Abstracts

Long talks

Polish Sign Language comprehension: effects of proficiency. A longitudinal fMRI study of hearing late learners.

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The neural plasticity underlying learning is a process rather than a single event, however the dynamics of training-induced functional reorganization are rarely examined. In the current study we focus on sign language acquisition in hearing adults who underwent an 8-months long sign language training with five functional magnetic resonance sessions. At each session, we tested whether growing proficiency leads to increased brain activity and a brain-wide reconfiguration of activity patterns due to the transition from sensory to linguistic processing. We also explored whether the above-mentioned processes are different depending on the level of linguistic processing (lexical or sentential). Language network reorganization occurred after 3 months of learning (second fMRI session), as reflected by increased activation in modality-independent perisilvian language-related network (e.g. left inferior frontal gyrus), together with modality-dependent parieto-occipital, visuospatial and motion-sensitive regions (bilateral superior parietal lobule and lateral occipital cortex). Such reorganization happens regardless of the linguistic complexity of the performed task. Moreover, despite further progress, no significant alterations in fMRI response were detected during the following months. Our results indicate that large-scale brain reorganization occurs during the first months of sign language acquisition, and further consolidation and learning proceeds in a stable, local manner.

SH3BP2 as a novel scaffold protein regulating muscle postsynaptic machinery

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Neuromuscular junctions (NMJs) are synapses formed between motor neurons and skeletal muscle fibers. Abnormalities in NMJ development lead to various neuromuscular disorders, which are often fatal. Despite their crucial role, the mechanisms that orchestrate NMJ development are still poorly understood. The Dystrophin-associated Glycoprotein Complex (DGC) is a major laminin receptor in the muscle required for proper development of the postsynaptic machinery by linking its components to the extracellular matrix and the actin cytoskeleton. One of the cytoplasmic DGC-associated proteins, α-dystrobrevin-1 (αDB1), was shown to play an important role in the organization of the NMJ postsynaptic machinery. For it's proper functioning αDB1 needs to be phosphorylated on its C-terminal fragment. To gain insight into the molecular mechanism of αDB1 function we have recently performed a biochemical screen for phospho-specific interacting proteins and identified SH3BP2 as a binding partner. SH3BP2 is a scaffold protein with unknown localization and function in skeletal muscles. We demonstrate that SH3BP2 is concentrated at the NMJ postsynaptic machinery and also at the muscle contractile machinery. Cultured myotubes depleted of

SH3BP2 had the impaired ability to cluster AChRs. A similar phenotype was observed at the NMJ upon muscles-specific deletion of SH3BP2. Protein complex purification experiments combined with mass spectrometry analysis revealed that SH3BP2 interacts with several postsynaptic proteins including Lrp4, AChR, and CK2, proteins of the muscle contraction machinery as well as several components of the DGC. Our results suggest that SH3BP2 acts as a scaffold protein involved in the organization of the NMJ postsynaptic specialization. This research was supported by the National Science Centre grants 2012/05/E/NZ3/00487, 2013/09/B/NZ3/03524, 2015/19/N/NZ5/02268 and 2016/21/B/NZ3/03638.

Is there anything specific in the way dyslexics read?

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Objective: Reduced activation to print in the left ventral, dorsal and anterior pathways has been implicated in readers with dyslexia (DR) but is also characteristic of typical, less proficient reading. As the majority of studies compared DR to their age-matched peers, the observed results could either represent a dyslexia phenotype or a developmental delay. We aimed to disentangle reading and dyslexia effects by employing two control groups: age and skill matched, and a longitudinal design. Method: We compared brain response for print in DR with typical readers (TR) who at the beginning of schooling (TP1, 6-7 years) read on average 3 words per minute, such as DR at TP1, but improved reading to an average level; and advanced readers (AR) who at TP1 read as well as DR two years later (TP3, 8-9 years). The TR and DR groups were tracked longitudinally to observe neurodevelopmental changes. Results: At TP1, no differences were observed between DR and TR. Only in TR did the neural circuit for reading in the left inferior frontal and fusiform gyri emerge along with reading development. At TP3, DR showed consistent hypoactivation in these areas compared to both age- (TR TP3) and reading-matched (AR TP1) controls. At TP3, TR hypoactivated left frontal and bilateral ventral occipital regions when compared to AR, but these effects were non-overlapping with hypoactivations present in DR and are partly accounted for by IQ. Conclusions: Decreased activation of the left fusiform and inferior frontal gyri to print in DR results from an atypical developmental trajectory of reading and cannot be explained solely by lower reading skills.

Detection of rare functional elements in proteins with machine learning – the case of π-helices

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Proteins play a crucial role in nearly all biological processes. Despite the variety of their functions, the chemistry of the protein world is rather simple, encoded by an alphabet of 20 building blocks – amino acids. The order of amino acids in a protein sequence determines its structure, which, in turn, dictates the function. To bridge the gap between the vast number of known protein sequences and the limited number of experimental structures, we develop machine learning methods that can predict properties of protein structures from sequence data

alone. I will present our recent tool for detection of π-helices in protein sequences. π-helices are short, unstable secondary structure elements present in 15% of all protein structures, often in functionally important regions. Given their similarity to α-helices, prediction of π-helices is a challenging task and none of the currently available secondary structure prediction methods tackle it. To fill this void, we have developed PiPred, a publicly available tool for predicting π-helices in protein sequences. Rigorous benchmarks showed that PiPred can detect π-helices with a per-residue precision of 47% and sensitivity of 45%. PiPred is freely available on the web at https://toolkit.tuebingen.mpg.de/#/tools/quick2d (as a part of the Quick2D tool). A standalone version is available for download at https://github.com/labstructbioinf/PiPred.

Role of stearoyl-CoA desaturase 1 in epigenetic control of pancreatic β-cells functional identity

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Type 2 diabetes (T2D) is a complex metabolic disorder, the worldwide prevalence of which is growing rapidly. Recent genetic and epigenetic studies indicate that not only β-cell demise, but also the loss of pancreatic endocrine cell identities are major causes of the development of T2D. The identity of insulin- and glucagon-secreting cells in pancreatic islets is maintained by dynamic control of specific transcription factors (TFs) expression. It has been shown that stearoyl-CoA desaturase 1 (SCD1), the rate-limiting enzyme in monounsaturated fatty acids synthesis, can regulate gene expression through changes in DNA methylation level. In the present study we tested the hypothesis that SCD1 affects expression patterns of TFs involved in maintenance of pancreatic β-cells identity via epigenetic mechanisms. The experiments were conducted ex vivo on Scd1 knock-out mice (SCD1 KO) and in vitro on INS-1E β-pancreatic cell line. Changes in SCD1 gene expression/activity in INS-1E cells were introduced using the SCD1 inhibitor (A939572), siRNA and plasmid vectors, respectively. Lipotoxicity was induced by palmitic acid (0.4 mM) treatment. Our data show that pancreatic islets of SCD1 KO mice are characterized by lower insulin secretion and different microarchitecture comparing with wild type mice. Furthermore we observed that SCD1 deficiency decreases protein level of TFs crucial for maintenance of β-cells identity (Pdx1, FoxO1, Isl1). In contrast SCD1 overexpression increases expression of Pdx1, FoxO1 and Pax6 in INS-1E cells. In addition, we noticed that inhibition of SCD1 activity as well as silencing of SCD1 gene expression, lead to global DNA hypomethylation and decrease in methyltransferase 1 (Dnmt1) protein level in INS-1E cells. It is also worth noting, that significant changes in gene expression of Pdx1 and MafA were accompanied by changes in methylation pattern within their promoter regions. Obtained results suggest that SCD1 affects expression of identity TFs in pancreatic islets via alterations in DNA methylation pattern. Therefore, SCD1 plays an important role in maintenance of pancreatic β-cells functional identities. Acknowledgements: This research was supported by National Science Centre, Poland, grant no UMO-2013/10/E/NZ3/00670 and UMO-2017/27/N/NZ3/01987.

Do Autistic Mice Dream Of Following Each Other?

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A novel method of sociability assessment in the IntelliCage™ system.

Neurogenesis and behavior – is there a link?

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The CREB/CREM/ATF transcription factors family have been found to play important role in the process of adult neurogenesis that is a generation and maturation of new neurons. The incorporation of these cells into neuronal network takes place in SVZ (SubVentricular Zone) and in SGZ (Sub-Granular Zone) of the hippocampus. This gene family includes a shortened variant of CREM protein - ICER (Inducible cAMP Early Repressor) very effective in repression of other family members activity and its own expression as well. In order to examine the influence of the ICER gene overexpression on CREB dependent gene expression in neurons we have developed the Syn-Flag-ICER II transgenic rat line. Paradoxically, we have detected increased levels of mRNA for CREB or CREM factors. According to this one of genes known to rely on CREB transcription factor – miR-132 – was also upregulated. Matrix metalloproteinase MMP-9 is a crucial element in active reorganization of extracellular matrix . This process inter alia allows for growth of neuronal dendrites and axons which guarantees successful incorporation of new born neurons into neuronal network in developing brain. As miR-132 targets mRNA for MMP-9 we have observed decreased activity of this proteinase in samples from male rat hippocampi. Additionally,Patch clamp recordings in the dentate gyrus of the hippocamus indicate higher excitability of neurons in transgenic male rats. As a consequence we have found that ICER II overexpressing rats showed reduced hippocampal neurogenesis. The number of the BrdU positive cells in the SGZ, where progenitor cells are located, was similar. However, in the granular layer we have noted a shortage of BrdU positive cells when compared to control animals. This observation may indicate difficulties in proper maturation and incorporation of new born cells. In the Morris Water Maze learning paradigm animals with affected neurogenesis employ different learning strategies than their control littermates. The results of this behavioral tests indicate that transgenic rats didn't differ from controls in their learning and memory capabilities, however they showed differences in strategies of finding the hidden platform. Male ICER rats more often were choosing imprecise strategies to find platform than control males. Obtained results demostrate that CREB dependent gene expression in neurons regulates a set of genes e.g. miR-132 that may in turn regulate translation of proteins involved in remodeling of extracellular matrix and affect adult neurogenesis. Such manipulation changes discrete aspects of animal cognitive behavior.

Sensitizing glioma cells to fatty acid oxidation inhibitor by modulation of SLC22A5 carnitine transporter activity.

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Gliomas are the most common primary malignant brain tumors in adults. Current treatment for glioblastoma patients is not sufficient – most patients will survive not more than 12-15 months from the time of diagnosis. Recent studies show that primary-cultured human glioblastoma multiforme cells depend upon fatty acid oxidation (FAO) for aerobic respiration and proliferation and that inhibition of FAO in vivo by etomoxir prolongs survival in the mouse model of malignant glioma. Etomoxir, an inhibitor of FAO rate-limiting enzyme – carnitine palmitoylotransferase 1 (CPT1), was tested in clinical trials which were terminated due to high hepatotoxicity. Carnitine is essential for the proper functioning of CPT1, and SLC22A5 is the only high-affinity carnitine transporter in the plasma membrane that is expressed in the central nervous system. Moreover, HEK293 cells overexpressing SLC22A5

show increased transport of several chemotherapeutics, the process that may inhibit carnitine transport into the cell. Thus this study is aimed at establishing the role of SLC22A5 in glioma cells and at assessing its potential for sensitizing them to lower, less toxic doses of etomoxir. Statistical analysis of SLC22A5 expression data from REMBRANDT database (Repository of Molecular BRAin Neoplasia DaTa) show that glioma patient-derived tissues show significantly higher SLC22A5 expression when compared to normal tissues. Similar results were obtained by Western Blot analysis of SLC22A5 in non-transformed astrocytes and several glioma cell lines. Transport of radiolabeled carnitine was measured to verify whether anti-cancer drugs transported by SLC22A5 are able to inhibit carnitine transport in U87-MG, LN229 and T89G glioma cells. Out of several tested drugs, vinorelbine and vincristine are most efficient in carnitine transport inhibition in all of the tested lines. The viability and proliferation, tested by MTT and CellTox assays, are significantly reduced when vincristine and vinorelbine are used with etomoxir. We conclude that proper carnitine delivery by SLC22A5 transporter is important for glioblastoma metabolism and that several chemotherapeutic drugs transported by SLC22A5 may induce cell death both by their regular mechanism of action and by inhibition of carnitine delivery to glioma cells, thus sensitizing them to lower, less toxic doses of CPT1 inhibitors This project is financed by grant 2016/23/N/NZ3/02430 from National Science Centre in Poland.

MMP-9 inhibition as antiepileptogenic strategy in mice intrahippocampal kainic acid model

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Introduction: Epilepsy is the most widespread neurological disorder (prevalence – 50 million) and still no therapies exist that can treat not only the symptoms – seizures, but the underlying causes of disease and prevent its development. The recent discoveries suggest that remodelling of the brain extracellular matrix, executed by extracellularly operating proteases, and MMP-9 in particular, may play a fundamental role in the pathogenesis of epilepsy. Aim: Our aim was to investigate the time and spacial pattern of MMP-9 activation in different brain regions upon intrahippocampal kainic acid injection and to analyse the ability of promising MMP-9 inhibitors to penetrate the blood-brain barrier(BBB) and exert its action. Methods: In animal model mice were injected with kainate at the left CA1 area of the dorsal hippocampus, MMP-9 activity was evaluated by qRT-PCR. In situ zymography and immunostaining techniques were applied to visualize gelatynolitic activity, neurons and astroglia. Compounds ability to penetrate BBB was analyzed in vivo by detecting its level in brain regions and blood serum by UPLC-MS/MS Results: Intrahippocampal kainic acid injection upregulated brain MMP-9 mRNA expression, the maximum increase (2,5 fold) was observed for ipsilateral hippocampus 24 h after kainate injection. Moreover, kainic acid induced significant boost of reactive astrocytes and microglia in CA1 and CA3 areas. MMP-9 inhibitors marimastat and IPR-179 were shown to penetrate the BBB in mice, with maximum concentration of 97 ng/g tissue and 24 ng/g for IPR-179 in hippocampus, respectively. Marimastat administration resulted in 40 % inhibition in MMP-9's activity in the brain. Conclusion: This study is the first attempt ever to investigate MMP-9 activation pattern in intrahippocampal kainic acid epileptogenesis model in mice. Obtained results, indicating the maximum MMP-9 increase 24 h after kainate administration accompanied by astrocytosis, are indispensable for optimization the model for testing potential MMP-9 inhibitors.

Serum Response Factor (SRF) is essential for dendritic spines maturation during development

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Dendritic spines, the locus for excitatory synapses, are morphologically heterogeneous, showing a correlation between spine structure and function. Re-arrangement of neuronal networks is essential for synaptic transmission and proper circuitry formation. During brain development, dendritic spines' shapes change from thin, elongated filopodia-like structures to stable mushroom spines. Neuronal remodelling is dependent on activity-regulated transcription of numerous genes. Serum Response Factor, one of the major transcription factors in the brain, plays a prominent role in various programs of gene expression in the adult brain, but its function during brain development is still unclear. The aim of our study was to investigate a potential new role of SRF in the regulation of spines' maturation. We found that SRF protein level changes within the course of postnatal development of mouse hippocampus in vivo. SRF knockdown in rat hippocampal primary cultures resulted in the increased number of immature filopodia-like protrusions and decreased number of mushroom spines with the general lack of changes in the overall density of dendritic spines. Moreover, low level of SRF during hippocampal development influences the number of functional synapses and their activity. The analysis of AMPAR-mediated miniature excitatory postsynaptic currents revealed reduction in the frequency and amplitude of mEPSCs. Obtained results are in agreement with our observation that SRF-depleted neurons have lower level of AMPAR GluR1 and GluR2 mRNA, as well as these receptor subunits total and surface levels. These findings indicate that SRF regulates transcription of genes essential for spine maturation and synapse formation during hippocampal development.

Angiomotins – a novel family of proteins involved in neuronal organisation and mice behavior

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Angiomotins family comprises of three scaffold proteins Amot, Amotl1 and Amotl2. All three angiomotins are known to regulate Hippo signaling pathway. The function of Angiomotins in CNS neurons, brain morphology and mice behavior has not been investigated. We have discovered that all three Angiomotin proteins are widely expressed in the brain and localize to the synaptic compartments in mature neurons. Our functional experiments on cultured neurons revealed that Amot regulates dendritic tree arborization. Mass spectrometry analysis of neuron-specific Amot interactors highlighted Yap, a transcription co-activator which plays a pivotal role in the Hippo signaling pathway. To study function of Angiomotins and Yap in the brain we generated mice with neuronal specific ablation of the protein. Amot or Yap mutant mice displayed similar phenotype: abnormal cerebellar morphology with defects in motor coordination. Additionally deletion of Amot or Yap caused abnormalities in the development of dendritic tree arbors in cerebellar Purkinje cells, similarly to the phenotype observed in our in vitro experiments. Although all three Angiomotin proteins are considered closely related, they appear to mediate different functions in the brain function and mice behavior. Deletion of Amotl1 did not affect motor coordination, but led to abnormalities in anxiety and social behavior. Collectively, our research identified a novel family of proteins that regulates neuronal organization and behavior of living animals. This research was

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Role of the mitochondrial mechanisms and nicotinamide N-methyltransferase in the LPS-induced endothelial dysfunction

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Endothelium is highly dynamic and active organ, constantly sensing and responding to the local extracellular environmental stimuli. Key endothelial role is vasoregulation, regulation of nutrient trafficking, homeostasis, inflammatory response and forming the blood barrier. Under septic conditions all of these functions may be impaired which can lead to the critical decrease of arterial pressure, refractory multi-organ dysfunction and finally – death. Our study aims to elucidate biochemical mechanisms explaining the relationship between NNMT activity and mitochondrial energy metabolism in LPS-treated Human Aortic Endothelial Cells (HAECs). In most cell types mitochondria are the main energy transducing and ATP delivering organelles and key metabolic regulators. According to the literature sepsis induces mitochondrial damage and downregulates transcription of mitochondrially encoded genes eventually leading to an impairment of the oxidative phosphorylation. However, due to very low mitochondrial content in the endothelial cells (only 2-6% of cytoplasm volume) a primary role of these organelles seems to be related to an intracellular signalling rather than ADP phosphorylation. We found that HAECs exposed to LPS showed dynamic, time-dependent changes in mitochondrial structure and the respiration rate while the total mitochondrial mass was not affected. Nicotinamide N-methyltransferase (NNMT) is an enzyme responsible for methylation of nicotinamide, forming the N1-methylnicotinamide (MNAM). Previously it was shown that NNMT regulates several metabolic pathways in liver, adipose tissue and cancer cells through the depletion of methyl donors and producing active metabolites but its role in the endothelium still remains unknown. Our experimental data clearly show that an incubation of HAECs with LPS for 6 and 24 hours results in an elevation of NNMT protein content. This observation may indicate that NNMT is involved in a defense response of endothelial cells exposed to LPS but the precise role of this enzyme under such challenging conditions needs to be elucidated.

Open chromatin landscape of rat microglia upon inflammatory or proinvasive polarization

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Rat is an important model organism in neuroscience, pharmacology and studies of brain tumors. Microglia are brain-resident, myeloid cells, that play important roles in health and brain pathologies. Glioma-secreted factors induce the pro-invasive and immunosuppressive activation of microglia, in which these cells promote tumor growth. Herein, we report the first comprehensive open chromatin (DNase-seq) data for the rat microglia. We compared open chromatin landscapes in untreated primary microglial cultures and cultures stimulated for 6 h

with lipopolysaccharide (LPS) or glioma-conditioned medium (GCM). The open chromatin landscape of the rat microglia consists of 126,640 reproducible DNase-hypersensitive (DHS) regions, of which 12357 and 2303 regions significantly (FDR 5%) change openness following the stimulation with LPS and GCM, respectively. Gene-bodies and +/10 kb gene-flanking regions are over-represented among the DHS regions, more so among the regions regulated by LPS, whereas the DHS regions regulated by GCM are enriched in regions more then 10kb away from gene bodies. GCM and LPS treatment differentially affect openness of (DHS mapped to) multiple genes in the functional KEGG pathways of Axon guidance and Toll-like receptor signaling, suggesting that these pathways are instrumental for glioma-induced proinvasive polarization of microglia. Mammalian-wide conserved transposable elements (TEs) are enriched in the open chromatin, whereas rat-specific TEs are strongly depleted – suggesting common regulatory roles of TEs across mammals. Different families of content retrotransposons that are present within gene-overlapping open chromatin regions, significantly classify host genes into distinguishably different functional classes. This observation emphasizes the importance of TEs in evolution of genes, their regulation and function. 912 lincRNA genes are associated with 1823 gene-overlapping DHS regions. Pvt1, which is known to regulate c-Myc gene, harbor the highest number of such regions (37) among all lincRNA genes. Genes classified by expression-specificity exhibit distinct DNase-I cut patterns around their TSSs. Active (microarray detected) genes possess constitutively open promoters.

Generation of silent synapses in dentate gyrus correlates with development of alcohol addiction

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The brain circuits and synaptic processes that underlie alcohol addiction are currently the subject of intensive research. Here we focus on hippocampal circuitry and show that chemogenetic inhibition of dentate gyrus (DG) during presentation of alcohol-associated cues has long-lasting effects on mice behavior. DG inhibition enhances alcohol seeking and drinking, suggesting that DG regulates addiction-related behaviors. To test this hypothesis, we perform whole-cell patch-clamp recordings from the granule cells of DG and look for electrophysiological correlates of alcohol addiction. We observe that presentation of alcohol-associated cue light that induces relapse to alcohol-seeking results in generation of silent synapses, that lack functional AMPA receptors. Furthermore, using human criteria of addiction, we differentiate mice controlling their alcohol consumption from those that undergo transition to addiction to discover that the levels of silent synapses induced by alcohol cues are specifically increased in the addicted mice. As the total level of dendritic spines that harbor synapses is constant at this time point, our data indicate that synapses of perforant path to DG are weakened during cue relapse. Finally we demonstrate that, acamprosate, a drug that limits alcohol drinking and seeking in addicts, prevents generation of silent synapses in DG upon presentation of alcohol-associated cues. Altogether, our data suggest that weakening of DG synapses upon cue relapse contributes to persistent alcohol addiction-related behaviors.

