. In the current studies we focused on CREB (cAMP response element-binding) we chose mouse neuroblastoma NB2a cells and applied two methods, dual luciferase reporter assay and Western blot analysis. The influence of CacyBP/SIP on CREB activity was analyzed after CacyBP/SIP overexpression or silencing.
We found that the transcriptional activity of CREB after CacyBP/SIP overexpresion was inhibited in NB2a cells by about 22,7%. In cells with diminished level of CacyBP/SIP the activity of CREB in NB2a cells was increased up to 360%. Furthermore, CacyBP/SIP overexpression correlated with a diminished level of phosphorylated CREB. Altogether, our results suggest the influence of CacyBP/SIP on CREB activity in neuroblastoma NB2a cells.

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P30:

The adhesion molecule CD44 regulates organization of dendritic tree of hippocampal neurons

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Dendritic arborization patterns define neuronal subtypes, and have important functional implications, determining how signals coming from individual synapses are integrated. Developing dendrites of neurons are responsive to extrinsic signals. Although several secreted proteins, cell surface receptors and adhesion molecules has been recently shown to be involved in dendrite morphogenesis, the role of extracellular matrix (ECM) components and molecular mechanisms of signal transduction from ECM to the neuronal cells involved in these processes are still poorly understood. The main component of the ECM in the brain is hyaluronan (HA). The major receptor for hyaluronan is CD44 adhesion molecule which mediates the response of cells to their extracellular microenvironment. The aim of this study was to investigate the role of CD44 in regulation of dendritic tree arborization. First, we examined the expression pattern of CD44 at the protein and RNA level in the rodent brain. All our experimental approaches clearly point to the neuronal localization of CD44, in addition to widely accepted presence in glia. Next, we investigated the role of CD44 in the primary neuronal cultures in vitro and organotypic hippocampal cultures using shRNA technology. The morphometric analysis showed that cells with diminished expression of CD44 have more complex dendritic tree then control cells. Golgi apparatus has been shown to be an organellum which is involved in development of proper dendritic tree of neuronal cells. Our results suggest that morphology of the Golgi apparatus is modified in neurons with changed CD44 expression. We observed more dispersed Golgi apparatus in cells with CD44 knock down in contrast to more condensed one in CD44 overexpressing cells in comparison to control or not transfected cells. The results of our experiments point to the importance of CD44 protein for the development of dendritic tree.

P31:

p53-independent pathways in the DNA-damage induced senescence of cancer cells

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Cancer cells upon DNA damage undergo so named stress-induced senescence (SIPS). In DNA damage-induced senescence the key protein is p53, which is activated by ATM kinase. In turn p53 transactivates CDKN1 encoding for cdk's inhibitor p21. Recently we have shown that not only p53 wild type (WT) but also p53 knock-out (KO) HCT116 cells undergo SIPS upon curcumin treatment, even though curcumin does not cause direct DNA damage (Mosieniak et al., 2012). Nonetheless, senescence was dependent on p21 expression. Now, we were interested whether other proteins than p53, namely NF-κB, which acts downstream of ATM, can activate CDKN1 and induce senescence in cells treated with DNA damaging agent. To this end we treated p53 WT and p53 KO HCT116 with Topo2 inhibitor (doxorubicin) for 7 days. We showed that both p53 WT and p53 KO cells treated with doxorubicin displayed hallmarks of senescence, such as increased size and granularity, β-galactosidase activity and cell cycle arrest. Both p53 WT and p53 KO cells exhibited senescence-associated secretory phenotype – they produced VEGF and IL-8. Interestingly, p53 KO cells produced even more IL-8 than p53 WT cells. Western blot analysis of the total and phosphorylated proteins of the NF-κB signaling pathway, such as IκBα, IKKα, IKKβ, and p65, unexpectedly showed no activation of NF-κB. It seems that NF-κB is not involved in DNA damage-induced senescence of HCT116 cells. The signalling pathways which could be activated after treatment with DNA damaging agents and lead to SIPS are under elucidation.

P32:

Localization of recent and remote memory traces for successful and impaired fear extinction

Szadzinska W., Rokosz K., Mikosz M., Sadowska J., Knapska E.

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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.