Epigenetic and Transcriptomic Profiling of Glioma Associated Microglia/Macrophages

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Glioblastoma is the most aggressive brain tumor characterized by massive infiltration of innate immune cells such as microglia and peripheral macrophages - up to 30% of the tumor mass. We previously showed that glioma-derived factors drive transformation of microglia and macrophages to glioma associated macrophages, which exhibit suppressed anti-tumor activity and promote tumor invasion and angiogenesis. Recent reports found that polarization of microglia is driven by specific transcription factors. The epigenetic landscape and networks of transcription regulatory elements can be modulated by microenvironment and tissue specific factors that may lead to altered immune response pathways. In the present study, we aim to identify epigenetic changes underlying transformation of tumor infiltrating immune cells to the pro-tumorigenic phenotype. We employ murine GL261 glioblastoma model to isolate microglia and macrophages from the tumor by flow cytometry (FACS), and next perform DNA and RNA sequencing. In this exploratory study we will perform ChIP-seq to profile the epigenetic landscape of key histone modifications: H3K4me3 and H3K27ac - active chromatin marks, H3K9me3 and H3K27me3 - repressed chromatin marks, and combine these data with the active chromatin regions (ATAC-seq) and transcriptomic profiles (RNA-seq) of immunosorted microglia/macrophages. The ChIP-seq protocol was optimized for low number of FACS sorted cells allowing us to yield high quality data. The analysis of H3K4me3 modification showed differential enrichment for immune response related genes and distal regulatory elements indicating altered modes of regulation. In the future, we would like to integrate the epigenetic profiling with single-cell RNA sequencing to decipher epigenetic regulatory mechanism in specific subpopulations of glioma-infiltrating immune cells.

The role of Arc/Arg3.1 protein in the regulation of alcohol seeking

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Alcohol addiction is a psychiatric disorder, characterized by compulsion to seek and take the alcohol, and high propensity for relapse after withdrawal. The glutamatergic system plays an important role in addiction due to its involvement in the brain reward system. Arc protein is involved in learning, plasticity and in the regulation of glutamatergic system due to control over AMPA receptor endocytosis. However, the function of Arc in alcohol addiction is unknown. Our data show that Arc KO mice drink as much alcohol as WT, but they are more persistent in alcohol seeking during alcohol withdrawal. Furthermore, Arc protein is upregulated at the synapses of Central Amygdala (CeA) in WT mice after withdrawal from long-term alcohol training. In order to analyze the role of Arc protein in the CeA in the regulation of addiction-related behavior we conducted longitudinal study in the IntelliCages. Our data indicates that mice with local deletion of arc in CeA by CrispR/Cas9 system are more persistent in alcohol seeking during cue-induced relapse. Moreover Arc deletion from CeA increases GluR1 expression compared to control after the Withdrawal. To confirm that Arc regulation was alcohol specific we trained mice in Intellicage for long term sucrose drinking. We found that mice with deletion of arc from CeA don’t differ in any tests compare to the control. In conclusions, we show the novel role of Arc protein in CeA as a specific regulator of alcohol seeking during relapse induced by alcohol-associated cues by regulation of the glutamatergic system.

Amot controls dendritic tree development through Yap1

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The Amot-Yap1 complex plays a major role in the regulation of cell contact inhibition, cellular polarity and growth in many cell types. However, the function of Angiomotin (Amot) and Hippo pathway transcription co-activator Yap1 in the central nervous system remains unclear. We demonstrate that Amot is a critical mediator of dendritic morphogenesis in cultured hippocampal cells. Amot function in developing neurons depends on interactions with Yap1, which is also indispensable for dendrite growth and arborization in vitro. Our results indicate that the function of Amot and Yap1 in dendrite growth does not rely on interactions with TEAD transcription factors or the expression of Hippo pathway-dependent genes. Instead, Amot and Yap1 regulate dendrite development through affecting phosphorylation of ribosomal protein S6.

Mapping of open chromatin and histone modifications in human gliomas

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Growing evidence indicates global dysregulation of epigenetics in gliomas as a driving force of transcriptional changes and pathogenic mechanisms. Gliomas, the most common primary brain tumors, are clinically divided by WHO into 4 grades according to their malignancy. Slowly growing, pilocytic astrocytomas (PA, grade I) are the benign and most treatable of the gliomas, with a cure rate of over 90 percent. Glioblastomas (GBM, grade IV) are aggressive tumors with poor prognosis due to diffusive nature and resistance to current therapies. We endeavored to provide a global view of epigenetic landscape of regulatory regions in combination with transcriptomes to identify gene regulatory networks that may contribute to tumorigenesis and tumor malignancy. To achieve these goals, we collected glioma samples obtained as fresh surgical resections, produced single cell suspension and on each tumor sample we performed multilayer genome-wide analyses to identify open chromatin areas (Assay for Transposase-Accessible Chromatin using sequencing, ATAC-seq) and histone modification patterns (Chromatin immunoprecipitation, ChIP-seq) in combination with transcriptome analyses (RNA-seq). Thereby, we provide annotations of functional regulatory sites in gliomas, notably active promoters and putative enhancers. Furthermore, we took advantage of publicly available Hi-C maps (Won H. et al., 2016) to predict enhancer-promoter interactions in our dataset. We have selected genes expressed differentially across glioma grades and focused on the genes with the highest significance of correlation between enhancer activation and gene expression. One of the most interesting genes that may be relevant in glioma pathobiology encodes a member of ANNEXIN II/ ANNEXIN II receptor axis that is know to regulate processes such as cell adhesion and migration. In conclusion, here we are showing the potential of this very first complex epigenomic study of low and high grade gliomas to identify novel target for future investigations. Supported by SYMFONIA 2015/16/W/NZ2/00314 grant from the National Science Center, Poland

Mechanisms of neuronal senescence – role of ATM and oxidative stress

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Cellular senescence can be broadly defined as an irreversible state in which cells cease to divide. The process of senescence is proven to be implicated in organismal ageing, including ageing of the brain. Historically, the term senescence has been used only to describe mitotic cells, however, according to our results and recent literature, it should also include non-dividing neurons. The aim of this study was to explore the role of ATM protein in senescence of neural cells. In particular, we examined if neurons lacking ATM underwent stress-induced senescence and we looked for underlying mechanisms. The rationale for the project was the observation that patients lacking ATM protein (ataxia-telangiectasia syndrome) demonstrate numerous signs of early ageing an neurodegeneration. The study was conducted using induced pluripotent stem cells-derived neuronal cells. Fibroblasts obtained from ataxia-telangiectasia patients and healthy subjects were reprogrammed using Yamanaka's cocktail. IPCs were then neuralized to neural progenitors and, in turn, driven into neuronal differentiation. Substantial differences were found in the level of senescence-associated β-galactosidase between A-T and normal cells. Further analysis of other markers of senescence showed elevated secretion of proinflammatory cytokines, accumulation of GATA4 transcription factor and increased level of Rubicon in ATM-lacking cells. DHE and 4-HNE tests revealed a high level of reactive oxygen species, which, to our interpretation, is a reason behind the senescence phenotype. Further results point to two sources of oxidative stress – increased level of NADPH oxidase 4 and accumulation of dysfunctional mitochondria. The latter seems to be a consequence of impaired mitophagy with a strong increase in the level of Parkin protein and other markers of impaired autophagic flux in general –LC3, p62 and Rubicon. In summary the results show the lack of ATM protein in neurons leads to the high oxidative stress which may induce senescence phenotype and impair mitophagy. That may provide an insight into the process of senescence in post-mitotic brain cells and contribute to understanding some aspects of ataxia-telangiectasia.

High-Throughput and Genomic High-Resolution Positioning of Genes

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The spatial chromatin organization within the interphase cell nucleus is still not completely understood. However, it is well established that chromosomes occupy distinct regions called chromosomes territories and the genes` positions are not random. Recently, biochemical methods that allow us to study spatial chromatin interactions revealed also a new structural and functional unit of organization – the topologically associating domains (TADs) or chromatin contact domains (CCDs) – average less than 1 Mb regions of frequent physical chromatin interactions. On the other hand, chromosome organization is a dynamic process and remains elusive at the lower level of organization. Therefore, we propose a novel tool combining high-throughput microscopy and HCR (Hybridization Chain Reaction) approach allowing visualization of specific short DNA sequences. Using signal amplification with HCR we can enhance DNA FISH (fluorescent in situ hybridization) signals by increasing the number of fluorophores. Using this tool we were able to capture regions as short as 4 kb. Moreover, in case of multicolor FISH we distinguished regions separated by the only 4 kb gap, showing cell-to-cell and copy-to-copy variability in the chromatin organization at this level. We propose also DNA HCR FISH combined with high-throughput microscopy as a novel approach to study interactions (DNA loops) revealed by the biochemical method, at the

single cell level and in a high-throughput manner. Acknowledgements: PT is supported by the Polish National Science Centre grants: 2014/15/N/NZ2/00379 and 2017/24/T/NZ2/00307.

Hemin and H2S Interplay in the Regulation of Mitochondrial Potassium Channel

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For many years, hydrogen sulfide (H2S) was considered as a toxic gas. However recently, together with nitric oxide (NO) and carbon monoxide (CO), this molecule gained the name of gasotransmitter because in low concentrations these gases cause beneficial effects in many physiological processes. Hypothetically, H2S could play an important role in cytoprotection by opening mitochondrial potassium channels. Mitochondrial large-conductance calcium-activated potassium channel (mitoBK) is one of the main potassium channels localized in the inner membrane of mitochondria. MitoBK channel is formed by a DEC splice variant of the KCNMA1 gene and it is a tetrameric protein composed of four α subunits. Each α subunit consists of a short N-terminus, seven transmembrane segments and a large C-terminus containing two RCK (regulating conductance of K+) domains located in the mitochondrial matrix. Various modulators of the activity of plasmalemmal BK channels are known, including inhibitor heme or its oxidized form hemin. Both plasmalemmal and mitochondrial BK channels contain the heme binding motif -CXXCH-. Heme is a receptor for gasotransmitters like CO and H2S, but unequivocal results concerning action of these gases on plasmalemmal BK channels were obtained. On the other hand, nothing is known about the effects of H2S on the activity of mitoBK channel. Mechanism of H2S action is complex and includes redox reactions, persulfide formation with -SH groups of cysteines and sulfide-metal interactions in heme proteins. In this study, I carried out the patch-clamp experiments to record single mitoBK channel activity from mitochondria of astrocytoma U-87MG cells. I applied NaHS as an H2S donor in combination with hemin in inside-out configuration of the channel. I observed that NaHS applied alone does not change the activity of mitoBK channels. However, it activates the hemin-inhibited mitoBK channels by reversible binding to the ferric ion of hemin. I also checked the effect of NaHS and hemin in the outside-out orientation of mitoBK channel, which seems to be similar to the effects observed in inside-out configuration suggesting the existence of an unknown extracellular hemin binding site. This work was supported by Polish National Science Center, grant no. 2015/17/B/ NZ1/02496

Effect of miRNA loss in adipose tissue

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MiRNAs are small, non-coding RNA molecules, able to regulate expression of genes, mainly by repressing protein translation or cleavage of the mRNA. This occurs in every tissue of the organism and regulates it's processes. Adipose tissue has been focus of many studies, because of the growing concern about overweight, obesity and following, health implications of those. There are two types of that tissue: white adipose (WAT), which serves as storage of energy surplus that is assimilated by overeating, and brown adipose (BAT), which main function is heat generation by dissipating flow of electrons in mitochondria. In our study we are using Phenomaster system to register metabolic parameters of mice and molecular techniques to detect hormonal and energy substrates changes during different types of food restrictions, to

establish dynamics of energy processes occuring in the tissue. We are looking also at which miRNAs in particular could be responsible for that dynamics. Lastly we are using transgenic animals as a tool for removal of miRNAs in the adipose and confront the effect with the known phenotype of food restricted mice.

Speed talks

Involvement of retrosplenial cortex in spatial learning and navigation

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The retrosplenial cortex (RSC) is a cortical area in the brain which is a part of a core network of brain regions involved in a range of cognitive functions including navigation in the environment, formation, storage and retrieval of spatial information. However its precise role in memory functioning has not been sufficiently explained. It has been postulated that especially dysgranular part of RSC might be responsible for processing of visual information about the external environment in order to allow orientation on the "mental map" generated by hippocampus. The main goal of our studies is to check whether RSC neurons form a memory trace. We use a rat animal model subjected to spatial memory training in a T-maze task, which requires the navigation based on external cues. Using controlled expression of viral opsins followed by the LED diodes implanted on the surface of RSC we want to activate or inhibit the entire neuronal population of this structure during specific phases of training. Comparison of learning curves between control and treated animals would result in the clear answer whether contribution of RSC is crucial for memory formation. We also use a Designer Receptors Exclusively Activated by Designer Drugs (DREADD) to detect whether the ligand-induced temporal inactivation of whole RSC influence solving the task. Using Fos protein level as an indirect marker of neuronal activity we could then answer the question whether temporal inhibition of RSC affects different brain areas which are very likely to take a part in solving a behavioral task. By using optogenetics and chemogenetics, two most powerful and valuable platforms for functional manipulations in vivo we are able to examine contribution of RSC in spatial memory formation. Furthermore complementing the T-maze behavioral tests of rats with mice model of two-photon imaging of the neuronal signal transduction to RSC during different time points of learning can lead to better understanding of crucial elements of the memory system that support navigation and spatial memory.

Molecular mechanisms of altered GSK-3β activity on synaptic plasticity

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Glycogen synthase kinase-3 beta (GSK-3β) is a serine/threonine kinase regulating functional and structural synaptic plasticity in the hippocampus, mostly because of its role in excitatory synaptic transmission or development of neuronal morphology. Improper changes in the morphology of dendrites and synapses are associated with different neuropsychiatric and neurodegenerative disorders. Several lines of evidence suggest the involvement of different microRNA and GSK3β in synaptic plasticity, however relationship between miRNA and GSK-3β in mouse models of neuropsychiatric disorders has not been studied to date. To understand the relationship between aberrant GSK-3β activity and synaptic plasticity we analyzed miRNAs expression levels in the hippocampus of mice overexpressing GSK-3β[S9A], which model some aspects of bipolar disorder. GSK-3β[S9A] mice were compared to WT littermates as controls. Illumina MiniSeq sequencing has shown different expression levels of 95 mature miRNA and isomiR between genotypes. We selected 4 mature miRNAs with the strongest differences in the expression level for qPCR validation. Following validation we will investigate role of those miRNAs in structural plasticity of dendritic spines.

Furthermore, we will analyze targets of those miRNAs to understand molecular mechanisms of aberrant GSK-3β activity.

SRF-dependent miRNAs in dendritic spines plasticity during brain development

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Synaptic plasticity is the activity-induced process leading to changes in the morphology of dendritic spines, the locus for excitatory synaptic transmission. During brain development dendritic spines` shape changes from thin elongated filopodia-like structures to stable mushroom dendritic spines. Re-arrangement of neuronal connections plays an important role in proper circuitry formation. Serum Response Factor (SRF) is a major transcription factor in the brain, responsible for regulating various programs of gene expression in the adult brain. In this study, we examine miRNAs regulated by SRF duringspines maturation. Dendritic spines formation was analysed in the hippocampal neurons from SRF flox/flox mouse transfected by CRE recombinase in early stages of neuronal maturation. Analysis of spines morphology showed that the lack of SRF expression decreases the spines density. Moreover low level of SRF causes increasing percentage of immature, filopodial and long spines. To identify transcription program regulated by SRF during neuronal development we performed microarrays analysis. We identified three miRNAs (miR-132-3p, miR-212-3p, miR-222-3p) with significant changes in the expression between control and SRF depleted hippocampal neurons. These findings suggest that miRNAs regulated by SRF might be involved in spines maturationduring development.

Prophylactic antidepressant effect of ketamine treatment in mice model of depression

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Ketamine, an NMDA antagonist, has gained wide attention due to its rapid antidepressant effects. Despite short biological half-life of ketamine even its single subanesthetic dose cause long lasting changes in brain structural plasticity what seems to correlate with sustained behavioral effects. Recently ketamine has also been recognized as prophylactic against depression in various animal models. Since chronic stress may leads to structural alterations of different brain regions that last beyond treatment related alleviation of symptoms, in this study we aimed at determining if prophylactic ketamine injection has long lasting impact on structural plasticity in brains of resilient and susceptible mice that had undergone chronic unpredictable stress procedure and went through recovery period. We found that administration of ketamine before stress procedure not only cause increase in the average sucrose preference and a number of resilient animals immediately after the cessation of stress but also seems to positively influence recovery of susceptible animals. Moreover, DiI staining of brain slices of stressed mice revealed that ketamine injected animals show significant differences in dendritic spine densities but not dendritic spine morphology compared to saline injected ones. These results confirm and expand previous observations concerning prophylactic action of ketamine. They also provide further evidence for association of ketamine antidepressant action with synaptic plasticity.

Towards a computational infrastructure for whole-brain mapping

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Methods for imaging entire brains at cellular resolution are becoming increasingly popular. As a result, there has been a surge in demand for tools capable of managing and analyzing voluminous imaging data, yet, adequate methods are still missing. We propose a data model for efficient storage and retrieval of imaging data, compatible with a pipeline for intra- and cross-modal registration of whole brain light sheet fluorescence microscopy (LSFM) images. The presented data model is based on the Hierarchical Data Format 5 (HDF5), specifically designed to efficiently handle large datasets. The container is capable of storing multichannel acquisitions of a single or multiple individuals, dense spatial transformations obtained from registration of specimen to an atlas, imaging metadata (voxel spacing, anatomical directions, etc.) and can be easily adapted to hold additional information. The core image data is deposited as a multi-resolution pyramid, enabling rapid access to subsets of image volume at different scales. These features allow one to perform atlas-based segmentation, or to export a specific region of the brain at desired magnification for further analyzes (e.g. cell counting). The format is also compatible with BigDataViewer, a 3D, memory-efficient image rendering software. The framework was applied to a set of LSFM images of unevenly cut slabs of five mice brains. First, images were denoised with Variational Stationary Noise Removal (VSNR) algorithm, then stored in the HDF5-based container. Registration to the Allen Institute Mouse Brain Atlas allowed for export of regions of interest for further analysis. This demonstrates high flexibility of the framework, even when dealing with difficult corner-cases. The project is supported by ERA-NET NEURON grant from the National Centre for Research and Development (ERA-NET-NEURON/17/2017) and by the G2631 grant from the National Science Center.

Influence of SGT1, a HSP90 co-chaperone, on α-synuclein aggregation

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α-Synuclein is a protein that may form cellular aggregates, called Lewy bodies, and it may thus contribute to the development of Parkinson's disease (PD). These aggregates are formed due to improper folding of α-synuclein mainly caused by disturbances in the level and/or activity of chaperone/co-chaperone proteins. Recently, it has been found that HSP90, HSP70 (Daturpalli S et al., J. Mol. Biol. 2013; Klucken J et al., J. Biol. Chem. 2004), inhibit α-synuclein aggregation in vitro and in animal models, so they can be considered as potential targets in clinical treatment of PD. The aim of this work was to analyze the effect of HSP90 co-chaperone, SGT1, on α-synuclein aggregation in HEK293 cells and in vitro. We found that the amount of α-synuclein aggregates was lower in SGT1-overexpressing HEK293 cells and higher in cells with diminished level of SGT1. By applying an in vitro assay, with the use of thioflavin T (Tht), we found that SGT1 had no influence on α-synuclein aggregation. Interestingly, when another Hsp90 co-chaperone, CacyBP/SIP, was checked in this assay it appeared that it reduced α-synuclein aggregation. Thus, at present we investigate the influence of CacyBP/SIP on α-synuclein aggregation in HEK293 cells. To sum up, the results obtained up to now suggest that HSP90 co-chaperones, SGT1 and CacyBP/SIP, may contribute to α-synuclein aggregation and PD pathology. The work is supported by the EU Horizon 2020, Marie Sklodowska-Curie grant no 665735, by Polish Ministry of Science and Higher

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Relationship between the number of HREs and the kinetics of hypoxia induced expression

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Hypoxia is a state of oxygen deprivation in a tissue, which results in a disturbance of cellular processes. Cellular response to hypoxia leads to activation of hypoxia inducible factors (HIFs), which bind to specific sequences in hypoxia response elements (HREs), localized within genes that promote angiongenesis, energy metabolism and cell survival. We discovered that the number of HRE instances in the promoter regions of the genes induced by hypoxia is associated with the faster kinetics of this induction.

A novel approach to language therapy in patients with aphasia

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The number of stroke patients increases every year. Epidemiological data indicate that in Poland there are over 60 000 cases per year. The most common consequence of stroke is a hemiparesis and an aphasia, i.e., the combination of speech production and / or speech comprehension difficulties resulting from a focal brain lesion in the language-dominant hemisphere. Traditional aphasia therapy is based on speech exercises used to directly improve deficient language functions. Nevertheless, it was evidenced that aphasic patients present deficits in non-language cognitive domains i.e., rapid auditory processing, executive functions, attention or working memory. These deficits may intensify the speech difficulties in aphasic subjects and hinder the process of speech functions restitution. Therefore, it is important to include exercises designed to improve non-linguistic functions in programs of aphasia therapy. The aim of the study is to compare the effects of traditional speech therapy and novel non-language cognitive training based on Dr Neuronowski® software. The preliminary results revealed that non-linguistic training improved not only trained non-language domain, i.e., rapid auditory processing, executive functions and working memory, but also untrained language functions. This result indicates that the training in non-linguistic functions is a promising direction in aphasia therapy. Supported by the Polish National Science Centre grant no. 2016/21/B/HS6/03775

Can factors secreted by senescent vascular smooth muscle cells influence the immune cells functions?

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Cellular senescence is proposed as a one of the mechanism that leads to organismal aging as well as age-related diseases. It was demonstrated that during progression of atherosclerosis, which is one of the most common age-related disease, vascular smooth muscle cells (VSMCs) undergo senescence within the atherosclerotic plaques. An important feature of senescent cells is secretion of soluble factors, known as senescence-associated secretory phenotype (SASP) which actively modulate microenvironment. However, so far there is no data concerning the characterization and the role of extracellular vesicles (EVs) released by senescent VSMCs, which are important particles playing role in communication between the cells. Thus, the aims of our studies were (i) to performed detailed unbiased proteomic analysis of both soluble factors and extracellular vesicles secreted by senescent VSMCs and (ii) to investigate the influence of senescent cells-conditioned medium (CM) on T cells functioning. We performed mass spectrometry analysis using tandem mass tags (TMT) of proteins to evaluate the quantitative and qualitative differences in the secretome of proliferating (control) cells, stress induced premature senescent (SIPS) VSMCs and VSMCs that underwent replicative senescence. We were able to distinguish the proteins most abundantly secreted by senescent cells as well as those that were underrepresented in comparison to secretome of control cells. Moreover we analyzed the influence of senescent cells-CM on T cells activation and proliferation. Our studies revealed that factors secreted by senescent VSMCs increase the expression of selected markers of CD4+ and CD8+ T cells activation however no influence on proliferation rate has been observed. This work is supported by grant National Science Centre UMO 2014/15/B/NZ3/01150.

Current Source Density Analysis in Multi Electrode Array recordings

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Using the kernel Current Source Density Method (kCSD) for analysis of potentials from multi-electrode arrays. With the use of the kCSD reconstruction method and independent component analysis (ICA) it is possible to analyze separately overlapping populations of neurons. https://github.com/Neuroinflab/kCSD-python

FAP69 is a novel Central Pair Protein

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Cilia are eukaryotic organelles that protrude from cell surface and perform sensory and locomotive functions. In case of motile cilia, the mechanochemical signals that initiate cilia beating originate at the central pair complex. The molecular mechanisms of this process is poorly understood. To better understand the role of central pair complex in cilia beating, we attempted to identify new proteins that build a central apparatus. We took advantage of the BioID assay that enables biotinylation of proteins positioned in close proximity (approximately 10 nm) to the protein of interest. We expressed Spef2, a C1b projection protein, fused with BirA* and identified biotinylated proteins by mass spectrometry. Among biotinylated proteins we found three novel highly evolutionary conserved proteins FAP69 (Flagellar Associated Protein), FAP246 and Androglobin-like protein. Tetrahymena cells with knocked out FAP69 gene swam significantly slower and had altered cilia beating pattern compared to the wild-type cells. The ultrastructural analyses using transmission electron

microscopy revealed that cilia in FAP69 knockout cells lack one large central pair projection, likely C1b projection. Interestingly, the level of Spef2, FAP246 and Androglobin was reduced in cilia of FAP69-KO cells compared to wild-type cells suggesting that FAP69 either docks or stabilizes these proteins in central apparatus complex. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No.665735 (Bio4Med) and from Polish Ministry of Science and Higher Education within 2016-2020 funds for the implementation of international projects (agreement no 3548/H2020/COFUND/ 2016/2).

Perineuronal net in spinal cord after transection: differential response in regions of glial scar formation and motor nuclei

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Perineuronal nets (PNNs) are mesh-like structures which in the spinal cord are particularly elaborated around inhibitory neurons and motoneurons. They are composed of chondroitin sulfate proteoglycans (CSPGs) and of Crtl1/Hapln1 link protein, which play an important role in maintaining stability and restricting plasticity of neuronal circuits. Whilst important for maintaining stable connections, they can have an adverse effect following insult to the spinal cord when accumulated, restricting the capacity for repair, where enhanced synapse formation leading to new connections could be functionally beneficial. Reported increase in CSPGs after injury prompted us to search for their molecular underpinnings in spinal regions with massive neuronal loss, where glial scar is formed, and regions where a massive denervation of lumbar motoneurons take place. To characterize quantitatively spatio-temporal changes in expression levels of the selected CSPGs (neurocan, phosphacan) and link protein genes in spinal segments after complete transection of the spinal cord (SCT). The CSPGs, Crtl1/Hapln1 and GFAP gene transcripts were quantified in adult rat spinal cord at the 2nd and 5th week after SCT. Assays were carried out in the spinal cord segments: Th 9/10 (lesion site), its vicinity; lumbar L1-L2 and L3-L6. To quantify gene expression qRT-PCR was carried out and expression levels were presented relative to internal control gene (GAPDH) as the scaled ratio value. SCT caused significant, 4-fold increase of neurocan matched by GFAP, and 9-fold decrease of link protein transcripts in the lesion site at early and chronic stage post-lesion, comparing to controls. In the lumbar segments the level of neurocan transcript was also increased, followed by an increase of phosphacan transcripts in L1-L2 segments at the chronic stage after SCT. Conclusion: a profound increase in neurocan and GFAP transcripts in the lesion site suggests that stimulation of neurocan gene expression occurs in reactive astrocytes during scar formation. Decrease in expression of the link protein (Hapln1/Crtl1) in the lesion and perilesion areas at 2 and 5 weeks after SCT indicates early and progressing destabilization of the PNNs. Increased expression of neurocan followed by phosphacan suggests extensive reorganization of PN network, of the structure altering in time. Support: NCN UMO-2013/09/B/NZ4/03306, UMO-2016/23/N/NZ4/03337NCN (K.G.) and Statutory for the Nencki Institute.

Temporal information processing and other mental functions in the elderly

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Aging is accompanied by decline of various mental functions, such as attention, executive functions and temporal information processing (TIP). TIP is responsible for perceiving and organizing incoming stimuli according to specific time patterns. Previous research has shown that decline in TIP (measured in the millisecond domain) is associated with deterioration of other cognitive processes, e.g. executive functions. The aim of this study was to verify whether divided attention mediates the relationship between TIP and executive functions. Forty two healthy elderly subjects aged from 62 to 78 years were tested. All of them had normal hearing level. TIP was measured using a temporal-order judgement task in which pairs of clicks were presented monaurally in rapid succession and the participants reported temporal order of these two sounds. Executive functions were assessed with Color Trails Test (CTT), and divided attention with a dual task from TAP battery. Results have shown that TIP was moderately correlated with both divided attention and executive functions ($r = 0.37$ and $r = 0.35$, respectively). Subsequently, a mediation analysis yielded significant results: $F(2, 39) = 5.66$, $p < 0.01$, i.e. after including divided attention in the model, previously significant relationship between TIP and executive functions became nonsignificant. These results indicate that divided attention plays a crucial role in the relationship between TIP and executive functions. Supported by National Science Centre grant no 2015/17/B/HS6/04182.

Associative learning increases intrinsic excitability of somatostatin-expressing cells in layer 4 of the mouse primary somatosensory cortex

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Strengthening of excitatory synapses as well as alternations in excitability of excitatory cells have been postulated to underlie learning and memory mechanisms. However, the GABAergic system is also plastic and takes part in learning and memory. Despite of that the mechanisms of the plasticity of inhibitory neurons and circuits are poorly understood, especially considering a huge diversity of inhibitory cells. The goal of our study is to analyze changes in the activity of somatostatin-expressing (SOM) interneurons in response to associative learning. This group of inhibitory interneurons have been implicated in experience-dependent plasticity, state-dependent modulation and their activity is regulated by neuromodulators. In our research, we used a simple model of sensory learning, where mice were subjected to the conditioning paradigm consisted of a paring whiskers stimulation with an electrical tail shock. Previous studies have shown that this model of learning results in expansion of cortical representation of stimulated row of vibrissae and in the increase of density of GAD+/SOM+ cells in the cortical representation of trained row of whiskers. Using transgenic mice with tagged SOM interneurons, we performed whole-cell patch-clamp recordings in slices of naïve and trained mice. We compared basic electrophysiological properties and intrinsic excitability of SOM cells located in layer 4 of the representation of the stimulated whiskers in the barrel cortex. Consistent with the literature, we found two main groups of SOM interneurons in layer 4: low-threshold spiking and irregular spiking cells. After conditioning paradigm, the excitability of low-threshold spiking SOM interneurons increased. Our findings indicate that sensory conditioning results in a long-lasting and selective enhancement of SOM interneurons activity due to changes in their intrinsic excitability. Hence, the presented study together with rising number of scientific reports

considering GABAergic system plasticity, suggesting that increases in inhibition are a common and important mechanism of learning and memory. Financial support: National Science Centre UMO-2015/18/E/NZ4/00721 to J.U.C.

MICROTUBULE-PAIR SLIDING CARRIED OUT BY KINESIN-1

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Kinesin-1 is a common motor protein present in many cell types, including neurons. It transports cargoes along microtubules (MT) by walking using its N-terminal domains, called heads. The cargo is attached to the kinesin tail, located at the C-terminus. Also, a second MT can serve as a cargo for kinesin-1 as a result of weak electrostatic interaction between positively charged kinesin C-terminal tail and negatively charged tubulin C-terminal tail. This phenomenon is called MT-MT sliding or MT pair sliding. For kinesin-1 it was observed recently in growing Drosophila neurons. Observations in cells allow to characterize a function of sliding but not to describe its mechanism or basic parameters. MT-pair sliding is quite difficult to recreate beyond the cells, using only proteins but our lab developed a reproducible assay to observe MT-pair sliding driven by kinesin-1 in vitro. Using this assay we measured basic parameters for MT pair sliding: – the velocity of the transport, the run length (the distance that the transport MT passed via kinesin); the influence of kinesin-1 concentration on the sliding, tubulin posttranslational modifications, ionic strength or kinesin-1 flexibility on MT-pair sliding. While performing the experiments we observed many unexpected properties of sliding that brought us closer to understanding this phenomenon that is necessary for normal development of nervous system. For example, it turned out that kinesin-1 is able to move both antiparallel and parallel MTs in contrast to another motor proteins that transport only antiparallel MTs. In my speed talk, I will briefly characterize MT-pair sliding, and then focus on orientation of MTs during sliding and also on kinesin-1 concentration influence. More details will be available during poster session.

Visual response enhancement in the rat visual system following sensory training

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One fundamental approach to induce plastic changes in the visual system is a behavioral stimulation through the repetitive sensory experience. In animal studies repeated exposure for a few days to specific visual stimuli (visual training) can modify neuronal network to improve the perception of these stimuli at the level of the primary visual cortex (VCx; Sawtell et al.,2003; Frenkel et al., 2006). In our study, we found that the shorter visual training, limited to several hours (3 hours) also induced strong enhancement of the magnitude of visual evoked potential (VEP) amplitudes not only at the cortical level but also at the subcortical level. There is still no sufficient explanation related to reinforcement of visual response after visual training at the subcortical level in the superior colliculus (SC). We considered two hypotheses: it might be a result of enhancement of the input synapse from retina to SC or the reinforcement of response in this midbrain structure may result from enhanced cortical input to the SC. To explain this issue in this study we performed visual training with VCx blocked since the beginning of training. In order to block the activity of the cortex during visual training, a well above the VCx was fulfilled with xylocaine solution (2.5%). Monocular visual

stimulation consisted of a series of 300 repetitions of light flashes separated by 2 s interval (0.5 Hz)) presented every 15 minutes through 3 hours. To investigate the effect of visual training, before and after visual training control recordings were carried out (100 repetitions of flash stimuli at 0.1 Hz). VEPs were recorded from the contralateral to the stimulated eye VCx and the SC. Comparison of the VEPs amplitude both in the VCx and SC indicated that repeatable visual stimulation significantly enhanced the magnitude of visual responses. Chemical inactivation resulted in strong attenuation of cortical VEP amplitudes, no significant difference between the magnitude of VCx responses in both control recordings was found. In the case of SC we observed an increase of VEP amplitudes during visual training with cortical inactivation, simultaneously the comparison of both controls showed enhancement of response after visual training.. Obtained results prompted us to hypothesize that the increase of responses in the SC is most likely due to the enhancement of the retino-tectal projection. " Supported by the National Science Centre Poland Grant 2017/25/N/NZ4/02914”

Plasma membrane transporter SLC6A14 is controlled by cytosolic heat shock proteins

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SLC6A14 is a member of solute carrier (SLC) family 6 of plasma membrane transporters specific towards amino acids, neurotransmitters, and osmolytes. SLC6A14 transports all neutral and basic amino acids in a Na/Cl – dependent way and it is overexpressed in many types of cancer. Both N- and C-termini of SLC6A14 are localized on the cytosolic side. Our analysis of SL6A14 interactome by mass spectrometry revealed, among others, the presence of cytosolic heat shock proteins (HSPs) and co-chaperones. We studied interaction of SLC6A14 with HSP90beta and HSP70 (HSPA14), identified as possible transporter partners. Immunofluorescence experiments demonstrated the strongest co localization of both HSPs with overexpressed rat SLC6A14 in transiently transfected HEK293 cells after 24 h. The direct interaction between HSPs and SLC6A14 was confirmed using the proximity ligation assay. Interaction of the transporter with HSP90beta was inhibited by radicicol, known to bind to HSP90 ATP-binding site, while interaction with HSPA14 was attenuated by its inhibitor - VER155008. Cell surface proteins biotinylation demonstrated a dramatic decrease of SLC6A14 presence in the plasma membrane upon treatment with either radicicol or VER155008, what resulted from the diminished level of the total transporter protein. Distortion of SLC6A14 proper folding by both HSPs inhibitors directed the transporter towards endoplasmic reticulum associated degradation, a process reversed by the proteasome inhibitor – bortezomib. These results indicate that a plasma membrane protein folding can be controlled not only by chaperones in the endoplasmic reticulum, but also those localized in the cytosol. Moreover, these observations may have a potential therapeutic significance, since the use of HSPs inhibitors could decrease amino acid supply to quickly proliferating cancer cells with a high expression of SLC6A14. This study was financed by a grant 2015/19/B/NZ3/00049 from the National Science Centre in Poland.

Ultrastructural changes in the human epileptic brain

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Epilepsy is one of the most common chronic neurological diseases affecting around 0.5-1.0% of the human population. Approximately 1/3 of the patients is not responsive to any anticonvulsant drug treatment, and therefore their only hope for recovery is to undergo a surgical removal of the brain area responsible for epileptic seizures. The source of most cases of epilepsy is unknown. Some occur as a result of brain traumas, strokes, brain tumors, brain infections, genetic mutations, or as birth defects. One of the most common causes of pharmacoresistant epilepsies, being a result of abnormalities of in utero brain development, is focal cortical dysplasia (FCD). It is a spectrum of focal developmental malformations characterized by the disruption of the normal cytoarchitecture of the cerebral cortex, i.e. tangential or radial dispersion or loss of laminar structure, and the presence of unique cell types such as balloon cells. Little is known about the ultrastructural changes accompanying epilepsy secondary to FCD. By using 3View electron microscopy technique and 3D reconstruction I want to describe, at the ultrastructural level, structural aberrations of the synapses of patients with focal cortical dysplasia. Brain samples from epilepsy surgeries undergo a neuronal spine recovery procedure and preparation for 3View electron microscopy imaging. During 3D reconstruction I am focusing on synapses and their surrounding astrocytic processes, as it was previously shown that astroglia may play an essential role in the pathology of that disorder.

Organization of Replication Factory

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Proliferating Cell Nuclear Antigen(PCNA), is a protein involved in DNA replication – it forms a clamp around DNA strand that acts as a scaffold on which DNA replication machinery is assembled. During S-phase PCNA in nucleus forms structures known as Replication Factories, which is where most of DNA replication takes place. Replicaton Factories are supossedly aggregations of of several replication forks. Using electron microscopy we were able to visualize interior organization of a Replication Factory

Plasma microRNAs at early stages of Alzheimer's disease

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Alzheimer's disease (AD) is the most common age-related dementia. One of the major challenges in the AD field is identification of easy accessible, blood-based biomarkers for early AD in patients with mild cognitive impairment due to AD (MCIAD). The aim of this project was deciphering the molecular signatures characteristic of early AD stages and understanding their function in development of AD. Using qRT-PCR we evaluated microRNA (miRNA) profiles in blood plasma collected from 15 MCIAD patients, whose neuropsychological diagnoses were confirmed by cerebrospinal fluid (CSF) biomarkers, 20 AD patients and 15 nondemented, age-matched individuals (CTR). In the first screening, 179 plasma miRNAs were compared between AD and CTR, and between MCIAD and CTR. 23 differentially expressed miRNAs reported earlier as AD biomarker candidates in blood were confirmed in the current study and 26 novel differential miRNAs between AD and CTR were

detected. The potential of these 15 miRNAs to be used as biomarkers was further verified in independent AD, MCIAD and CTR groups. Finally, 6 miRNAs (3 novel in AD context and 3 reported) were selected as the most promising biomarker candidates differentiating early AD from controls with the highest fold changes (from 1.32 to 14.72), consistent significance, specificities from 0.78 to 1 and sensitivities from 0.75 to 1), (patent pending, PCT/IB2016/052440). The miRNA panel is promising for diagnostics of early AD. The TargetScan, MirTarBase and KEGG database analysis indicated putative protein targets of the differential miRNAs. In particular, this analysis indicated that BACE1 and PSEN2, two key proteins in the pathogenesis of AD, can be regulated by mir-200a-3p and miR-30b-5p, two differential miRNAs in AD blood identified in our screen. To verify this prediction, I designed and constructed: plasmids containing 3'UTR fragments of genes of interests containing candidate binding site (WT), constructs that have ideal binding site for miRNAs (PM) and constructs with mutated binding site (MUT). Then I performed luciferase assays and confirmed the interaction between miRNA and binding sites in BACE1's and PSEN2's 3'UTRs. Given that the luciferase results confirmed miR-200a-3p mimic:BACE1 3'UTR and miR30b-5p:PSEN2 3'UTR interactions, the next step will be the verification of the impact of miR-200a-3p and miR-30b-5p on BACE1 and PSEN2 mRNA and protein levels in the cells.

AUDITORY PERCEPTION OF TEMPORAL ORDER IN THE ELDERLY

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Recent studies have suggested that the perception of temporal order is an essential component of human cognition. It can be measured using Temporal-Order Judgement (TOJ) paradigm and indexed by Temporal-Order Threshold. The aim of the study was to investigate the pattern of performance on two different TOJ tasks in elderly subjects. We tested 40 healthy participants aged from 62 to 78 years using two TOJ tasks which differ in the stimulus presentation modes. In the spatial task two identical clicks were presented monaurally in rapid succession, while in the spectral task two tones with different frequencies were presented binaurally. All participants performed both tasks twice in two sessions. Our results indicated different patterns of performance in these two tasks in elderly subjects: in the spatial task TOT values are stable across two sessions, while in the spectral task TOT values are significantly lower in Session 2 than in Session 1. Such variation can be a consequence of different perceptual strategies engaged in each task. Obtained results indicated that performance in the spatial task is more based on sequencing abilities and analytic strategies, while performance in the spectral task depends more on the auditory streaming and frequency modulation strategy. National Science Centre Grant No. 2015/17/B/HS6/04182

Inhibition of the central amygdala circuits involved in social interaction suppresses motivation for food reward.

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Appetitive motivation system evolved to mediate a wide array of adaptive behaviors aimed at providing resources such as food or social contacts. The question whether motivation to approach various types of reward involves different neuronal mechanisms is still largely unanswered. In particular, it is not known whether neuronal circuits controlling social motivation are uniquely social, i.e., do they apply only to social domain and are not utilized

by other non-social motivational processes? To address this question, we inhibited activity of the central amygdala (CeA) circuits activated during either instrumental conditioning for food reward or interaction with a partner. CeA has been implicated in generating incentive motivation for food and drugs. Using *c-fos*-driven targeting with halorhodopsin we were able to inhibit the respective neuronal subpopulations in the CeA during the Skinner box session, in which motivation was assessed in the progressive-ratio schedule of food-pellet reinforcement. To obtain food pellets rats had to press the lever. The number of responses required to get reinforced increased when the reward was obtained. Motivation was measured as the highest number of responses performed to obtain the food reward. We observed that inhibition of either the social or food neuronal circuits in the CeA resulted in significantly decreased motivation for food reward, suggesting that, social and food motivation depends on, at least partially, the same neuronal circuits.The project was supported by European Research Council Starting Grant (H 715148) to EK.

Palmitoylation of plasma membrane raft proteins in LPS-stimulated cells

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My studies focus on signaling pathways triggered in macrophages by bacterial lipopolysaccharide (LPS). The LPS-induced signaling leads to production of pro-inflammatory mediators, such as cytokines, chemokines and interferons, thus launching the immune response to the bacterial infection. The mechanism of LPS recognition in macrophages is complex and involves binding of LPS to CD14 protein which is localized on the surface of plasma membrane nanodomains, named rafts. One of the most important mechanisms which control association of proteins with rafts is the reversible modification - palmitoylation. Hence, protein palmitoylation can control the LPS-induced signalling. Obtained results directed my attention to flotillins which are palmitoylated adaptor proteins anchored in rafts. We hypothesize that flotillins can control raft organization which is crucial for LPS-induced signalling.

Characteristics of mitochondrial potassium channel formed by the BK-DEC splice variant

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Ischemia of brain or heart tissue is the one of the most common causes of death in most Western countries. In the inner mitochondrial membrane several potassium channels have been identified to have cytoprotective function during ischemic event. One of them is mitochondrial large conductance calcium activated potassium channel (mitoBKCa). It was found that activation of mitoBKCa preserves brain and heart muscle cells. Recently, the molecular identity of the mitochondrial BKCa channel was described. The BK-DEC splice variant of BKCa-type channels α subunit has been demonstrated to localize in mitochondria. However it is not known whether this isoform is able to form a functional pore in mitochondria. In our study we used HEK293T cells transiently transfected with cDNA encoding the BK-DEC splice variant. Electrophysiological recordings with use of mitoplast isolated from transfected cells revealed the presence of the large conductance and voltage dependent ion channel. This type of channel was not present in mitoplasts isolated from untransfected cells. We found that the recorded channel showed all basic pharmacological

properties typical for the mitoBKCa channels described previously. The channel was Ca2+ sensitive and inhibited by a well known mitoBKCa channel inhibitor - paxilline. Additionally, kinetics and conductance of the observed channel were very similar to the mitoBKCa channel's. Based on that data we conclude that the BK-DEC splice variant forms a functional channel in the inner mitochondrial membrane of HEK293T cells. This work was supported by the Polish National Science Centre grant No.2015/18/E/NZ1/00737 and the Nencki Institute of Experimental Biology.

microRNA mediated regulation of Alzheimer's disease associated genes: search for disease driving pathomechanism

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the main cause of dementia in the elderly. Although well-established hallmarks of the disease such as oligomers and the aggregates of amyloid beta (Aβ) peptides, and intracellular deposits of hyperphosphorylated tau protein, known as neurofibrillary tangles (NFTs) are known for decades, the cause that drives these AD pathomechanism hallmarks are unknown. microRNAs (miRNAs) are known to regulate key genes studied in AD. Our recent study identified 15 miRNAs which show altered levels in blood in patients at the early AD stage. The miRNAs characterized by the highest fold change was hsa-miR-483-5p. We aimed to elucidate the function of hsa-miR-483-5p in AD pathomechanism. To achieve this goal, first we predicted possible binding targets for hsa-miR-483-5p using bioinformatics tools (TargetScan). This approach suggested that hsa-miR-483-5p may regulate such genes associated with AD as Microtubule Associated Protein Tau (MAPT) and Mitogen-Activated Protein Kinase 3 (MAPK3/ERK1) known to phosphorylate Tau, and we further verified this predictions experimentally. We used quantitative real-time polymerase chain reaction (qRT-PCR), Immunoblots and luciferase assays to study the mRNA expression levels, protein levels and the binding of miRNA-3'UTR seeding region of the mRNA, respectively. Our results suggest that hsa-miR-483 binds ERK1, causing reduction in both the ERK1 mRNA expressions and ERK1 protein levels. Also, we showed novel interaction between 3'UTR of MAPT and hsa-miR-483 in luciferase assay. Our future perspective is to investigate the role of hsa-miR-483 in modulating AD pathological hallmarks, especially on the hyperphosphorylation of tau in in-vitro cell models.