P33:

Regulation of dendritogenesis by ZBP1 depends on its phosphorylation at Ser181

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Zipcode binding protein 1 (ZBP1) is one of several RNA binding proteins found in ribonucleoparticles (RNPs) and dendritic processing bodies (P-bodies), structures involved in mRNA silencing and transport - both required for local protein synthesis, a mechanism present in various types of polarized cells. In neurons, local protein synthesis enables proper axonal growth cone and spine formation, as well as appropriate dendritic arborization - features that define electrical properties of a neuron. Recently we showed that dendritogenesis relies on ZBP1-dependent dendritic transport of β-actin mRNA and its local translation. 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 (i) dendritic arborization and (ii) cellular distribution of ZBP1. To address these questions we constructed non-phosphorable (S181A) and phospho-mimicking (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 mutants was more even than the 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.
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P34:

Biogenesis of mitochondria-localized superoxide dismutase 1

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Mitochondria are the main site of reactive oxygen species production that is harmful for the cells. Sod1 as a major superoxide-scavenging enzyme in the eukaryotic cell is localized in the cytosol and in the intermembrane space (IMS) of mitochondria. To be entrapped in mitochondria in the fully functional form, Sod1 requires the Ccs1 chaperone that inserts Cu^{2+} and transfers disulfide bonds to Sod1. Surprisingly, we found Sod1 in the mitochondria lacking Ccs1 and in a fully reduced form. Furthermore, the amyotrophic lateral sclerosis-linked

versions of Sod1 were also efficiently localized to mitochondria despite their inability to be oxidized by Ccs1. Interestingly, the localization of the reduced forms of Sod1 was reliant on Mia40, an essential component of the MIA pathway, which is responsible for the biogenesis of IMS proteins. Thus, we suggest that the MIA pathway is a novel factor influencing pathology caused by Sod1.

P35:

Role of microtubule severing proteins in motile cilia

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Microtubule-severing proteins are widely distributed in all Eukaryotes. The first enzyme with an ATP-dependent microtubule-severing activity, was purified in 1993 and named katanin. Together with later identified katanin-like proteins, spastin and fidgetin, katanin belongs to the group of closely related microtubule-severing enzymes within AAA (ATPases Associated with various cellular Activities) family of proteins. Microtubule-severing proteins generate internal breaks in microtubules. This activity is essential in several cellular processes such as: movement of chromosomes in mitotic spindle, cell migration, formation of cortical microtubules in higher plants and branch formation in neurons.
Some data suggest that katanin severing activity is implicated in the assembly of motile cilia. Depletion or mutation of katanin or its regulatory subunit p80 in Chlamydomonas and Tetrahymena, affects formation of the central pair microtubules and cause cilia or flagella immotility. However, the mechanism of this process remains unknown.
To better understand the mechanisms of katanin function, we expressed katanin, its regulatory subunit, p80 and katanin-like protein, as HA tagged fusion proteins and performed systematic analysis of their localization at the cellular and at the ultrastructural level in Tetrahymena cells.
Next, because it was shown that in mammalian cells spastin preferentially associates with glutamylated microtubules, we decided to investigate if changes in the level of tubulin glutamylation would affect localization of analysed proteins in Tetrahymena cells, especially in cilia and basal bodies. It turned out that the changes in the level of tubulin glutamylation in microtubules indeed affect localization of katanin and katanin-like protein but do not have effect on the distribution of the katanin regulatory subunit, p80.

P36:

Molecular Mechanism of Mutant p53 Stabilization: The Role of HSP70 and MDM2

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Numerous p53 missense mutations possess gain-of-function activities. Studies in mouse models have demonstrated that the stabilization of p53 R172H (R175H in human) mutant