ELOVL3 deficiency affects energetic metabolism in skeletal muscle

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ELOVL3 is one of the members of elongase family, which initiates synthesis of saturated and monounsaturated C20–C24 fatty acids. Loss of ELOVL3 leads to a reduction of lipid storage and resistance to diet-induced obesity, hyperplasia of the sebaceous glands, reduced hepatic lipogenesis and triglycerides synthesis in mice. Moreover, it has been shown to play a crucial role of ELOVL3 expression for muscle-specific satellite cells differentiation. White gastrocnemius (WG) muscle isolated from ELOVL3-/- mice was used to analyze the role of ELOVL3 in regulation of lipid and glucose metabolism. Performed studies showed that ELOVL3 deficiency decreases cellular fatty acid uptake, de novo lipogenesis, lipolysis and increases β-oxidation in skeletal muscle. Moreover, ELOVL3-/- mice were characterized by

elevated protein level of glucose transporter 1 what indicates elevated glucose utilization in WG. It have been shown that high glucose concentration promotes hyperacetylation of histones and a decrease in histone deacetylases (HDACs) protein level. Obtained results showed decreased level of HDACs of class II and III: HDAC4 and sirtuin 1, whereas HDAC1 protein level was increased. High-glucose conditions are also associated with acetylation and subsequent activation of β-catenin and connected with oxidative stress Poly (ADP-ribose) polymerase 1 (PARP1) activation. Accumulation of an active form of β-catenin and increased level of PARP1 in WG of ELOVL3-/- mice was observed when compared to wild type mice. Summarizing, presented data suggest the crucial role of ELOVL3 in the plasticity of energetic metabolism and epigenetic reprogramming in skeletal muscle.

ADP-evoked purinergic response of dystrophic myoblasts (mdx); immortalized cells versus primary ones

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Duchenne muscular dystrophy is the most often hereditary neuromuscular disorder which is caused by mutation in the dystrophin-encoding gene located in the X chromosome. Previously we found that some metabolic changes related to this mutation are visible as soon as in myoblasts. Among them there is an aberrant susceptibility to purinergic stimulation dependent on both ionotropic and metabotropic receptors. Stimulation of immortalised mdx myoblasts with ADP under calcium free conditions results in substantially stronger Ca2+ release from the endoplasmic reticulum than it is in a case of w/t cells. This is additionally supported by Western blot data indicating an elevated amount of P2RY1 in mdx myoblasts. These observation tempts to generalize that this is a common feature of dystrophic myoblasts. However similar experiments with primary myoblasts isolated from various muscles of mice (tibialis anterior, gastrocnemius, soleus and FDL) clearly show big differences between them concerning their susceptibility to ADP-induced stimulation. Thus the question is how far can we conclude on a basis of experiments with immortalized myoblasts about situation in a whole animal. And how long do myoblasts isolated from particular muscles “remember” their origin.

Role of stearoyl-CoA desaturase 1 in control of thyroid hormone-dependent DNA methylation in the heart

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Epigenetic modifications and thyroid hormones (TH) are involved in pathogenesis of several cardiovascular diseases, including hypertrophy and heart failure. TH modulate expression of many genes through regulation of DNA and histone methylation level, what affects chromatin structure. Aforementioned epigenetic modifications are regulated by the enzymes, including DNA methylotransferases (e.g. DNmt1 and DNmt3a) and histone methyltransferases/demethylases (e.g. lysine-specific demethylase 1 [LSD1] and histone H3 lysine 9 methyl transferase [H3K9HMTase]). Furthermore, it has been shown that stearoyl-CoA desaturase 1 (SCD1) one of the main enzymes involved in lipid metabolism and whose gene expression is under TH control, affects also the level of DNA methylation in 3T3 adipocytes. Therefore, the aim of the presented study was to investigate the role of SCD1 and TH in regulation of DNA methylation in the heart. Wild type (WT) and SCD1-/- mice were

injected with triiodothyronine (T3) in order to induce a hyperthyroidism. We used HL1 cardiomyocytes pre-incubated with T3 and SCD1 inhibitor as an in vitro model. Performed analyses showed that SCD1 deficiency decreases the level of global DNA methylation. Moreover, we observed decline in DNMT1 and DNMT3a protein levels in SCD1 deficient heart and HL1 cardiomyocytes after inhibition of SCD1 activity. Additionally an increase in LSD1 protein level in SCD1 deficient heart was reported. Furthermore, hyperthyroidism leads to an increase in DNmt1 and DNmt3a, and a decrease in LSD1 and H3K9HMTase protein levels in WT mice heart. Interestingly, in the heart of SCD1-/- mice hyperthyroidism lead to a decrease in DNmt3a and an increase in LSD1 and H3K9HMTase protein levels when compare to WT littermates. Additionally, those changes were linked to elevated global DNA methylation level in hyperthyroidism in SCD1 deficient heart. Taken together, obtained results emphasize the important role of SCD1 expression in control of DNA methylation level in the heart and suggest that SCD1 is a key player during response to TH action in the heart.
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SHORT PEPTIDE BINDING GM-CSF INTERFERES WITH GLIOMA-MICROGLIA ENVIRONMENT AND INHIBITS GLIOBLASTOMA PROGRESSION.

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Glioblastoma (WHO grade IV, GBM) is a malignant, very aggressive, primary brain tumor which due to lack of efficient therapy caused by the heterogeneity of genetic abnormalities of tumor cells remains incurable. Tumor microenvironment plays an important role in growth, metastasis and response to treatment in many tumors, also GBM. Therefore, an approach to target tumor microenvironmet gained recently an increased attention. Brain resident immune cells – microglia and peripheral macrophages accumulate in malignant glioma and constitute 30-50% of the tumor mass. Glioma cells overexpress and secrete protein that reprogram microglia and peripheral macrophages into cells which potentiate tumor invasion and growth, furthermore suppress antitumor immunity. Glioma-derived granulocyte macrophage colony-stimulating factor - GM-CSF (Csf-2) induces accumulation and protumorigenic activation of microglia/macrophages. We designed and identified a humanized peptide that selectively binds to GM-CSF, blocks its binding to respective receptors on microglia, and inhibits activation of the receptors and downstream signaling pathways resulting in inhibition of glioma invasiveness. First, we designed peptide library containing 27 short peptides. Next, we identified the peptide binding GM-CSF using peptide microarrays, enzyme-linked immunosorbent assay (ELISA) and a technique based on surface plasmon resonance (SPR). Subsequently, we selected peptide (G7) with most potent capacity for inhibition of U87 MG and LN18 glioma cell invasiveness in the presence of human and mouse microglia cell line using the Matrigel Matrix cell invasion assay. We also confirmed that this peptide blocks binding of GM-CSF to its receptor using a method based on SPR technique and Ligand Tracer technology. Antitumor activity of G7 peptide in vivo was confirmed in orthotopic xenograft mouse model. We designed and identified G7 peptide which binds GM-CSF and blocks GM-CSF binding to its receptor. G7 has a potent capacity for inhibition of glioma cell invasiveness induced by the presence of microglia and exhibits antitumor activity in vivo. Intracranial delivery of this peptide inhibitor omits blood-brain barrier and minimizes nonspecific toxicity. Therapeutic strategy is under patent granting procedure.

Liprin-α-1 and role of microtubules in clustering of acetylcholine receptors

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Neuromuscular junctions (NMJs) are specialized synapses that connect motor neurons to skeletal muscle fibers and orchestrate proper signal transmission from the nervous system to muscles. The efficient formation and maintenance of the postsynaptic machinery that contains acetylcholine receptors (AChR) are indispensable for proper NMJ function. Abnormalities in the organization of synaptic components often cause severe neuromuscular disorders, such as muscular dystrophy. The dystrophin-associated glycoprotein complex (DGC) was shown to play an important role in NMJ development. We recently identified liprin-α-1 as a novel binding partner for one of the cytoplasmic DGC components, α-dystrobrevin-1. In the present study, we performed a detailed analysis of localization and function of liprin-α-1 at the murine NMJ. We showed that liprin-α-1 localizes to both pre- and postsynaptic compartments at the NMJ, and its synaptic enrichment depends on the presence of the nerve. Using cultured muscle cells, we found that liprin-α-1 plays an important role in AChR clustering and the organization of cortical microtubules. Our studies provide novel insights into the function of liprin-α-1 at vertebrate neuromuscular synapses.

A novel probe for tracing glycogen in living cells.

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Glycogen is a branched, very compact structure of coiled polymer of glucose. It is the primary carbohydrate storage form in many cell types. The compactness of glycogen allows large amounts of carbon energy to be stored in a small volume, with little effect on cellular osmolarity. But branched nature of glycogen structure makes it hard to characterize. It is known that the amount of glycogen in cells can be estimated. It is possible due to isolation of glycogen and enzymatic digestion, periodic Shiff staining, staining by glucose derivative – 2-NBDG or by Baba antibody. However, imaging of glycogen deposits in living cells is currently not available. Thus a probe to visualize glycogen particles is needed. For this purpose, we established a probe lacking enzymatic activity per se which may be used to bind to glycogen granules in living cells. Established probe contains carbohydrate binding membrane 20 (CBM20), found in human starch-binding domain containing protein 1 (STBD1) tagged to fluorescent protein mNeptune. Investigations were performed with the use of eHap cell line and its genetically modified (CrispR/Cas9 method) derivatives: knockouts of GYS1 (KO GYS1) and knockouts of GBE1 (KO GBE1), and U-2OS cell line with stable expression of the glycogen probe. Presented data indicates that p-mNeptune-C1-CBM20 probe may be used to visualize glycogen particles. However, during investigations many additional questions arose. Acknowledgments: This work was supported from the source of the National Science Centre according to the decision number DEC-2013/08/W/NZ1/00687

CCDC147A and CCDc147B form a heterodimer position in close proximity to N-DRC

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Cilia are highly conserved microtubule-based protrusions built of several hundred proteins. Proper beating of motile cilia powers a sperm motility, and in multicellular organisms, enables a shift of fluids or particles along the surface of the ciliated cells lining the Fallopian tubes, brain ventricles or respiratory tracts. In human dysfunction of motile cilia or asynchronous beating of motile cilia cause primary ciliary dyskinesia, genetic disorder that affects 1:10 000 individuals. The molecular mechanisms that regulate cilia beating is unclear partly because the knowledge of cilia protein composition is incomplete. To understand the molecular mechanisms that control cilia beating I analyze as-yet uncharacterized ciliary proteins that are indispensable for proper cilia beating. I focus on highly evolutionarily conserved, coiled-coils-containing protein encoded by a single gene in mammals and by two orthologous genes, CCDC147A and CCDC147B in a ciliate Tetrahymena thermophila. In Tetrahymena cells lack of CCDC147Ap or CCDC147Bp impairs cells motility and alters the swimming paths. The biochemical and genetic studies suggest that CCDC147A and CCDC147b are positioned in close proximity and that the ciliary localization of CCDC147A and CCDC147B is interdependent. The CCDC147Ap was not targeted to cilia in CCDC147B-KO cells and reverse, CCDC147Bp was absent in cilia isolated from CCDC147A-KO cell. Interestingly, based on BioID data, CCDC147A and CCDC147B proteins are positioned in close proximity to main ciliary regulator, N-DRC (nexin-dynein regulatory complex). Thus, it is possible that CCDC147A and CCDC147B form a heterodimer that interacts with N-DRC complex. Supported by National Science Centre, Poland (Harmonia 6, 2014/14/M/NZ3/00511)

Somatic mutation history of glioblastoma patients with recurrent tumors

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Glioblastoma (GBM, WHO grade IV) is a common and most lethal primary brain tumor which remains largely resistant to current therapies. Despite tumor resection and the following treatment of GBM patients with radiotherapy and chemotherapy, GBM frequently recurs. Dissection of the GBM genetics and understanding intrinsic mechanisms of tumor recurrence may lead to more targeted and effective treatments. Here we report the results of targeted next-generation sequencing of cancer- and epigenetics-related genes in 16 fresh frozen glioma samples of WHO grade IV, collected from Polish population. We employed a second generation DNA sequencing target enrichment design comprising a 600 cancer-related gene panel and 100 epigenetics-related genes, comprising the exomes +/- the promoter regions. The target region spanning 7 MB (1x106 base pairs) was designed to cover meaningful portion of genomic, cancer-related sites with a strong emphasis on epigenetic regulators (histone modifiers, chromatin modelers, histone chaperons). Additionally, RNA sequencing was performed to better understand the transcriptomic profiling in the malignant progression of GBM. Targeted sequencing of GBMs demonstrated different genetic drivers (including well known EGFR, TP53, PDGFR and PTEN mutations) and numerous genetic alterations in genes responsible for histone modifications, chromatin remodeling and DNA damage repair.

In recurring GBMs we observed only a subset of mutations coming from primary tumors, suggesting a specific path of clonal evolution.

Integrative analysis of epigenetic landscape of glioblastoma infiltrating immune cells

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Glioblastoma is the most common and aggressive primary brain tumor. Its molecular diversity and structural heterogeneity makes it an extremely difficult target for a successful therapy. We perform bioinformatics analyses to better understand transcriptional and epigenetic mechanisms present in tumor cells and in its microenvironment. We attempt to discover changes in immune cells in the microenvironment that support tumor progression. Creating molecular characteristics of glioblastoma and tumor-infiltrating cells requires integrative analysis of the high throughput Next Generation Sequencing data generated using diverse methods. Chromatin immunoprecipitation (ChIP)-seq data and Assay for Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq, a method for mapping chromatin accessibility) data help to determine how changes in the chromatin structure influence phenotype of the cells. Bulk RNA-seq data provides an overview of the changes in gene expression patterns between normal and tumor cells. We can additionally observe global transcriptomic response of the cells to a given treatment. Gene expression values in bulk RNA-seq data are however averaged over all cells present in the sample. Using single-cell RNA-seq data we focus on heterogeneity of the tumor microenvironment and on understanding differences between cell subpopulations (in particular microglia). Single-cell RNA-seq data significantly increases resolution of the transcriptome analyses and extends possibilities of exploring cells diversity.

Endothelial dysfunction in in vitro model of dyslipidemia

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Hyperlipidemia is a state of elevated level of lipids in the blood and is the most common form of dyslipidemia. Apart from genetic background it is associated with obesity and sedentary lifestyle. If untreated it may lead to type 2 diabetes and cardiovascular disease. Some literature data suggest that maternal obesity and dyslipidemia may influence umbilical endothelium and finally affect fetal metabolism of lipids and mitochondrial. Endothelium plays an important role as a barrier and the first cellular layer directly contacting with the circulating blood. Mild palmitate treatment of endothelial cells seems to be a good in vitro model of hyperlipidemia, mimicking high level of blood fatty acids. Under experimental conditions an incubation of HUVEC cells with palmitate resulted in a reduced sensitivity to insulin and an elevated nitric oxide generation while any pro-inflammatory response was detected. The aim of this study is elaborate the effects of palmitate on various endothelial cells function including von Willebrand factor exocytosis and to explain a role of annexins in various signaling pathways in endothelium.

Epigenetic regulation of EDC cluster gene expression in keratinocyte differentiation

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The research concentrates on epigenetic regulation of keratinocyte differentiation. In particular, we study histone modifications of genes located within the Epidermal Differentiation Complex (EDC) gene cluster, which encodes proteins involved in the process of epidermis formation. Within EDC, we especially focus on the locus containing LCE gene subgroup-1 (encoding Late Cornified Envelope proteins) as their expression rises significantly during epidermal differentiation. So far, we have examined changes in selected histone modifications in several LCE gene promoters by means of chromatin immunoprecipitation. This analysis revealed a significant decrease in histone H3 lysine 9 (H3K9) trimethylation upon differentiation. To verify the importance of this modification, we have employed the CRISPR-Cas9 strategy to knock-out the Suv39H1 methyltransferase that trimethylates H3K9. We have obtained Suv39H1 knock-out keratinocytes (HaCaT cell line) and demonstrated that Suv39H1 deficiency results in a lower level of H3K9me3 modification in the cell. Currently, we check the effect of SUV39H1 knock-out on the course of epidermal differentiation in general, and expression of LCE genes in particular.

Knowledge of stimulus contingency matters in observational fear conditioning.

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Learning how to avoid threats often happens indirectly, through observation of others. The involved processes can be studied in humans using the observational fear learning paradigm proposed by Haaker and Olsson. In order to improve its ecological validity, instead of using pre-recorded videos, we recruited pairs of friends, one of whom (demonstrator) was asked to perform an aversive differential conditioning task while being observed by the other (observer) through a video stream. In the conditioning task, two different visual cues were presented on a computer screen in a randomized order. Presentation of one of them co-terminated with a highly unpleasant electric shock to the forearm with 50% probability. After the observation phase, the observer was informed that he will perform an identical task, but in fact no aversive stimuli were administered to him. The acquisition of conditioned fear can be measured through physiological responses, including the Acoustic Startle Response - a primitive defensive reflex, manifesting in a twitch of facial muscles elicited by a loud noise. The startle response is known to be amplified when the noise occurs during the presence of a cue that has been previously paired with an aversive stimulus (this is called startle potentiation). It is debatable whether participant's declarative knowledge of stimulus contingency (i.e. knowing which of the cues predicted an electric shock) is required for fear conditioning to occur in various experimental protocols. However, dual-system theories, which propose an automatic associative system independent of cognition, are commonly held as valid in most cases. In our experiment, we observed that the startle response was potentiated towards the previously reinforced cue only in those observers who were able to correctly indicate the stimulus contingency. This suggests that declarative knowledge of stimulus contingency might play an important role in observational fear conditioning.

How social environment impacts an ability to understand intentions of others

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Social interactions are important part of our everyday life. They shape our behavior and lead to changes in the functioning of the brain. Theory of mind (ToM) is an ability to read one self's or other people mental states and is needed for communication with others. The Reading the Mind in the Eyes-Test (RMET) is a test used to measure ToM. In this test, adjectives describing mental states need to be attributed to photographs of eyes region of adults. The Nencki Children Eyes Test (NCET) is a similar test, which instead uses photographs of eyes region of children. NCET was developed in our department, as an experimental procedure for studying certain clinical populations. In our study this test was used to clarify if our social environment change our behavior and brain functioning. Therefore, we recruited 20 (9 males) childless, young adults who are, or were working with children (WWC) and 19 (9 males) childless, young adults, who have never worked with children (NWC). The participants performed NCET and RMET during functional Magnetic Resonance Imaging procedure. The WWC group showed better accuracy in NCET than NWC group. The WWC also group showed increased activation in the left inferior frontal gyrus than the NWC group, when performing NCET. Moreover the WWC group showed increased activation in the left inferior frontal gyrus, when comparing NCET to RMET. This result suggest that specific social interactions induce changes in the functionality of the brain, similar to acquiring new skill or language.

KLF4 regulates the expression of genes from the Grainyhead-like (GRHL) family

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Development of many types of cancer is often accompanied by changes in the levels of expression of the genes from the Grainyhead-like (GRHL) family. Thus, the knowledge about the mechanism of their regulation could provide novel and valuable insights into the molecular mechanisms of cancer development. To date, only fragmentary studies investigated the regulation of expression of these genes by transcription factors. In order to fill this gap in our knowledge we are performing a systematic analysis of promoter regions of the GRHL genes in order to identify and characterize transcription factors binding to these promoters. We examined KLF4 binding in the promoter regions of GRHL1, GRHL2 and GRHL3 genes and determined KLF4 role in the regulation of expression of these genes. The results suggest that KLF4 associates with putative binding sites in the promoter regions of GRHL1, GRHL2 and GRHL3 genes and it is responsible for regulating their expression. This work is supported by the National Science Centre grant 2016/21/B/NZ1/00279

The cellular organization of the opossum cerebellum

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The gray short-tailed opossum (Monodelphis domestica) is an omnivorous, pouchless, South American marsupial. Opossum pups are born at very immature stage with nervous system comparable to 11 day old mouse embryo, therefore opossums are useful for ex utero studies on early stages of mammalian development. This research is focused on cerebellum development at the cellular level. Part of the opossum pups were injected with bromodeoxyuridine (BrdU) in different time points and sacrificed at postnatal day (P) 90 to study migration or final destination of proliferating cells. Brains were cut and immunostained for BrdU. The second part of animals was sacrificed at different ages to identify cellular organization of the developing cerebellum using molecular markers for various type of cells. I have found that Purkinje cells generation occurs between P1-P7 with peak at P3. The deep cerebellar nuclei formation starts at P5 in the nuclear transitory zone. Granule cells generation starts at P17 with proliferation peak at P30-P40 and continues even till P90. Development of the opossum cerebellum is slower and occurs later than in placental mammals.

In Search for Reliable Markers of Glioma-Induced Polarization of Microglia

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Immune cells accumulating in the microenvironment of malignant tumors are tumor educated and contribute to its growth, progression, and evasion of antitumor immune responses. Glioblastoma (GBM), the common and most malignant primary brain tumor in adults, shows considerable accumulation of resident microglia and peripheral macrophages, and their polarization into tumor-supporting cells. There are controversies regarding a functional phenotype of glioma-associated microglia/macrophages (GAMs) due to a lack of consistent markers. Previous categorization of GAM polarization toward the M2 phenotype has been found inaccurate because of oversimplification of highly complex and heterogeneous responses. In this study, we characterized functional responses and gene expression in mouse and human microglial cultures exposed to fresh conditioned media [glioma-conditioned medium (GCM)] from human U87 and LN18 glioma cells. Functional analyses revealed mutual communication reflected by strong stimulation of glioma invasion by microglial cells and increased microglial phagocytosis after GCM treatment. To define transcriptomic markers of GCM-activated microglia, we performed selected and global gene expression analyses of stimulated microglial cells. We found activated pathways associated with immune evasion and TGF signaling. We performed computational comparison of the expression patterns of GAMs from human GBMs and rodent experimental gliomas to select genes consistently changed in different datasets. The analyses of marker genes in GAMs from different experimental models and clinical samples revealed only a small set of common genes, which reflects variegated responses in clinical and experimental settings. Tgm2 and Gpnmb were the only two genes common in the analyzed data sets. We discuss potential sources of the observed differences and stress a great need for definitive elucidation of a functional state of GAMs.