protein, by currently unknown factors, is a prerequisite for its oncogenic gain-of-function phenotype such as tumour progression and metastasis. Here we show that MDM2-dependent ubiquitination and degradation of p53 R175H mutant protein in mouse embryonic fibroblasts is partially inhibited by increasing concentration of heat shock protein 70 (HSP70/HSPA1-A). These phenomena correlate well with the appearance of HSP70-dependent folding intermediates in the form of dynamic cytoplasmic spots containing aggregate-prone p53 R175H and several molecular chaperones. We propose that a transient but recurrent interaction with HSP70 may lead to an increase in mutant p53 protein half-life. In the presence of MDM2 these pseudoaggregates can form stable amyloid-like structures, which occasionally merge into an aggresome. In cancer cells, where endogenous HSP70 levels are already elevated, mutant p53 protein forms nuclear aggregates without the addition of exogenous HSP70. Aggregates containing p53 are also visible under conditions where p53 is partially unfolded: 37°C for temperature-sensitive variant p53 V143A and 42°C for wild-type p53. Refolding kinetics of p53 indicate that HSP70 causes transient exposure of p53 aggregate-prone domain(s). We propose that formation of HSP70- and MDM2-dependent protein coaggregates in tumours with high levels of these two proteins could be one of the mechanisms by which mutant p53 is stabilized. Moreover, sequestration of p73 tumour suppressor protein by these nuclear aggregates may lead to gain-of-function phenotypes.

P37:

Different response to apoptotic stimulation can distinguishes lymphocyte from sporadic and familial Alzheimer`s disease patients

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It has been shown that in AD patients, molecular changes occur not only in in neurons, but also in peripheral blood cells such as lymphocytes. The main goal of this study was to compare apoptotic response to oxidative stress in B-lymphocytes from healthy individuals and from patients with familial (FAD) and sporadic form of AD (SAD). Our study was conducted using EBV-immortalized B-lymphocytes from patients with SAD and FAD in comparison to cells from two age-matched nondemented control groups, adequately S-CTR and F-CTR. We investigated response of lymphocytes to oxidative stress evoked by reducing sugar 2-deoxy-D-ribose (2dRib).
Role of mitochondria in response to the oxidative stress was investigated by measuring changes in mitochondrial membrane potential (MMP). In these assay we used cationic dye JC-1 staining, commonly used as an indicator of MMP. We also examined apoptosis after 2dRib- induced oxidative stress by flow cytometry measurement of SubG1 level (identification of DNA fragmentation as cells with fractional DNA content on histograms).
24h after 40 mM 2dRib treatment, JC-1 assay showed the higher apoptotic rsponse of SAD lymphocytes comparing to FAD cells. Accordingly, in the same conditions, SAD lymphocytes showed increased percentage of cells in SubG1 phase when compared to FAD cells. Under basal conditions without 2dRib stimulation, each of the assays showed no significant changes between cells from all four groups. Altogether, our work shows that changes in the apoptotic response can distinguish cells from SAD and FAD patients.

P38:

DNA methylation regulates expression of pro-epileptic protease MMP-9 during epileptogenesis

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Epilepsy is one of the most common neurological disorders in humans. A disturbance of epigenetic mechanisms is just started to be revealed as one ofimportant factors underlying a development of seizures. Matrix Metalloproteinase-9 (MMP-9) is a pro-epileptic protein which expression is regulated mainly on the mRNA level. It is involved in a formation of aberrant brain neuronal networks during epileptogenesis, what finally leads to a development of seizures.
Our recent data suggest that MMP-9 gene transcription in brain neurons is regulated via depolarization-dependent epigenetic mechanisms. We have studied epigenetic changes occurring on the Mmp-9 promoter during pentylenetetrazole (PTZ)-induced kindling in the rat hippocampus, which is a model of an epileptogenesis. We found that MMP-9 activity as well as MMP-9 mRNA expression are gradually upregulated during PTZ-induced epileptogenesis in the rat hippocampus. Moreover, we noticed that the induction of MMP-9 gene expression during epileptogenesis is associated with a progressive association of DNA demethylase Gadd45β (growth arrest and DNA-damage-inducible) and simultaneous dissociation of DNA methyltransferase Dnmt3b from the proximal Mmp-9 promoter during epileptogenesis in the rat hippocampus in vivo. Consequently, these phenomena were accompanied by a successive demethylation of the proximal Mmp-9 promoter during epileptogenesis in the rat hippocampus in vivo. Additionally, we showed that intraperitonneal administration of anti-epileptic agent dizocilpine(MK-801) inhibits PTZ-induced kindling and MMP-9 expression as well as it blocks MMP-9 proximal promoter demethylation. These results altogether strongly suggests that MMP-9 promoter demethylation is an important factor driving the upregulation of MMP-9 expression during epileptogenesis in the rat hippocampus in vivo.