New insights into the role of BLM helicase in malignant gliomas

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Glioblastoma (GBM) is the common and most aggressive primary brain tumor, with a median patient survival 14 months. Genome wide studies revealed numerous genomic alterations that are linked to pathogenesis of gliomas. Using Next Generation Sequencing and Ultra-Deep Sequencing of glioma samples we found small deletions in the BLM gene in 5 out of 37 GBMs. BLM encodes Bloom helicase, the member of RecQ helicases family, which is involved in DNA replication and repair of DNA double-strand breaks, predominantly by the homologous recombination pathway. Mutations in BLM are linked to Bloom syndrome (BS), which is a rare recessive human chromosome breakage disorder. The detected deletions in the BLM gene identified in our cohorts may affect the BLM expression. The analysis of TCGA (the Cancer Genome Atlas) data shows overexpression of BLM in gliomas of higher grades. Using tissue microarrays we found up-regulated expression of BLM in gliomas. Localisation of BLM was both nuclear and cytoplasmic. The aim of further study was to delete the BLM gene in glioma cells of LN18 and L229 lines using a CRISPR/Cas9 approach and analyse the effect of BLM knockout on basal functions and sensitivity towards DNA alkylating agents. Moreover, we performed a functional analysis of interactions between BLM and its functional partner RecQL4 in glioma cells and effects of manipulation of BLM levels on other helicases.

L1 tauopathic mice show cholinergic impairment without neuronal degeneration.

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The sites of the greatest concentration of tau neurofibrillary tangles in the AD are the axons of cholinergic neurons. The aim of this study was to find out whether progression of tauopathy coincides with a functional deterioration in the brain cholinergic system. A transgenic line 1 tau mice (model of Alzheimer's Disease) was used, which expresses truncated tau (amino acids 296-390 of the longest human 441 isoform). In AD-L1 and wild-type (WT) NMRI mice, the following brain stainings were performed: immunohistochemistry against the microtubule-binding domain of tau (tau), tau phosphorylated on Ser-404 (p-tau), choline acetyltransferase (ChAT), histochemical staining of acetylcholinesterase (AChE) activity and Fluoro-Jade C staining. Mice were aged 3-3,5, 8-9 and 12 months old. In AD-L1 mice there was an increase in tau staining in the neuronal cytoplasm in cortex and hippocampus (CA1 and CA4 field) as compared to WT (NMRI). Moreover, microscopic analysis revealed a change in intracellular localization of p-tau in hippocampus and cortex of 9-mo-old AD-L1 mice as compared to WT. The greater intensity of cytoplasmic p-tau immunoreactivity in AD-L1 mice suggests its sequestration from the axodendritic compartment to neuronal somata. Furthermore, in 3- and 9-mo-old AD-L1, there was a decline in anti-ChAT staining, decreased number of immunoreactive-cells and atrophy of these cholinergic neurons in comparison with control mice. In parallel, the intensity of AChE staining in cortex and hippocampus in AD-L1 was significantly lower than in WT at both ages. However, we did not detect any neuronal degeneration in hippocampus and cortex with the Fluoro-Jade C staining. In AD-L1 tauopathic mice, cholinergic neurons were not undergoing degeneration. However, there was a loss of cholinergic phenotype and impairment of projection to hippocampus and cortex. This

deficiency in cholinergic function may be related to the presence of pathological short tau oligomers in AD-L1 mice.

Cortical vasoactive intestinal polypeptide (VIP) interneurons in learning-induced plasticity.

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VIP interneurons constituting about twelve per cent of all cortical inhibitory interneurons, are an enigmatic group of cells and their role in brain circuitry and cognitive functioning is highly unexplored. A few studies have shown that VIP interneuron group is a key part in disinhibitory circuit that regulates adult visual cortical plasticity or assosciative learning in the auditory cortex. Here, using a chemogenetic approach we aimed to study a role of VIP interneurons in plasticity induced by fear learning in primary somatosensory cortex of mice. With immunofluorescence labeling we validated selectivity of inhibitory DREADDs expression in VIP interneurons. Next, under optical imaging recording we transduced cortical representation of row B whiskers with viral vectors expressing designer receptors. During fear learning, in which stimulation of vibrissae paired with tail shock results in plastic modification of the barrel cortex activation, we blocked the activity of VIP interneurons by activation of inhibitory designer receptors with CNO (1 mg/kg). After the conditioning [14C]-2-deoxyglucose brain mapping was performed. On autoradiograms of brain sections functional representation of the conditioned row (B) of whiskers and contralateral row on the other side of the snout were compared. Our preliminary results suggest that blocking of VIP interneurons can enhance plasticity in fear learning and the effect can be mediated by increased activity of somatostatin interneurons.

Posters

Drebrin as a novel protein of the postsynaptic machinery at the neuromuscular junction

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Drebrin is an actin-binding protein mostly known for its role in dendritic spine dynamics in the CNS. Morphological plasticity of synapses is crucial for high cognitive processes, such as learning and memory and is thought to be regulated by cytoskeleton organization1. Together with the scaffolding protein Homer, Drebrin recruits F-actin, PSD-95 and other synaptic proteins, forming a polymeric network that controls spine structure and synapse function. On the other hand, Drebrin and Homer have been described to play different roles in non-nervous cell types, including cell differentiation and myoblast fusion in the muscle. Finally, Drebrin has been detected in the contractile machinery of Caenorhabditis elegans, where it stabilizes and promotes actin bundle formation within the sarcomere. In the present study, we describe the role of Drebrin in the neuromuscular junction (NMJ). We have performed in vivo localization and in vitro functional experiments which suggest that drebrin contributes to the organization and maturation of acetylcholine receptor (AChR) clusters in the mammalian NMJ.

Live observation of a friend undergoing aversive conditioning influences fear learning

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Observational fear learning is a process which enables indirect acquisition of threats and has recently been described as an experimental protocol for human studies (Haaker et al., Nature Protocols, 2017). We modified the methodological framework proposed by the authors, aiming at improving its ecological validity. Instead of using pre-recorded videos, we recruited pairs of friends, one of whom (demonstrator) was asked to perform an aversive differential conditioning task while being observed by the other (observer) through a video stream. After the observation phase, the observer was asked to perform an identical task (direct phase), but no aversive stimuli were administered to him. We assumed that the observer should develop a conditioned response to the stimuli used without directly experiencing the aversive stimulation and that the acquisition of fear would be reflected in fear potentiated startle (FPS) and skin conductance responses (SCRs). The results show that the observational unconditioned stimuli evoked strong skin conductance responses in the observers. Elevated skin conductance level was observed throughout the whole experiment. In direct phase of the experiment there was no significant difference in SCRs to the previously reinforced and non-reinforced stimuli. The startle responses were significantly higher during the direct phase compared to observation and potentiated towards both conditioned stimuli, with no significant difference between them. Declarative knowledge of the CS/US contingency was low, but in participants who correctly identified it, physiological reactions did differ between the CSs. This interaction effect was found only in FPS responses though. Furthermore, an interesting result suggesting a counterintuitive relationship between a tendency for emotional contagion and FPS differentiation was observed in the direct phase. These results suggest that the modified version of the observational fear learning paradigm was effective in eliciting

emotional contagion, but learning effects were weaker and more complex than expected. An fMRI study using this paradigm is in preparation.

Enhancement of S-palmitoylation in chronic stress

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Chronic stress is the environmental factor linked with development of neuropsychiatric disorders such as major depression and anxiety. A number of studies have demonstrated association of chronic stress with divergent changes in the specific brain regions and aberrant activity of synaptic proteins, however the molecular mechanisms underlying these psychopathologies are not fully understood. One of the best described mechanisms of synaptic proteins regulation are post-translational modifications (PTMs). Multiple PTMs occurs in neurons, and are able to modulate proteins in different subcellular compartments. Protein S-palmitoylation (S-PALM) is a lipid modification where palmitate (C16) is being attached to cysteine via thioester bond. S-PALM is reversible and tightly regulated by palmitoyl acyl transferases and palmitoyl thioesterases. In order to understand the role of S-PALM in psychopathological changes we applied animal model in which wild-type C57BL/6J mice were subjected to chronic restraint stress. In the following steps of our study we performed Acyl-Biotin Exchange (ABE) method coupled with Western blot analysis. In this method S-Palmitoylated proteins are purified using selectively replaced palmitoyl groups with biotin and selective enrichment only S-palmitoylated peptides. To get deeper insight into synaptic functions we analyzed only postsynaptic density proteins (PSD). We found that S-PALM of numerous proteins is significantly increased in PSD fraction isolated from brains of mice subjected to chronic restraint stress. Moreover, for non-targeted proteomic identification of cysteines which are modified by S-PALM we applied mass spectrometry LC-MS/MS combined with ABE method. We identified 806 S-PALM peptides assigned to 451 proteins including prominent synaptic proteins such as Homer, Shank or PSD-95. In the group of 451 identified S-PALM postsynaptic density proteins, 3 were present only in control mice and 60 were exclusively present in mice exposed to chronic restraint stress. All of these results indicate that our methods are well established and allow detection of even subtle changes in the modification of proteins that may play key roles in various neuronal signaling pathways.

Ultrastructural correlates of postsynaptic scaffolding in memory remodeling - the role of PSD-95 trafficking.

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Structural plasticity of synaptic connections is thought to underlie memory formation and remodelling, processes that are still not well understood. Part of the excitatory synapse, termed postsynaptic density (PSD), is responsible for receiving of the incoming neurotransmitter signal. One of the main scaffolding proteins of the PSD is PSD-95. In vitro overexpression of PSD-95 leads to formation of multiinnervated dendritic spines (Nikonenko et al. 2009). What is more, PSD-95 phosphorylation on Serine 73 (S73) dissociates it from the PSD region (Gardoni et al. 2007, Steiner et al. 2008). In this study we asked whether inhibition of this dissociation would impair memory remodelling in vivo and what are the ultrastructural effects of such impaired trafficking in the CA1 field of the hippocampus. To this end we have used a hippocampus-dependent task, contextual fear memory extinction, in animals overexpressing either control protein (mCherry), wild-type PSD-95 fused with

mCherry (PSD-95WT_mCherry) or non-phosphorylable PSD-95 (PSD-95S73A_mCherry) in the CA1 area of the hippocampus. We show that PSD-95 overexpression slightly impairs fear memory acquisition and that overexpression of mutated form of PSD-95 impairs memory extinction, thus remodelling. We use 3D Serial Block-Face Scanning Electron Microscopy (SBEM) to investigate synaptic structures in stratum oriens of CA1 area to show how PSD-95 and PSD-95S73A overexpression affects PSD structures and whether it leads to formation of multiinnervated dendritic spines in vivo. An on-going correlative light-electron microscopy study protocol is also displayed. Overall this study indicates crucial role of flexible synaptic scaffolding in learning processes.

INTERACTION BETWEEN PRION PROTEIN AND TAU PROTEIN

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An important role for prion protein (PrP) in the pathogenesis of Alzheimer's disease (AD) has been previously reported. Although there are some data suggesting interaction between PrP and Tau protein, it is however not much known about its consequences. Our studies are focused on the influence of PrP on the amyloidogenesis of Tau protein as well as the effects of PrP on Tau-induced neurotoxicity. Moreover, since Tau is abnormally phosphorylated in AD, we have checked whether this modification could affect Tau-PrP interaction. We have identified the binding sites for Tau protein on the PrP molecule and have demonstrated that the short PrP sequence 23-50 is crucial for Tau-PrP interaction. We have also analyzed the influence of PrP and its fragments on the aggregation of Tau protein and we have shown that PrP inhibits formation of amyloid aggregates of Tau protein. Tau alone formed numerous amyloid fibrils, while in the presence of PrP the amount of Tau fibrils was apparently reduced. Furthermore, we have found that phosphorylation of Tau by GSK-3b or PKA leads to significant increase in binding of PrP to Tau. Noteworthy, PrP is able to affect oligomerization process of PKA-phosphorylated Tau. Phosphorylated Tau incubated alone has formed small globular oligomers while in the presence of PrP it has assembled into amorphous aggregates. Moreover, we have shown that oligomers of phosphorylated Tau are the main neurotoxic species of Tau and that PrP is able to reduce toxicity of these oligomers in the primary culture of hippocampal neurons. Our studies provide important evidence for the impact of PrP on the amyloidogenesis of Tau protein as well as point out potentially protective role which PrP may play in tauopathies. S.B. has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 665735 (Bio4Med) and the funding from Polish Ministry of Science and Higher Education within 2016-2020 funds for the implementation of international projects (agreement no 3548/H2020/COFUND/2016/2). The study has been supported by grant 2016/21/B/NZ4/00181 from the Polish National Science Centre to K.N.

Annexins and fetuin-A regulate the bone mineralization

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Bone mineralization is initiated by matrix vesicles (MVs), cell-derived structures released into the extracellular matrix (ECM) which are nucleation sites for hydroxyapatite (HA) formation. It is suggested that annexins are mineralization-stimulating membrane proteins that exhibit ion channel activity and facilitate the influx of Ca2+ into MVs. The process is also regulated via enzymatic degradation of inhibitory pyrophosphate by tissue-nonspecific alkaline phosphatase (TNAP). Another layer of control is exerted by circulating, mineralization-inhibiting protein fetuin-A. The objective of our study was to examine the roles of TNAP, annexins and fetuin-A in MVs function during physiological and pathological mineralization. We used two human cell lines: osteoblastic hFOB 1.19 and osteosarcoma Saos-2. These cells were stimulated for mineralization for 7 days by osteogenic factors (50 mg/mL ascorbic acid and 7.5 mM β-glycerophosphate) treatment. We compared cell morphology, intracellular distribution of proteins and formation of HA in control and levamisole (TNAP inhibitor) or K-201 (a calcium channel inhibitor) treated cells. We detected calcium nodules by Alizarin Red-S staining of cell cultures. We then isolated MVs from these cells by collagenase digestion and determined TNAP activity using pNPP as a substrate. Finally, we used Western blot method to identify differences in annexins and fetuin-A profile and expression in examined cell lines. Understanding of the role of annexins and fetuin-A as biomarkers in TNAP-regulated function of MVs may provide novel insights into the mechanisms of physiological and pathological mineralization and may help to develop therapeutic strategies on the basis of the use of TNAP and calcium channel inhibitors to prevent pathological mineralization.

Astrocyte-specific CD44 knock-out mouse model as a new tool in revealing CD44 protein functions

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CD44 adhesion molecule is highly expressed in astrocytes but its role in these cells is unknown. We are working on revealing new functions of CD44 protein in mouse brain, especially in astrocytes. To study the function of CD44 in vivo, we used double transgenic mouse model ERT2-EF1-GFAP-Cre/CD44fl/fl with inducible CD44 knock-out in astrocytes. CD44 knock-out is initiated after administration of tamoxifen (TAM) which activates cre/lox system. However, due to the ineffective silencing of CD44 expression in double transgenic mice we decided to use an alternative experimental approach - Cre recombinase insertion into the brain of CD44fl/fl mice with the use of viral vectors. Using AAV-GFAP-GFP-Cre we were able to effectivelly silence CD44 in astrocytes in molecular layer of dentate gyrus in mouse hippocampus.

Up-regulation of PI3K-Akt-mTor signaling pathway in neurons leads to temporary improvement of cognitive functions.

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Aims: PI3K-Akt-mTOR pathway plays important role in neuronal plasticity. In normal conditions activity of this pathway is controlled by Pten phosphatase. We used two Pten knock-out mice models to analize long-term activation of mTOR influence on cognitive functions. . Methods: Mice model: Mutation of Pten gene was investigated in 2 models: Pten/CaMKCreERT2 and Pten-flox. Modification in Pten/CaMKCreERT2 was restricted to forebrain neurons and induced by tamoxifen. To avoid lethal phenotype we injected AAV-CaMKCre construct directly into the hippocampal neurons of Pten-flox line. Behavioral testing:Pten/CaMKCreERT2 mutants and respective controls were tested in learning and memory test in IntelliCage. We measured spatial learning with appetitive reinforcement using water with 10% sucrose solution. Place preference learning task assessed the animals ability to learn the specific location of reward in cage. Pten-flox/AAV and controls were examined in Contextual Fear Conditioning task. We measured animals ability to associate an aversive stimulus with neutral environmental cues. CFC was assessed 24 h by scoring freezing behavior in the same context chamber. Results Life span: Long-term activity of PI3K-Akt-mTOR pathway led to increased mortality of Pten/CaMKCreERT2 mutants. Compared with controls, their lived about 13 weeks. IntelliCage: We discovered the better performance of Pten/CaMKCreERT2 mutants in PL task. The memory improvement lasted to even 24 hours before the death. CFC task: Mice developed stronger aversive memory than controls, manifested as increased freezing behavior in the training context. Conclusions Pten/CaMKCreERT2 mice showed enhance memory of rewarded place compared to control mice. Pten/CaMKCreERT2 mice showed decrease life span Pten-flox/AAV-CaMKCre mice developed enhanced contextual fear memory compared to control group

Cap2 involvement in NMJ organization

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Actin dynamics are involved in many important biological processes including embryonic morphogenesis, immune surveillance, tissue repair and regeneration. The transition between globular actin (G-actin) and filamentous actin (F-actin) represents a central feature of actin-mediated remodelling1. Cyclase-associated proteins (CAPs) are a family of high-conserved actin-binding proteins, which regulates actin dynamics by sequestering G-actin and severing F-actin. In higher eukaryotes, two CAP proteins have been identified: CAP1 and CAP2. While CAP2 has been studied in cardiac muscle and neurons, little is known of its function in skeletal muscles and more specifically at the neuromuscular junctions (NMJs) – a specific type of synapses form between motor nerve terminal and muscle fibers2. We demonstrate that Cap2 ablation results in abnormal Acetylcholine receptors (AChR) organisation at NMJ. In Cap2 KO muscles many NMJs were noticeably fragmented, which is known to be a hallmark of disease and premature aging of synapse. At the same time other synapses exhibit dramatic reduction in size while other were strongly enlarged. Thus, although the mechanism by which Cap2 is involved in the organization of postsynaptic machinery is still unknown, we suspect that actin remodelling medited by Cap2 is crucial for NMJ stabilization.

Imaging the mouse brain in a photostroke model using Optical Coherence Microscopy (OCM)

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To visualize cortical vessels in the region of the brain ischemia we used Optical Coherence Microscopy (OCM), a new developing brain imaging tool. This method bases on the detection of infrared light scattered on the examined structures. OCM provides access to real-time detailed three-dimensional information about the brain vascular system up to the level of capillaries across superficial layers of neocortex. In our experiments we monitored changes of the vascular network due to photostroke in the mouse brain in vivo. We used thromboembolic stroke model involving application of photoactive Bengal Rose and precise green laser illumination of the Medial Cerebral Artery branch. We present angiographic maps of the stroke-affected brain region enabling in-depth insight to the process of development of the disorder. To verify photostroke infarct and revile degenerating neurons we perform histological hematoxylin-eosin (HE), Fluoro-Jadge (FJ) and TTC staining.

Alterations in mitochondrial dynamics in primary fibroblasts derived from patients diagnosed with sporadic form of Alzheimer's disease.

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Mitochondria are multifunctional, dynamic organelles, which are continuously undergoing fusion and fission, turnover (biogenesis and clearance of mitochondria), as well as movement along the cytoskeleton. Proper dynamics is crucial for maintenance of vital functions of the cell. Disturbances of mitochondrial dynamics were observed in most common neurodegenerative disorders. Detailed mechanisms of these impairments are still lacking. In our study, conducted on primary fibroblasts derived from patients with sporadic form of Alzheimer's disease (AD), we showed diminished mitochondrial turnover, changes of the level of proteins involved in mitophagy and decreased level of factors engaged in biogenesis. Moreover mitochondria in AD cells were functionally older and created diverse mitochondrial network (less fragmented, longer branch length, different number of junctions). Additionally the level of fission proteins were reduced. Investigations of mitochondrial dynamics seems to be important for better understanding the pathogenesis of Alzheimer's disease.

Modified Multi-Source Interference Task (MSIT+): preliminary behavioural and EEG results from extended version of the test

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MSIT is a validated tool used in fMRI research that robustly activates the cingulo-fronto-parietal attention network (CFP). The original test contains two stimuli conditions: Easy, with spatially Congruent location of target digit (EC) and Hard one, with Incongruent locations and flanker distractors (HI). We aimed to modify MSIT to gradually increase its difficulty and possibly observe resulting progressing engagement of CFP network in fMRI and EEG studies. Two conditions were added to standard MSIT: Easy, with Incongruent (EI) digit locations (introduced for the first time); and Hard, with flanker distractors but Congruent digit locations (HC). Reaction times (RT) were measured to assess task difficulty. Pilot study included 7 people tested behaviourally with added HC condition and 6 people who performed full MSIT+ during high-density EEG recording (4 subjects underwent both tests). Average RT ranged from 582ms to 788ms with the shortest observed in EC and the longest in HI condition. New conditions of MSIT+ brought intermediate RT values: EC (M=582ms) < EI (M=665ms) < HC (M=690ms) < HI (M=788ms), ($F(2,18)=12.9$, $p<0.001$, $n=6$). Post-hoc Student's t-test have shown significant RT differences for EC-HC, EC-HI, EI-HI, HC-HI comparisons ($p<0.05$). Four subjects, who performed MSIT+ twice, did not show learning effects. Observed scale of RTs indicate a gradual increase of difficulty in proposed four MSIT+ conditions. Flanker-interference task seems to require higher cognitive load than spatial incongruence interference. Neural basis underlying these differences will be further studied with EEG and fMRI. EEG spectral characteristic was significantly modulated by task conditions: theta band, which is supposed to increase over frontal-midline region when we are dealing with demanding tasks (e.g. Cavanagh et al., 2015), was stronger during Simon interference (FS and S0) then during Flanker interference (F0). Alpha band pattern mirrored that of theta. The discrepancy between RT and theta results may contradict ACC time on task theory or question the theta as ACC signature, however these are only preliminary results which needs further evaluation. Future plans: study on a larger group (40 participants with equal ratio of F/M) and additional psychological battery tests. [Supported by National Science Centre, Poland, UMO-2016/20/W/ZN4/00354]

Epigenetics changes in chromatin structure and gene expression during replicative and premature senescence in vascular smooth muscle cells; the role of HP1α.

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Cellular senescence is a natural phenomenon accompanying organismal aging. It is characterised as a permanent state of cell cycle arrest. Although cells cease to divide, they are

still metabolically active. This in turn, can lead to general health deterioration and pose a risk of developing age-related diseases. One of the most frequent is atherosclerosis caused, among others, by accumulation of senescent vascular smooth muscle cells. Senescence can be either triggered by different agents (resulting in premature senescence - PS) or occur as an effect of telomeres' shortening (named replicative senescence - RS). This process is associated with number of morphological and physiological changes, including nucleus and chromatin structure. Chromatin is highly flexible and can assume two distinct conformations depending on the post-translation modifications, i.e. relaxed euchromatin or highly condensed heterochromatin. Euchromatin is often observed in senescent cells, where compaction of chromatin gradually decreases throughout the lifespan of a cell. In young dividing cells, however, chromatin usually assumes the second conformation, i.e. heterochromatin. Moreover, this structure can also be present in premature senescent cells as senescence associated heterochromatin foci (SAHF). Our preliminary results showed that changes in chromatin compaction depend on the type of senescence. In RS the level of heterochromatin marks, especially H3K9me3, decrease along with HP1α as compared to the control cells. In contrast, prematurely senescent cells do not display significant loss of neither H3K9me3 nor HP1α. The latter change its organization within the nucleus in the same manner as in RS, although higher number of foci was observed in PS than RS. Further analysis of chromatin modifications and their influence on gene expression in senescent vascular smooth muscle cells can contribute to understanding the role of cellular senescence in the process of atherosclerosis. It may also help in developing new markers of senescence and aid the identification of new potential therapeutic targets.

Targeted sequencing of cancer- and epigenetic-related genes in glioblastoma reveals a deep deregulation of epigenetic mechanisms

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Recent whole genome studies demonstrated that epigenetic enzymes, histones and chaperone proteins harbor mutations that may result in gross alterations of the epigenome leading to genome instability. Such mutations are common in pediatric hematopoietic and solid tumors, and are targets of innovative treatments with epigenetic enzyme inhibitors. Glioblastoma (GBM, WHO grade IV) is a common and most lethal primary brain tumor in adults, and remains incurable by conventional therapies. Greater understanding of GBM genetics may lead to more targeted and effective treatments. Here we report the results of targeted next-generation sequencing of cancer- and epigenetics-related genes in 118 fresh frozen glioma samples of grade II, III, and IV collected from Polish (n=97) and Canadian (n=21) populations. We employed a second generation DNA sequencing target enrichment panel comprising 600 cancer-related genes and 100 epigenetic-related genes. The target region spanning 7 MB (1 MB = 1x106 base pairs) was designed to cover meaningful portion of genomic, cancer-related sites with a strong emphasis on epigenetic regulators (histone

modifiers, chromatin modelers, histone chaperons). Several filtering steps were used to eliminate variant calling errors: mapping quality > 35, each variant coverage > 20x, the penetration of each variant > 20%. Targeted sequencing of GBMs demonstrated mutations in different genetic drivers (including well known EGFR, TP53, PDGFR and PTEN mutations) and numerous genetic alterations in genes responsible for histone and chromatin modifications, chromatin remodeling and DNA methylation. Newly discovered variants were confirmed by ultra-deep sequencing. Funding: TEAM TECH CORE FACILITY FNP: Development of comprehensive diagnostics and personalized therapy in neuro-oncology

Short-term elevation of BDNF expression in the spinal cord leads to partial protection of the NMJ in the rat soleus muscle from denervation after complete spinal cord transection

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Introduction: Complete spinal cord transection (SCT) leads to loss of motor control due to disruption of supraspinal tracts and altered functioning of both central and peripheral synapses. We showed that SCT at low thoracic segments causes deficiency in cholinergic input to ankle extensor (soleus) motoneurons (Skup et al, 2012), whereas brain-derived neurotrophic factor (BDNF) overexpression below the lesion site increases markers of spinal neurotransmission and improves locomotor performance (Ziemlinska et al, 2014). These findings raise the question if SCT impairs also integrity of peripheral synapses in soleus muscle and if BDNF can counteract lesion effects. Aim: To disclose the impact of SCT and BDNF overexpression on pre- (VAChT and S-100) and postsynaptic (nAChR) components of neuromuscular junction (NMJ) in soleus muscle. Methods: VAChT and S-100 were detected immunohistochemically and acetylcholine receptors were visualized with fluorescently labeled bungarotoxin on free-floating muscle fibers 2 weeks after SCT and intraspinal injection of PBS (n=6) or BDNF (n=7). Images acquired on Zeiss confocal microscope were deconvoluted with Huygens Professional and analyzed with 3D Imaris Software to evaluate NMJ morphology. Results. SCT reduced the number of contacts of normal morphology to 39% which was accompanied by decrease in NMJs size. BDNF overexpression resulted in preservation of 73% of normal contacts, but did not prevent NMJ shrinkage. VAChT-labeled motoneuron terminals were visibly more dispersed after SCT than in controls. BDNF did not affect this dispersion. Conclusions. Spinal BDNF overexpression partially prevents NMJs from denervation, albeit does not counteract the reduced size of NMJ. It needs further investigation whether motor improvement is the effect of direct neuroprotective role of BDNF on NMJs or the result of altered signaling at central synapses. Supported by grants: National Science Centre 2013/09/B/NZ4/03306, statutory for the Nencki Institute.

Cognitive abilities and neuronal plasticity of laboratory mice divergently selected for Basal Metabolic Rate: a test of the "Expensive Tissue" hypothesis

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Large brains (encephalisation) and associated behavioral complexity of homeotherms are fueled by energy expenditures an order of magnitude higher than those of ectotherms. How the energetic costs of evolution of large brains were overcome, and what sort of energetic, anatomic and physiological trade-offs and/or inherent positive associations were involved? The 'Expensive Tissue' posits that an increased encephalization was primarily possible thanks to 'financing' metabolic costs of enlarged brain by a reduction in energetically demanding gut parts, which in turn was possible because increased cognitive abilities allowed for more efficient foraging for food of better quality. We tested the ET hypothesis using two lines of mice divergently selected for low and high basal metabolic rate (BMR) and control (C) lines. Using a battery of behavioral tests in the IntelliCage system we demonstrated that mice having high BMR associated with larger internal organs (heart, liver and kidneys) performed better in cognitive tasks, despite the lack of between-line type differences in brain size. Most importantly, we also found that mice having high BMR and large guts are also characterized by increased neuronal plasticity, quantified as amplitude of long-term potentiation (LTP) at the CA3-of neurons of CA1 hippocampal pathway. Taken together, our results contradict the ET hypothesis, and suggest that the first stages of evolutionary increase of cognitive abilities were primarily driven by mass-specific re-organization of structure and function of the brain, without concomitant increase of its mass. Project is financed by the National Science Centre grant (NCN 2015/17/B/NZ8/02484).

Ttyh1 overexpression contributes to pyramidal neuron morphogenesis and susceptibility for epileptogenesis in PTZ-induced kindling

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Introduction: Ttyh1 (Tweety homolog1) protein is a presumed volume-regulated Cl− channel and may be involved in neuronal function. Aim: We aimed to examine morphogenesis of CA1 and CA3 neurons following Ttyh1 overexpression and influence of increased Ttyh1 expression on PTZ-induced epileptogenesis. Methods: Rat organotypic hippocampal slices were co-transfected with Ttyh1-GFP-Synapsin and RFP-β-actin constructs, using biolistic transfection. L-measure was used for morphometric analysis of CA1 and CA3 neuronal reconstructions. Spine morphology was studied with SpineMagick!. Transgenic rats with Ttyh1 overexpression and non-transgenic littermates were subjected to PTZ (30mg/kg) induced epileptogenesis three times a week. After injection, rats were observed for 30 min and seizure severity was scored according to five-stage scale. Results: L-measure revealed that CA3 neurons overexpressing Ttyh1 showed increased average branch length in the seventh ($P<0.05$) branch order of apical dendrites and increased number of branches in the third ($P<0.01$) branch order of basal dendrites. CA1 pyramidal neurons overexpressing Ttyh1 showed reduced average branch length in the third ($P<0.05$) and fourth ($P<0.001$) branch orders of basal dendrites. Ttyh1 overexpression resulted in increased number of stubby spines on CA1 neurons (apical proximal and distal dendrites: $P<0.01$; basal dendrites: $P<0.05$) and

CA3 neurons (apical proximal dendrites: $P<0.05$). Ttyh1 overexpression led to decreased percentage of mushroom spines (CA3 apical proximal dendrites: $P<0.01$; CA3 basal dendrites: $P<0.05$) and long spines on CA1 neurons (apical proximal and distal dendrites: $P<0.01$) and CA3 neurons (apical proximal dendrites: $P<0.05$). In the kindling experiment, transgenic rats with Ttyh1 overexpression showed a tendency to increased latency to the onset of stage-5 seizures and kindling acquisition compared with non-transgenic littermates. Conclusions: Ttyh1 protein may be involved in neuronal plasticity and patricipate in susceptibility for epileptogenesis and seizure propagation. Research was supported by Polish National Science Centre Grant: 2011/03/NZ4/00302 and 2015/19/N/NZ3/03268.

Activity-driven chromatin remodeling in neurons is dependent on HDAC1 deacetylase

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Despite the growing evidence that 3D chromatin structure is crucial for transcriptional regulation [1], very little is known about its role in the nervous system. Spatial organization of chromatin can be particularly important in neurons, as these terminally differentiated cells severely change their transcriptome upon the external stimuli [2]. Our recent studies showed that chemically induced Long-Term-Potentiation (cLTP) causes global reorganization of the chromatin of in vitro cultured rat's hippocampal neurons. Such phenomenon has never been described before and occurs not only in vitro, but is also apparent in the amygdala neurons of rats subjected to fear conditioning. It is fast, and reversible, and takes place long before the expression of immediately early genes, and independently of transcription. Observed chromatin remodeling, which is not an outcome of cell death, is accompanied by changes in the distribution of posttranslational histones modifications, such as methylation and acetylation. Moreover, it leads to rearrangements of spatial distribution of chromosome territories. To determine a possible mechanism underlying activity-driven chromatin structural changes we inhibited and silenced histone deacetylases and showed HDAC1 contribution to this process. The epigenetic modifications of the chromatin can have long-lasting effects on neuronal function and thereby represent still largely unexplored, molecular substrate for neuronal plasticity. Project is supported by SonataBis5 National Science Centre grant nr: 2015/18/E/NZ3/00730. References: [1] Mercer, T.R. and J.S. Mattick, Understanding the regulatory and transcriptional complexity of the genome through structure. Genome Res, 23(7), 1081-8 (2013). [2] Benito, E. and A. Barco, The neuronal activity-driven transcriptome. Mol Neurobiol, 51(3), 1071-88 (2015).

Gene expression and protein localization of polysaccharide metabolism enzymes in healthy brain and after stroke induced photochemically.

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Hyaluronic acid (HA) and chondroitin sulfate (CS) compose polysaccharide backbone of the brain extracellular matrix (ECM) structure. As crucial part of tetrapartite synapse, ECM play important role in regulation of neuronal plasticity. In injured brain substantial alterations of ECM are observed that may create environment favorable for synaptic rearrangement. However, enzymes of ECM polysaccharide metabolism, which activity may alter ECM formation and restructuring, are hardly recognized in healthy brain and in pathological condition. In this study, we investigated spatiotemporal expression of genes coding for enzymes involved in HA and CS metabolism in the perilesional and remote areas at early (1 hour - 7days) and late time points (1 and 3 months) after unilateral photothrombotic stroke. Immunohistochemical staining was used to analyze protein cellular localization of selected enzymes in the brain tissue and in vitro. Expression levels of enzymes involved in HA synthesis and HA degradation were concurrently up-regulated as soon as 24h after stroke in the perilesional area. Increased mRNA level of HA synthase 3 was noticeable at later time points, 1 month and 3 months post-stroke. In stroke animals, CS synthesis enzymes expression was in most cases decreased, however prominent bilateral elevation was detected for beta-1,3-glucuronyltransferase 2. Stroke induced CS sulfotransterases/sulfatases mRNA level alterations in both investigated areas of interest. Proteins of investigated enzymes were widely expressed in various types of cells (Hyal1 in astrocytes, B3gat2 in neurons) in healthy condition and after stroke. Moreover, single enzyme could be localized in more than in only one type of brain cell (Hyal2, Gusb, Has2, C4st1, C6st1). Following stroke, modifications of protein expression level and number of cells expressing the enzyme were noticeable. Most evident changes comprised perilesional area and glial scar formation as potent regulator of ECM polysaccharide content in stroke brain. Our results indicate stroke-induced alterations in polysaccharides metabolism enzymes expression with distinct regulatory mechanisms of transcription activation depending on the distance from the cortical lesion. Proteins of selected enzymes were shown to be expressed in specific brain cell types that may work in tandem to regulate ECM polysaccharide conversions. This work was supported by National Science Centre (Poland) grants:2012/05/B/NZ3/00851, 2015/17/N/NZ3/02244

The role of ZF5 motif in the regulation of genes with circadian rhythm disrupted in epileptic animals

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Our recent unpublished data indicate that oscillations in gene expression occur in normal hippocampus and these oscillations are disturbed in experimental model of epilepsy. And the promoters of these genes that belongs to individual clusters shows interesting common features, overrepresentation of ZF5 (ZFP161) motif. Our goal is to identify transcription factors that can alter gene expression in epileptic brain in connection with circadian rhythm in hippocampus. We hypothesize that orchestrated oscillations in expression of gene ensembles and changes in their expression patterns are governed by transcription factors. Within the frame of this project, our first approach is to search for direct transcriptional targets of ZFP161 within genes with epilepsy-related disruption in oscillations. We first used immunohistochemistry and western blot to characterise the expression pattern of ZFP161 in

the hippocampus. We have used eight different antibodies against ZF5. ARP33497_P050 and sc-514298 were failed on IHC and WB. ab110904 had expected nuclear staining however we also observed unspecific astrocytic staining in white matter and WB with this antibody had several unspecific bands. Immunoprecipitation (IP) and chromatin immunoprecipitation (ChIP) techniques were also tried on ab110904, but we couldn't get any successful results. In May 2018, producing company is discontinued ab110904. Another promising antibody HPA050758 has unique pattern in IHC and WB that we observed before with ab110904. While some neurons had cytosolic staining, others had both cytosolic and nuclear staining. IHC and WB are performed with SAB2106303, SAB1400299, ARP38308_P050 and SAB1402396. All four needs further optimization. We also worked on another overrepresented motif, LRF, with the same approach. IHC, WB and IP is done sc-33683. It works very well with IHC and WB. IP needs to be optimized for sc-33683. IHC and WB is done with A300-549A and ab1759018. IHC is not working with these antibodies. In WB with A300-549A, there are clear two bands - one at level of protein of interest and the other is lower level. It needs to be tested for IP. In WB with ab1759018, it has one very faint band at lower level than expected. To conclude, we are trying to characterize expression pattern of ZF5 and LRF motifs with available antibodies on market. Meanwhile, we are using in house methods by dr. Michał Dąbrowski to select candidates of ZF5 and/or LRF targets.

Role of MDM2 oncogene in DNA damage response in breast cancer cells

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MDM2 protein is an E3 ubiquitin ligase with a multidomain structure. It interacts with many proteins involved in all kinds of cellular pathways. Among them most important is p53, known as the guardian of the genome. MDM2 is a negative regulator of p53. It has been shown that MDM2 protein is involved in proper folding of wild-type p53 demonstrating a chaperone-like activity. This activity requires the binding of ATP by MDM2, however does not depend on its E3 ubiquitin ligase function. Many studies suggest that both p53-dependend and independent activities of MDM2 are involved in cancer development and progression. Also clinical data suggest that MDM2 overexpression could be a negative prognostic marker for cancer patients. Our bioinformatics analysis performed on The Cancer Genome Atlas data set confirm that elevated level of MDM2 transcript correlates with worse prognosis for survival of breast cancer patients. Our research is focused on p53-independent role of MDM2 protein in DNA damage response and its implications in breast cancer cells drug sensitivity. Utilizing a panel of breast cancer cell lines with different p53 status we demonstrated by co-immunoprecipitation the interaction between MDM2 and MRN (Mre11-Rad50-NBN) complex. To determine the impact of MDM2 on activation of DNA damage response pathway we performed a screening experiment with clinically used DNA damaging agents such as Camptothecin, Etoposide, Doxorubicin and analyzed the phosphorylation level of histone H2AX. We have shown that, upon MDM2 silencing with specific siRNA, phosphorylation of histone H2AX and other proteins of homologous recombination pathway was impaired. Moreover, cells with low MDM2 level were more sensitive to tested drugs in cell viability assay. Reduction of MDM2 expression results in features similar to homologous recombination deficiency. Thus, our findings suggest that MDM2 could be an important modulator in homologous recombination pathway and potentially prognostic marker for selection of cancer chemotherapy.

Complete spinal transection differentially affects receptors on ankle extensor and flexor motoneurons

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Spinal locomotor network is controlled by the balance between excitatory and inhibitory neurotransmission in the spinal cord. Temporal and spatial summation of postsynaptic excitatory and inhibitory potentials generated by activating respective groups of receptors on motoneurons (MN) mediate the activity of the motor circuit controlling locomotion. Complete spinal cord transection (SCT) leads to a long-term decrease in markers of excitatory (Glutamatergic) and inhibitory (GABAergic) interneurons in the lumbar network (Ziemlinska et al., 2014) and a deficiency in the modulatory cholinergic (Skup et al., 2012) and glutamatergic proprioceptive (Gajewska et al., unpublished) inputs to ankle extensor (Gastrocnemius lateralis; GL) but not flexor (Tibialis anterior; TA) MNs. These responses to the injury prompted us to ask whether postsynaptic receptors in these two pools of MNs adapt to presynaptic changes. We hypothesize that the postsynaptic receptors will respond similarly to maintain the balance between excitatory and inhibitory signals. Rats injected with neurotracers (Cholera Toxin Subunit B-Alexa Fluor™ 594 Conjugate and Cholera Toxin Subunit B-Alexa Fluor™ 488 Conjugate) were spinalized at Th9-10. After 2-weeks, RNA was isolated from L3-6 segments or from MNs dissected by LCM and transcripts were measured (qRT-PCR). SCT led to a moderate increase in NR1 and a marked decrease of NR2A, M2, Glra1 and Gabrg2 transcript levels in L3-6 segment. The transcript level of GluR2 showed a tendency to decrease. In MNs of control animals, transcript levels of all receptors were higher in GL than in TA MNs except for NR1 and NR2B which were lower in GL than in TA MNs by around 35%. After SCT, transcript levels in MNs of all receptors were higher in GL than in TA MNs except for Glra1 which was lower in GL than in TA MNs by 35%. SCT caused a profound increase of GluR1 transcript level and marked decreases of NR1, NR2B, M2, Gabrg2 and Glra1, while GluR2 and NR2A did not change. Concluding, the decreased levels of glutamatergic NMDAR, cholinergic M2 and GABAergic receptors in GL MNs indicates that the postsynaptic changes do not compensate the presynaptic deficits in response to SCT. Besides, SCT results in a change of AMPAR composition (the ratio of GluR1/GluR2 was increased) in both GL and TA MNs, which may act as a mechanism to regulate the MN function after SCT. Support: 665735 Bio4Med H2020 MSCACOFUND 2014, National Science Centre 2013/09/B/NZ4/03306 grants.

The role of temporoparietal junction in updating object-based attention

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Stimuli in the focus of attention are detected faster and more accurately than those outside of the current spot. Spatial orienting of attention is commonly investigated with use of validly and invalidly cued target locations - invalidly cued targets increase activity in the temporo-parietal junction (TPJ), a region ascribed to ventral attention network (VAN). Recently, several works proposed that TPJ & VAN may be responsible for comparing external input with internally prepared templates and mediate both spatial and non-spatial reorienting of attention (e.g. Dombert et al. 2016, Geng and Vossel 2013). These conclusions were, however, poorly justified. In our study we aimed to verify the hypothesis that TPJ region is activated at the appearance of behaviourally relevant, unexpected object, but the process of updating does not reside in activation of ventral attention network. Twenty-five healthy adults

participated in an fMRI study. We modified Posner paradigm (with valid, neutral and invalid cueing) to engage feature rather than spatial attention. The cue (word) represented the likely category (face/house) of the upcoming target and the task consisted in detecting pictures from these pre-defined categories within the rapid serial visual presentation (RSVP) of item pictures, serving as a search background. The task also included trials without target ("search only trials"), which allowed for the assessment of the processing of cue information alone. Reaction times revealed consistent benefits and costs of valid and invalid cueing (+/- 20 ms comparing to the neutrally cued targets). Activation of higher visual areas showed that behavioral effects might arise from decisive components at target identification rather than from increased sensitivity to the stimulus category throughout the search period. Formal evaluation of network affiliation showed that activity involved in directing and updating object-based attention (in vicinity of TPJ region) could not be sufficiently described by reference only to VAN, but it pertained also to engagement of fronto-parietal and default mode networks. Operations within these networks may relate to the necessity of evaluating the meaning of incoming stimuli and disengaging from task-irrelevant information. These results allow reinterpreting a number of studies, by acknowledging the role of DMN in updating process. The study was supported by the Polish National Science Centre grant 2015/19/N/HS6/02364.

Consequences of ESCRT-I dysfunction for autophagy and NF-κB response

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INTRODUCTION. The endosomal sorting complex required for transport (ESCRT) machinery is involved in cargo sorting and membrane deformation, two important processes in the endolysosomal degradation pathway. Although an additional role for ESCRT proteins in autophagy and autolysosomal biogenesis was suggested [1], molecular mechanisms underlying this function are ill-defined. Autophagy may also be transcriptionally regulated by calcium-dependent nuclear translocation of transcription factor E (TFE) proteins, TFEB or TFE3 [2, 3]. However, whether ESCRT dysfunction affects the TFE activity is not known. Our recent study showed that depletion of ESCRT-I subunits results in accumulation of cytokine receptors on enlarged endosomes inducing a proinflammatory response via the NF-κB pathway activation [4]. Here we study a possible involvement of autophagy and TFE in the proinflammatory response evoked upon ESCRT-I depletion. RESULTS. Our RNA-Seq analysis indicates changes in expression of autophagy-related genes after depletion of Tsg101 and Vps28 (two ESCRT-I subunits). Additionally, lack of ESCRT-I leads to inhibition of autophagic flux. To investigate the involvement of autophagy in NF-κB signaling upon ESCRT-I depletion, we inhibited autophagosome formation by depletion of ATG7 protein (Autophagy Related 7). We observed that NF-κB activation in ESCRT-I depleted cells is independent of accumulation of autophagic structures. To study the role of TFE in proinflammatory response induced upon ESCRT-I depletion, we firstly analyzed TFE intracellular localization. We found that ESCRT-I silencing leads to nuclear translocation of TFE. Despite that, simultaneous depletion of single TFE genes and ESCRT-I does not prevent the activation of the NF-κB pathway. Nevertheless, calcium chelation, which inhibits nuclear translocation of both, TFEB and TFE3, decreases non-canonical NF-κB response in ESCRT-I-depleted cells. Further experiments will aim to address whether calcium signaling is important for the regulation of the inflammatory response evoked upon ESCRT-I depletion.

MITOCHONDRIAL PHYSIOLOGY IN PARKIN-MUTANT FIBROBLASTS DERIVED FROM PATIENTS WITH PARKINSON'S DISEASE

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The parkin gene is one of the most frequently mutated genes in familial form of Parkinson's disease (PD). Its protein product belongs to E3-ubiquitine ligase family, and due to its enzymatic activity mediates numerous cell processes, including mitochondrial turnover and dynamics. It is known that dysfunction of mitochondria is involved in pathophysiology of PD. Therefore, we investigated mitochondrial physiology and dynamics in three fibroblast cell cultures derived from patients with clinically diagnosed PD carrying mutations in the parkin gene (in exon 3-4 region) in comparison to three control fibroblast cell cultures derived from healthy age-matched individuals. Mitochondrial properties were estimated by measurements of reactive oxygen species production level, mitochondrial membrane potential, mitochondrial mass, and levels of main proteins involved in mitochondrial turnover and dynamics.

Regulation of mitoBKCa channel by cardioprotective flavonoids.

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Potassium channels such as KATP, BKCa or Kv1.3 have been found in the inner mitochondrial membranes of many types of cells. It is considered that potassium channels regulate the mitochondrial membrane potential, respiration, matrix volume and Ca2+ ion homeostasis. There are suppositon that mitochondrial BKCa channels play an important role in ischemic preconditioning. It was also shown that mitochondrial potassium channels are potential targets for some flavonoids in the anti-ischemic strategies. Our pervious study showed functional properties of the mitoBKCa channel in mitochondria of endothelial cells (EA.hy 926). Large conductance (270 pS), voltage dependence, a high open-state probability at positive potentials, sensitivity to Ca2+, NS1619 (a BKCa channel opener) and paxilline (BKCa channel inhibitor) indicate similarity to the mammalian BKCa channel. Previously, these channel was discovered in glioma, brain, skeletal muscle and cardiac. In this studies, single channel activity of the mitoBKCa channel was measured with patch-clamp technique of the mitoplasts isolated from EA.hy 926 endothelial cell line. We have shown data describing regulation of the mitoBKCa channel by different cardioprotective flavonoids (luteolin, quercitin and cyanidin).

L-CANAVANINE INCREASES UNFOLDED PROTEIN RESPONSE UNDER ARGININE DEPRIVATION ON HUMAN GLIOBLASTOMA U251MG AND U87MG CELLS

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The endoplasmic reticulum (ER) plays a vital function that has to be strongly regulated to carry out the proper cellular processes. Nowadays, there is a big interest in the study of ER-stress as an attractive target for anti-cancer therapy. Also, arginine starvation is being increasingly recognized as a promising approach for controlling malignant tumor growth. One of the drugs that could be potentially used in combination with arginine deprivation is L-canavanine, an arginine analogue of plant origin. It has been shown that L-canavanine can effectively incorporate into proteins instead of L-arginine and this incorporation causes disturbances in the normal function of the ER, which may induce the unfolded protein response (UPR). We analyzed the effects of canavanine on the ER of human U251MG and U87MG glioblastoma cells. Staining with the ER-specific dye, ER Tracker Blue/White DPX, revealed an increase in the fluorescence intensity under arginine deprivation as well as formation of the ER Tracker Blue/White positive vesicular structures in the ER after 48 hours of treatment. The more, combination treatment with L-canavanine resulted in disintegration of the ER. We also observed that the treatment caused accumulation of the marker of ER-stress, glucose-regulated protein (GRP78), which is indicative of an increase in the level of misfolded proteins within the ER lumen. We showed that under arginine starvation L-canavanine could lead to inhibition of protein synthesis as well. Moreover, we noticed pro-apoptotic response as an increase in the level of the transcription factor C/EBP homologous protein (CHOP) and in the phosphorylation of SAPK/JNK kinase was observed. Thus our findings show that human glioblastoma cells are more sensitive to L-canavanine under arginine starvation as it promoted more significant changes in the UPR. These observations indicate that this combinational treatment might be potentially used for the development of an effective anticancer therapy. This work was supported by European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No.665735 granted to the Nencki Institute.

Neurodevelopmental and behavioral abnormalities in Zebrafish model of Tuberous Sclerosis Complex

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Tuberous Sclerosis Complex (TSC) is an autosomal dominant genetic disorder, caused by mutations inactivating genes for proteins hamartin (TSC1) and tuberin (TSC2) and subsequent overactivation of the mTORC1. TSC manifests itself by the presence of benign tumours (hamartomas) in various organs (ex. brain, retina, heart, skin), although the neurological symptoms are the most influential in mortality and morbidity of this disease. In many cases, clinical manifestations of TSC include epilepsy, cognitive deficits and autism spectrum disorders (ASD). We use tscvu242 Zebrafish mutant line [1] in which truncating mutation in tsc2 gene leads to lack of Tsc2 protein. It results in highly elevated mTORC1 activity as seen by phosphorylation levels of S6 protein in tsc2-/-. Live imaging of tsc2 mutants showed more frequent, spontaneous embryonic coiling, which may be in connection with epileptic

symptoms or improper muscle contractions. Behavioural tests performed on tsc2vu242 indicate decreased motor function, despite confirmed ability to see and move. Other tests point to increased anxiety levels, which can be intermediate cause of general movements problems. Some of pathological features, observed in tsc2 mutants, can be partially reverted/rescued by rapamycin (inhibitor of mTORC1) treatment. Results based on brain commissures development and GABAergic neurons location in tsc2-/- suggest problems with axon elongation and neuronal migration. Along with gene expression analysis for GABAergic signalization pathway, these results may suggest general issues with brain inhibitory transmission. Imbalances between excitation and inhibition in neurons have been already implicated in autism spectrum disorders and epilepsy in humans. Therefore, presented observations are a good basis for further investigation of TSC disease in zebrafish. [1] Kim, Seok-Hyung, et al. "Zebrafish model of tuberous sclerosis complex reveals cell-autonomous and non-cell-autonomous functions of mutant tuberin." Disease Models and Mechanisms 4.2 (2011): 255-267. This work was supported by a SONATA grant no.2015/17/D/NZ3/03735 from National Science Centre.

MICROTUBULE-PAIR SLIDING CARRIED OUT BY KINESIN-1

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Kinesin-1 is a common motor protein present in many cell types, including neurons. It transports cargoes along microtubules (MT) by walking using its N-terminal domains, called heads. The cargo is attached to the kinesin tail, located at the C-terminus. Also, a second MT can serve as a cargo for kinesin-1 as a result of weak electrostatic interaction between positively charged kinesin C-terminal tail and negatively charged tubulin C-terminal tail. This phenomenon is called MT-MT sliding or MT pair sliding. For kinesin-1 it was observed recently in growing Drosophila neurons. Observations in cells allow to characterize a function of sliding but not to describe its mechanism or basic parameters. MT-pair sliding is quite difficult to recreate beyond the cells, using only proteins but our lab developed a reproducible assay to observe MT-pair sliding driven by kinesin-1 in vitro. Using this assay we measured basic parameters for MT pair sliding: – the velocity of the transport, the run length (the distance that the transport MT passed via kinesin); the influence of kinesin-1 concentration on the sliding, tubulin posttranslational modifications, ionic strength or kinesin-1 flexibility on MT-pair sliding. While performing the experiments we observed many unexpected properties of sliding that brought us closer to understanding this phenomenon that is necessary for normal development of nervous system. For example, it turned out that kinesin-1 is able to move both antiparallel and parallel MTs in contrast to another motor proteins that transport only antiparallel MTs. In my speed talk, I will briefly characterize MT-pair sliding, and then focus on orientation of MTs during sliding and also on kinesin-1 concentration influence.

Hamamelis virginiana and Salix alba aqueous extracts prevent the action of Shiga-like enterotoxins

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Shiga and Shiga-like toxins (STXs) belong to a family of AB5 enterotoxins actively secreted by two bacterial species, Shigella dysenteriae and Escherichia coli, respectively. The structures of these toxins are similar. They consist of toxic subunit A and pentameric, non-toxic subunit B responsible for binding to glycosphingolipids. STXs recognize and bind to glycosphingolipid Gb3 and Gb4 at the plasma membrane of host cells. Outbreaks of S. dysenteriae occur mostly in overcrowded areas in the third world countries with poor sewerage systems and lack of clean, uncontaminated water. In developed regions, the largest threat are E. coli species producing Shiga-like toxin (STEC). The recent outbreak was in Germany in 2011, where 3,950 people were infected and 48 died. Antibiotic treatment during STEC infection should be restricted. The bactericidal activity of antibiotics results in the immediate release of Shiga-like toxin by dying bacteria, which can lead to hemolytic-uremic syndrome and the death of the infected person. The possible solution to this problem is to find a treatment that can kill bacteria and, at the same time, inactivate the released toxins. To follow this direction we were employing plants extracts traditionally used to treat diarrhea. We analyzed the antimicrobial activity of selected plant extracts. We focused on the ability of plant extracts and their secondary metabolites, mainly polyphenols, to block toxin endocytosis in host cells. We investigated if the interaction between STX and plant extracts can stop the toxins from binding to immobilized Gb3 and receptors located on Vero cell plasma membranes. Our results allowed us to identify several plant species, including Salix alba and Hamamelis virginiana, which aqueous extracts can simultaneously inhibit bacteria growth and toxin binding to glycosphingolipids. We suggest that likely mechanism of inhibition the toxin binding to its receptor is related to the ability of plant polyphenols to induce toxins aggregates. We hope this knowledge will be useful to create new tools in fighting diarrheal diseases caused by STEC infection. This work was supported by grant no. 2016/23/N/NZ1/02449 from NCN, Poland and by NCBR, POWER 3.2. Work implemented as a part of Operational Project Knowledge Education Development 2014-2020 co-financed by ESF.

Role of central amygdala circuits in responding to social cues about danger

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To avoid danger, animals need to regulate exploration based on emotional signals emitted by conspecifics. In order to study this process, we used two experimental paradigms in which rats interact with a fear conditioned partner. In the first one (imminent danger) an 'observer' watched his cagemate (‘demonstrator’) undergoing contextual fear conditioning. In the second paradigm (remote danger) the observer could freely interact with a recently conditioned demonstrator in the safe environment of a home cage. Both paradigms elicit robust but different reactions of observer rats - freezing and rearing, respectively. They are also

accompanied by different patterns of ultrasonic vocalizations (centered around 22 vs. 50 kHz). As central amygdala (CeA) is crucial for single subject defensive behaviors, we hypothesised that it also plays role in regulating reactions to social signals about threats. To verify this, we used viral vectors in which channelorhodopsin (ChR2) or halorhodosin (NpHR) sequence was linked to c-fos promoter. This approach allowed us to selectively manipulate the subpopulations of CeA cells which were activated by either type of social interaction. Twenty four hours after the behavioral paradigm, observer rats received light stimulation either in the open field or during a free interaction with the cagemate. The results indicate that the population of CeA cells activated by imminent danger paradigm controls passive defensive reactions – such as hiding in dark areas and reduced exploration - whereas the one activated by remote danger has an opposite role, promoting active reactions including rearing and escape from the light. Suprisingly, stimulating none of the populations had direct effect on the observer social behaviors, suggesting that the main function of the studied circuits is regulation of exploratory strategy.

Visual response enhancement in the rat visual system following sensory training

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One fundamental approach to induce plastic changes in the visual system is a behavioral stimulation through the repetitive sensory experience. In animal studies repeated exposure for a few days to specific visual stimuli (visual training) can modify neuronal network to improve the perception of these stimuli at the level of the primary visual cortex (VCx; Sawtell et al.,2003; Frenkel et al., 2006). In our study, we found that the shorter visual training, limited to several hours (3 hours) also induced strong enhancement of the magnitude of visual evoked potential (VEP) amplitudes not only at the cortical level but also at the subcortical level. There is still no sufficient explanation related to reinforcement of visual response after visual training at the subcortical level in the superior colliculus (SC). We considered two hypotheses: it might be a result of enhancement of the input synapse from retina to SC or the reinforcement of response in this midbrain structure may result from enhanced cortical input to the SC. To explain this issue in this study we performed visual training with VCx blocked since the beginning of training. In order to block the activity of the cortex during visual training, a well above the VCx was fulfilled with xylocaine solution (2.5%). Monocular visual stimulation consisted of a series of 300 repetitions of light flashes separated by 2 s interval (0.5 Hz)) presented every 15 minutes through 3 hours. To investigate the effect of visual training, before and after visual training control recordings were carried out (100 repetitions of flash stimuli at 0.1 Hz). VEPs were recorded from the contralateral to the stimulated eye VCx and the SC. Comparison of the VEPs amplitude both in the VCx and SC indicated that repeatable visual stimulation significantly enhanced the magnitude of visual responses. Chemical inactivation resulted in strong attenuation of cortical VEP amplitudes, no significant difference between the magnitude of VCx responses in both control recordings was found. In the case of SC we observed an increase of VEP amplitudes during visual training with cortical inactivation, simultaneously the comparison of both controls showed enhancement of response after visual training.. Obtained results prompted us to hypothesize that the increase of responses in the SC is most likely due to the enhancement of the retino-tectal projection. "
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Identification of signaling pathways activated by the addition of PDE10A inhibitor in the striatal neurons in vitro.

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Phosphodiestrase 10a (PDE10a) is a dual-specific enzyme that can hydrolyze both Cyclic Adenosine-3 ', 5'-monophosphate and Cyclic guanosine-3 ', 5'-monophosphate . PDE10 regulates striatal outputs by its effect on both this (cAMP and cGMP) pathways, that results in changes in phosphorylation of a variety of intracellular proteins. It is predominantly expressed in striatal medium spiny neurons at high levels. Activity of PDE10a modulates both corticostriatal and nigrostriatal transmissions. Disruption of these pathways leads to aberrant neuronal activity in the cerebral cortex. As striatal dysfunction is implicated in the pathophysiology of various CNS diseases, including schizophrenia, Huntington's disease, Parkinson's disease, addiction, and obsessive-compulsive disorder, PDE10 is seen as a promising target of therapeutic intervention in diseases with striatal hypofunction. In our study we aim at testing whether pharmacological inhibition of these phosphodiesterases can potentially treat psychotic disorders. In the present study we analyzed signaling pathway modulated by CPL-500-036-02, a novel PDE10a inhibitor. We used three weeks old, primary striatal rat cultures. Neurons treated with the were analyzed by Western Blot. PDE10a inhibition led to increased phosphorylation of proteins like AMPA receptor subunit GluR1 and extracellular signal–regulated kinases, ERK1 and 2. We observed increased phosphorylation of these proteins, suggesting activation of the cAMP pathway. Since both D1 and D2 neurons express high level of PDE10A, we used striatal cultures from transgenic mice (drd1a-tdTomato and drd2-GFP) to compare the effects of PDE10A inhibition in both type of striatal neurons. Using immunofluorescence staining we monitored the level of histone 3 phosphorylation in response to stimulation. Our preliminary results show that after PDE10A inhibition higher level of histone 3 phosphorylation occurred in D2 neurons. The results indicate that administration of PDE10A inhibitor increased phosphorylation of proteins by affecting cAMP signaling pathways in D1 and D2 neurons.

Shape defined by motion discrimination task allows to delineate peripheral from central visual processing.

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After central retinal loss in macular degeneration (MD), the description of perceptual deficits is limited to the central vision attributes (e.g. acuity). However, surprisingly in MD the peripheral motion processing is reinforced (Burnat et al., 2017). We propose new visual testing approach, enabling measuring motion and shape perception simultaneously. We report in control subject's psychophysical measurements performed in full visual field as opposed to restricted to 10 deg viewing condition and in three patients with Retinitis Pigmentosa (RP) and two patients with Stargardt Disease. 5 controls, 3 RP patients, and 2 STGD patients (age range 27-50, both sexes) were examined. The positive stimulus, S+ was a circle and the negative stimulus, S- was an ellipse surface matched with S+. The difficulty of the task depended on a staircase procedure in which the aspect ratio of the ellipse's dimensions, depending on the subject's response, could vary from 0,2 to nearly 1. The initial level of the

difficulty was determined using plain grey S+/- on the bright background (Bcg). In shape from motion tasks, S+/- were built from the random dot kinematograms (RDKs) placed on the Bcg differing from the S+/- in one of the motion cues: coherence (S+/- 0%, Bcg 100%), direction (S+/- upward, Bcg horizontal leftward) and velocity (S+/- 10 deg/s, Bcg 20 deg/s). Two luminance sets of RDKs were tested: black dots on white background or reverse. Discrimination of motion defined shapes independently of motion cue was more difficult when motion signal was carried by black dots, as compared to white. Coherence cue was the easiest for all subjects. The velocity cue was the most difficult, and differentiated in control subjects central 10 deg viewing condition from the full view. At the full viewing condition, black motion signal was significantly more difficult as compared to white motion, as shown by the thresholds calculated for the difference between ellipse height and circle diameter (for black: 1,7 – 0,22 deg, white: 0,32 – 0,09 deg). We could differentiate RP from control subjects only with dark motion signal at 10 deg. visual condition.Peripheral motion stimulation by high contrast dark signal has strong influence on central processing. Tasks measuring simultaneously central and peripheral vision allow full assessment of vision loss. We are grateful for providing contacts to patients to prof. dr hab. n. med. J.Szaflik and dr T. Gałecki from Ophthalmological Clinic (SPKSO)

Plasma membrane transporter SLC6A14 is controlled by cytosolic heat shock proteins

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SLC6A14 is a member of solute carrier (SLC) family 6 of plasma membrane transporters specific towards amino acids, neurotransmitters, and osmolytes. SLC6A14 transports all neutral and basic amino acids in a Na/Cl – dependent way and it is overexpressed in many types of cancer. Both N- and C-termini of SLC6A14 are localized on the cytosolic side. Our analysis of SL6A14 interactome by mass spectrometry revealed, among others, the presence of cytosolic heat shock proteins (HSPs) and co-chaperones. We studied interaction of SLC6A14 with HSP90beta and HSP70 (HSPA14), identified as possible transporter partners. Immunofluorescence experiments demonstrated the strongest co localization of both HSPs with overexpressed rat SLC6A14 in transiently transfected HEK293 cells after 24 h. The direct interaction between HSPs and SLC6A14 was confirmed using the proximity ligation assay. Interaction of the transporter with HSP90beta was inhibited by radicicol, known to bind to HSP90 ATP-binding site, while interaction with HSPA14 was attenuated by its inhibitor - VER155008. Cell surface proteins biotinylation demonstrated a dramatic decrease of SLC6A14 presence in the plasma membrane upon treatment with either radicicol or VER155008, what resulted from the diminished level of the total transporter protein. Distortion of SLC6A14 proper folding by both HSPs inhibitors directed the transporter towards endoplasmic reticulum associated degradation, a process reversed by the proteasome inhibitor – bortezomib. These results indicate that a plasma membrane protein folding can be controlled not only by chaperones in the endoplasmic reticulum, but also those localized in the cytosol. Moreover, these observations may have a potential therapeutic significance, since the use of HSPs inhibitors could decrease amino acid supply to quickly proliferating cancer cells with a high expression of SLC6A14. This study was financed by a grant 2015/19/B/NZ3/00049 from the National Science Centre in Poland.

MSIT+ (Modified Multi-Source Interference Task) for fMRI Environment - preliminary results

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The MSIT is a validated tool for strong ACC activation, though it had several limitations, such as being inherently block-design. It is also of interest to possibly obtain gradual activity of ACC in different condition. For that reason we modified MSIT with additional uni-source interference effects (Simon and Flanker) and restructured the scheme to mixed-design. Current study aimed to investigate whether modified MSIT+ task successfully activated ACC in gradual manner. During the fMRI session participants performed modified MSIT+. The MSIT+ consisted of four conditions: no interference; spatial interference only flanker interference only; multi-source interference. The examination took 22 minutes with 11 minutes for each of two identical runs. GLM analysis and contrasts was performed between the: a) Simon effect; b) Flanker effect; c) MS effect. Preliminary results of fMRI examination show strong and reliable activation of ACC with MS effect contrast (Flanker and Simon positive vs Flanker and Simon negative) ($t=6.5$, FWD corr. $p<0.05$) and weaker, yet present, activation of ACC with Flanker effect ($t=5.3$,FWD corr. $p<0.05$) and less so Simon effect ($t=3.9$,$p<0.0001$). In summary, obtained results point out that our modified MSIT+ task is a successful adaptation of a tool for reliable ACC activation. Further examination will explore the gradual activation of ACC in different interference source. [Supported by National Science Centre, Poland, grant UMO-2016/20/W/ZN4/00354]

Formation of RNA binding protein complexes regulates BRCA1 mRNA translation in chronic myeloid leukemia cells.

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Chronic myeloid leukemia (CML) is characterized by the presence of BCR-ABL oncoprotein, which demonstrates constitutive tyrosine kinase activity and plays a major role in the development and progression of the disease. We have previously shown that increase in BCR ABL activity leads to downregulation of BRCA1 tumor suppressor protein resulting in affected chromosome segregation and increased aneuploidy. However, precise mechanism of altered BRCA1 expression in CML cells and the role of BCR-ABL in this process remain unknown. There are various reasons for BRCA1 deficiency in cancer cells, including mutations in BRCA1 gene in case of breast and ovarian cancers, as well as hypermethylation of BRCA1 promoter region or microRNAs activity. Here we show, that despite high level of BRCA1 mRNA in CML cells, BRCA1 protein level is decreased due to inhibited translation and lower protein synthesis caused by formation of RNA binding protein (RBPs) complexes. Mass spectrometry and co-immunoprecipitation analyses have revealed that those multiprotein complexes consist of TIAR, HuR, FMRP and other RBPs, known to aggregate into cytoplasmic structures called stress granules. Formation of stress granules in BCR-ABL expressing cells was confirmed using TIAR immunostaining. We have also found out that BCR-ABL activity promotes cytosolic localization of TIAR and HuR and increases FMRP protein level. IP-RT-qPCR has shown elevated BRCA1 mRNA association with TIAR and HuR proteins. Silencing and overexpression studies have confirmed that all found RBPs forming BRCA1 influencing complexes play a pivotal role in the regulation of BRCA1 expression. Altogether, we have shown a novel mechanism affecting BRCA1-dependent

signaling in CML, in which BCR-ABL expressing cells modulate translation of BRCA1 mRNA leading to protein downregulation.

Leukemic extracellular vesicles as new modulators of Foxp3+ regulatory T cell function in chronic myeloid leukemia

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Regulatory T cells (Treg) constitute a significant immunosuppressive factor in chronic myeloid leukemia (CML), as they participate in inhibition of effector immune response against leukemic cells. Immune cells can be regulated by extracellular vesicles (EVs) secreted by cancer cells, as demonstrated in solid tumors. Leukemic EVs have been shown to influence stromal and endothelial cells in the bone marrow niche, but their immunomodulatory role has not been explored. To study role of leukemic EVs in differentiation and function of thymic Treg (tTreg), we used ex vivo and in vitro cellular models using cells from C57BL/6 and B6.Cg-Foxp3tm2Tch (co-expressing Foxp3 and EGFP) mice. For analysis thereof we used multicolor flow cytometric analysis of cell properties like: phenotype, proliferation, viability and level of intracellular proteins (Foxp3). EVs released by murine 32D BCR-ABL+ cells were isolated from the conditioned medium by differential ultracentrifugation and characterized by transmission electron microscopy and nanoparticle tracking analysis (NTA). Expression of EV markers was verified by western blotting and EVs association with thymocytes analyzed using flow cytometric tracking of fluorescently labelled EVs. Mature, sorted tTreg exposed to CML derived EVs exhibit higher suppressive activity. They also express significantly higher level of Foxp3 transcription factor, suggesting global regulation of tTreg function, rather than of single molecules responsible for suppressive activity. We also show that even though CML-derived EVs do not increase tTreg differentiation ex vivo, naïve thymocytes exposed to EVs during ex vivo culture and eventually differentiated into Tregs demonstrate higher level of Foxp3. Collectively, our data show contribution of leukemic extracellular vesicles to suppressive activity of both differentiating and mature thymic regulatory T cells, thus suggesting a novel immunosuppressive mechanism in chronic myeloid leukemia. This work was supported by National Science Center grant 2013/10/E/NZ3/00673 to K. Piwocka

Novel central pair proteins in Tetrahymena thermophila

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Central pair (CP) complex is essential for proper cilia beating. Early analyses showed that CP is composed of at least 25 proteins. To better understand the role of CP in cilia beating generation and regulation we attempted to identify new CP proteins. We expressed Spef2p (subunit of C1b projection) tagged with biotin ligase BirA* in Tetrahymena cells in order to identify proteins located in close proximity (up to 10 nm) to Spef2. Among biotinylated proteins we found three as-yet uncharacterized evolutionarily conserved proteins (Tt400p, Tt650p, Tt850p) and one ciliate specific protein (Tt170p). Those proteins and Spef2 were also biotinylated if BirA* was fused to either Tt400p or Tt650p, or Tt850p. Lack of either of these proteins (gene knock-out) reduced cells motility and changed cilia beating. Normal swimming

rate was restored if wild-type copy of the gene was introduced (rescue). TEM analyses showed that at least one large projection is missing in Tt650-KO and Tt850-KO cells. Expression of V5-tagged Spef2 and new CP proteins in their native loci in wild-type, Tt650-KO and Tt400-KO cells revealed significant reduction of the level of those proteins in mutant cilia. Thus, likely Tt400p, Tt650p, Tt850p and Tt170p are subunits of the C1b or neighboring projections. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 665735 (Bio4Med) and from Polish Ministry of Science and Higher Education within 2016-2020 funds for the implementation of international projects (agreement no 3548/H2020/COFUND/2016/2).

Plasma membrane proteins are differentially expressed in iPSC-derived human neural stem cells with TSC1 knockdown

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Objectives During brain development, the emerging complex neural network is a result of precisely regulated processes of neurogenesis, which involves neural stem cells (NSCs) proliferation and migration. Many proteins that regulate NSCs proliferation and migration localize on the cell surface in order to allow communications with adjacent cells or responses to environmental cues. The presence of these proteins at the cell surface is partly determined by endocytosis that controls their internalization. The abnormalities in these processes may lead to brain lesions and subsequent neurological symptoms observed in tuberous sclerosis complex (TSC). Thus, the aims of the study is to investigate if disturbed endocytosis of proteins crucial for proper neural stem cell migration and proliferation contributes to brain lesions development in TSC. Methods The mass spectrometry, biotinylation assay and Western blot analysis were used to identify proteins differentially expressed at the plasma membrane of iPSC-derived human neural stem cells with TSC1 knockdown. The transferrin uptake assay was used to determine the level of endocytosis in TSC1 knockdown cells. Results The obtained data pointed out the differences in the presence of proteins at the plasma membrane between control cells and cells with TSC1 knockdown. These proteins are crucial for central nervous system development that encompass such processes as: adhesion, migration, proliferation, growth, cell shape, differentiation, extracellular matrix (ECM) organization, cell-cell contacts, apoptosis, axon guidance, neurite outgrowth, receptor trafficking, cytoskeleton organization. Moreover, the results showed that endocytosis process is significantly disrupted in TSC1 knockdown cells. Conclusions Taking together, the significant changes were observed at the level of endocytosis and plasma membrane proteins of TSC1 knockdown cells and control cells. Both are crucial for proper central nervous system development and may contribute to brain lesions development in TSC. This work has been financed by National Research Centre grant no. 2016/23/N/NZ3/00108

Three dimensional (3D) visualization of immediate-early genes (IEGs) structure upon neuronal activation

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It is well established that three dimensional changes of chromatin organization play a crucial role in biological processes. So far it has been well documented in dividing cells. Recently, there have also been studies showing the meaning of this organization in terminally differentiated cells, such as neurons. Importantly, chromatin rearrangement contributes to the gene activity regulation like immediate transcription in response to neuronal stimuli. This rapid activation and transcription is provided by Immediate-Early Genes (IEGs), to which c-Fos and Bdnf belong. The results obtained in our laboratory showed that activation of Bdnf gene is associated with its detachment from nuclear lamina toward the nucleus center, where Bdnf colocalizes with transcriptional factory (Walczak, 2013, J.Neurosci.). A gene relocation is hypothesized as a process of chromatin looping. Presumably, chromatin rearrangement connected with gene expression is a time consuming process, while some of IEGs response rapidly. Hence the idea to divide these genes based on a reaction time. Rapid IEGs (e.g. c-Fos) which are transcribed within minutes upon stimulation are associated with RNA Polymerase II stalling in the proximity of their promoters (Saha and Dudek, 2013, Neuroscience). This suggests that they do not need to be repositioned to be activated, in contrast to Delayed IEGs (e.g. Bdnf), which products are not detected until later in the hour. For studying this phenomenon I used a novel method developed by us – 3D-EM-ISH. The technique combines three dimensional electron microscopy (3D-EM) and DNA in situ hybridization (ISH) and reaches down to 5 nm lateral resolution. The studies were performed on the rat model of very strong pharmacological neuronal stimulation leading to seizures. My preliminary data suggest that not only the structure of Bdnf and its neighborhood is reorganized upon stimulation, but surprisingly also the vicinity of rapid IEG - c-Fos is significantly remodeled. Taken together, activation of IEGs is a very complex and still not completely understood process. However, novel superresolution morphological studies combined with biochemical ones, bring us closer to understanding the basic principles of neuron-specific gene expression. Acknowledgements: The studies were supported by the Polish National Science Centre grant (2014/15/N/NZ2/00379) and international HFSP grant (RGP0039/2017).

Optimization of potassium channel ROMK1/2 expression and purification

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ROMK2, a splice variant of the KCNJ1 gene product, has been identified as a key subunit of the mitochondrial mitoKATP channel. ROMK2 is shorter by N-terminal 19 amino acids than ROMK1 - a splice variant located in the plasma membrane. It is known that ROMK1/2 proteins form homotetrameric channels. Each of their subunits is characterized by the cytoplasmic domain composed of the short N- and the long C-terminus and by the core region of two transmembrane helices flanking the pore-forming segment. To obtain purified ROMK1/2 channels for biochemical and biophysical studies, we decided to express the ROMK1/2 protein in Escherichia coli cells. In the first step, the codon-optimized ORFs encoding for ROMK1, or chimera between cytoplasmic N- and C-termini of ROMK1, and the transmembrane part of ROMK1 bacterial homolog KirBac1.3 were fused to sequences of several expression tags. These tags, such as SUMO, pOmpF, and MISTIC, could assist in

membrane insertion and folding of eukaryotic proteins in bacteria. Fusion proteins contained an affinity tag for purification (C- and N- terminal 6-His tag). In the next step, the best performing construct, ROMK1 with C-terminal 6xHis tag, was chosen for further studies. This construct exhibited the highest membrane expression level and low degradation. Next, we screened several detergents and the best one (n-Dodecyl β-D-maltoside – DDM) was used for ROMK1 protein purification. A large amount of E. coli expressed ROMK1/2 protein gives the opportunity to reconstitute this channel in membranes and membrane nanodiscs for functional studies. This work was supported by the Polish National Science Center, grant no. 2015/19/B/NZ1/02794.

Is RECQL4 a novel player in glioblastoma pathogenesis?

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Introduction Glioblastoma (GBM), the common and aggressive human primary brain tumor (WHO grade IV), is highly resistant to standard radio- and chemotherapy. This is partly due to numerous genetic alterations in oncogenes and DNA damage repair systems. Despite progress in understanding the molecular background of GBM and advances in treatment modalities, survival of GMB patients is only 14 months post-diagnosis. RECQL4 belongs to RecQ family of ATP-dependent DNA helicases and plays an important role in genomic integrity and stability maintenance via involvement in DNA replication, repair, recombination, transcription and telomere maintenance. Mutations in the human RecQ genes are linked with cancer predisposition and/or premature aging. Of all five human helicases, only RECQL4 is found in mitochondria. We explored if alterations of RECQL4 expression or functions contribute to pathogenesis of human GBM. Materials and methods We determined the RECQL4 expression in various tumor specimens (tumor samples, human primary and established glioma cell cultures) by qPCR and Western Blotting. We determined the effect of RECQL4 depletion on cell viability, proliferation and GBM sphere formation using MTT metabolism, BrdU incorporation and tumor sphere forming assays, respectively. Results We found the upregulated expression of RECQL4 in GBM at mRNA and protein levels when compared to non-transformed human astrocytes. This finding was corroborated by TCGA data analysis. Fractionation of mitochondrial and cytosolic fractions from human glioma cells revealed the presence of RECQL4 in mitochondria. Downregulation (by siRNA) or genetic depletion of RECQL4 (by CRISPRCas9 knockout) in human glioma LN18 and U87-MG cells impaired cell viability and proliferation. We found upregulation of RECQL4 expression in GBM sphere cultures, enriched in glioma stem cells. Transient knock-down of RECQL4 significantly affected tumor sphere formation as evidenced by decreased numbers and sizes of cultured spheres. Conclusions These data indicate that deregulation of RECQL4 expression or function may play an important role in GBM pathobiology. Our results provide a rationale for further studies of RECQL4 role in gliomagenesis. Supported by National Science Centre grants 2015/19/N/NZ3/02374 (SKK) and 2016/22/M/NZ3/00679 (BK) and the Foundation for Polish Science TEAM-TECH Core Facility project „NGS platform for comprehensive diagnostics and personalized therapy in neuro-oncology” (AK).

DYNAMICS OF HP1β, RFC AND PCNA IN S-PHASE HELA CELLS

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Heterochromatin protein 1β(HP1β) is a protein involved in regulation of chromatin structure by forming and sustaining heterochromatin and translocating heterochromatin to regions near nuclear envelope. Recently it has been shown that HP1β interacts with Proliferating Cell Nuclear Antigen(PCNA), which is a protein involved in DNA replication – it forms a clamp around DNA strand that acts as a scaffold on which DNA replication machinery is assembled. We are also examining Replication Factor C(RFC), which is a complex responsible for loading of PCNA trimer onto the DNA strand. Our data analysis allowed for characterisation of stechiometry of HP1β – PCNA complex as well as size evaluation of subpopulations of those proteins directly engaged in complex. We also assessed in this way subpopulations of RFC and RFC deprived of the ability to interact with DNA

BK-DEC splice variant forms a functional BKCa channel in the inner mitochondrial membrane

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Ischemia of brain or heart tissue is the one of the most common causes of death worldwide. In the inner mitochondrial membrane several potassium channels have been identified whose activation lead to cytoprotection during ischemic event. It was found that activation of mitochondrial large conductance calcium activated potassium channel (mitoBKCa) preserves brain and heart muscle cells. Recently, the molecular identity of the mitoBKCa channel was described. A BK-DEC splice variant of BKCa-type channels α subunit has been demonstrated to localize in mitochondria. However it is not known whether this isoform is able to form a functional channel in mitochondria. In our study we used HEK293T cells transfected with cDNA encoding BK-DEC splice variant. Electrophysiological recordings with use of mitoplast isolated from transfected cells revealed presence of the large conductance and voltage dependent ion channel. This type of channel was not present in mitoplasts isolated from untransfected cells. We found that recorded channel showed all basic pharmacological properties typical for the mitoBKCa channels described previously. The channel was Ca2+ sensitive, its activity was stimulated by potassium channel opener NS1619 and inhibited by paxilline, well known mitoBKCa channel inhibitor. Additionally, kinetics and conductance of observed channel were very similar to the mitoBKCa channel. Based on collected data we conclude that BK-DEC splice variant forms a functional channel in the inner mitochondrial membrane of HEK293T cells. This work was supported by the Polish National Science Centre grant No.2015/18/E/NZ1/00737 and the Nencki Institute of Experimental Biology.

Characterization of intracellular trafficking and signaling of lymphotoxin β receptor (LTβR)

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Tumor Necrosis Factor Receptor Superfamily (TNFRS) members are responsible for maintenance of the immune system homeostasis. One of them, lymphotoxin β receptor (LTβR) mediates inflammatory responses in various cell types. Binding of specific ligands: lymphotoxin (LT) α1β2 and LIGHT, leads to activation of the NF-κB pathway. There are two branches of the NF-κB pathway: canonical and non-canonical, employing RelA/p50 and RelB/p52 transcription factors, respectively. It was proposed that upon binding of a ligand, LTβR is internalized into endocytic vesicles, leading to activation of the non-canonical branch of the NF-κB pathway1. Moreover, it was demonstrated that under some conditions accumulation of the receptor on endosomes results in activation of NF-κB signaling, in ligand-independent manner2. However, it is still unclear by which routes LTβR is internalized into the cell upon the stimulation with a ligand, and how it is trafficked inside the cell. Furthermore, there is still no clear data on how trafficking of the ligand-bound receptor might affect NF-κB signaling. We characterize the time course of ligand-dependent LTβR internalization and NF-κB signaling activation. We also show that these processes are clathrin-, dynamin- and caveolae-dependent, and that depletion of clathrin or dynamins lead to enhanced expression of NF-κB target genes, while affecting the caveolae-dependent internalization leads to stronger activation of the NF-κB pathway at the protein level. We hypothesize that the impairment of internalization can lead to accumulation of the receptor at the plasma membrane, which results in over-activation of the NF-κB pathway upon ligand stimulation.

Functional hierarchy for tactile processing in the visual cortex of sighted adults

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Perception via different sensory modalities was traditionally thought to be supported by largely separate brain systems. However, growing number of studies show that the visual cortex in typical adults can be recruited for tactile and auditory perceptual task, crossing the boundaries between modalities. Here we investigated how tactile input is incorporated in the visual network using transcranial magnetic stimulation applied at various time windows (chronometric TMS). We taught Braille reading to sighted subjects and enrolled them in a tactile letter reading task. During reading, TMS was applied to their early visual cortex, visual word form area (VWFA) and early somatosensory cortex, at five time windows from 20 to 520 ms after Braille letter presentation. Subjects' response accuracy decreased when TMS was applied to the early visual cortex, at an intermediate time window (120-220 ms post-stimulus), and to the VWFA, at a late time window (320-420 ms post-stimulus). TMS applied to the early somatosensory cortex did not have a time-specific effect on the accuracy of Braille letter reading but caused a general slowdown in this task, which might reflect different types of computations occurring in somatosensory and visual cortices. This temporal double dissociation indicate that in a non-deprived functioning visual cortex, tactile stimuli can follow a canonical visual processing hierarchy, with lower-level features being processed in the early visual cortex and more complex computations occurring in the high-level visual

areas. Our findings are compatible with metamodal theory of brain organization and show that potentially the whole visual cortex might operate in a task-specific sensory-independent manner.

Insight into Drp1 mediated mitochondrial fission from FCS calibrated imaging

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Dynamin related protein 1 (Drp1) is involved in the process of mitochondrial fission, which is very important in maintaining cell homeostasis, as well as in apoptosis and cell division. Upon fission signals in the cell, Drp1 intermediate assemblies form oligomeric ring-like structure which wraps around mitochondria and then constricts thanks to the energy gained from GTP hydrolysis. This results in mitochondrial fragmentation. Precise state of Drp1 protein population in the cytosol is not yet known. Also the detailed understanding of the Drp1 dynamics in the fission complex is lacking. Fluorescence Correlation Spectroscopy (FCS) and length scale dependent viscosity model allow us to quantify the diffusion coefficient of Drp1 and the extent of its aggregation. This then can be translated into the size of Drp1 oligomer in the cytosol. Our results suggest that Drp1 likely exists in the cytosol in tetrameric form. What is more, thanks to CRISPR-Cas9 gene editing technique we have obtained a cell line with GFP sequence attached to Drp1 gene, what then let us to visualize Drp1 cellular distribution and dynamics at endogenous expression level. Moreover, observations from Spinning Disc microscope give us supplementary data concerning dynamics of Drp1 in the cytosol and on mitochondria during the fission event. These observations showed that Drp1 forms stable large oligomers which can move along mitochondria, but the Drp1 ring formation does not necessarily lead to mitochondrial fission. We also observed that in the case of successful mitochondrial fragmentation, just before the fission event the fluorescence intensity of GFP-Drp1 spot rapidly increases, what can be a trigger signal for this process. Thanks to FCS calibrated images we can quantify the amount of Drp1 molecules which take part in mitochondrial fission event at different stages of this process. Research was supported by The Polish National Science Centre grant (decision number DEC-2013/08/W/NZ1/00687)

The whole-genome search for regulatory mutations in laryngeal squamous carcinoma.

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Laryngeal squamous cell carcinoma (LSCC) is the second most prevalent malignancy of the upper aerodigestive tract in United States. Also in Europe the incidence is about 7 deaths per 100,000 people per year. However, the molecular mechanism contributing to this type of tumor is still unclear. The aim of this project is to identify potential mutations within cis-regulatory regions (regulome) of LSCC. Our definition of regulome is based on Ensembl

Regulatory Build filtered with three principles: possibility to correlate a feature with expression data – hence focus on promoters, mappability to genes relevant for head and neck cancer and 50 Mb size limit of the sequencing target. We assume that besides known alterations of cancer related genes, alterations of regulatory regions of these genes also occur. We assume also that to some extent these changes may contribute to the development of LSCC. To reach this goal Next Generation Sequencing of target sequence containing proximal promotors and enhancers will be performed for single nucleotide variant call. Also the expression of candidate genes will be moreover determined by microarray data. Finally, all results will be combined and compared to Nencki Genomic Database (NGD). This approach can shed a new light on the role of regulome in pathogenesis of LSCC.

Methyl-CpG binding domain 3 promoter activity in a rat model of seizure evoked by intraperitional injection of pentylenetetrazole (PTZ)

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Animal models for seizures and epilepsy have played a fundamental role in advancing our understanding of basic mechanisms underlying epileptogenesis and epilepsy. During epileptogenesis and epilepsy, several molecular and cellular changes occur, including alterations in gene and protein expression. MBD3 (Methyl-CpG binding domain 3) protein is a reader of DNA methylation marks which play important role in epileptogenesis. Our previous data (Bednarczyk et al., 2016) showed that MBD3 gene and MBD3 protein are expressed in the brain in neurons in both normal and epileptic rat brains. This project was conducted to test the hypotestis that there are changes in MBD3 protein expression after acute seizure in the rat brain. Animals were kept in enriched environment and were subjected to the handling procedure. Single interperitional injection of pentylenetetrazole (PTZ, 40mg/kg) was used to evoke tonic-clonic seizure. Control rats (n=16) which were injected by saline and rats after PTZ administration were observed for an hour after injection. To examine changes in protein expression animals were sacrificed in selected time points: 1h, 4h, 8h and 24h after injection. For RNA and protein analysis fresh frozen tissue was collected. Changes in MBD3 expression was verified using protein extracts from the hippocampal and entorhinal cortex sections. Protein extracts was tested using Western Blot with anti-MBD3 anibody (#A302-528A, Bethyl). Data was validated by ImageJ. Moreover, changes on RNA level was verified using Real Time PCR. No signifficant differences was observed in RNA level at hippocampal and entorhinal cortex structure during 24h after injection. Western Blot showed that pentylenetetrazole (PTZ) did not affect on MBD3 protein expression in the hippocampal and entorhinal cortex structure 4h, 8h and 24h after injection. This work is supported by the Polish Ministry of Science and Education grant G1401.

Activity-dependent trafficking of PSD-95 after cLTP and cLTD

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The ability of the nervous system to learn and form new memories, hence adapt, is believed to be based on activity dependent modifications of synaptic connections. These are accompanied by their morphological alterations. It is not yet known what exact molecular mechanisms underlie these morphological changes. PSD-95, a major scaffolding protein of the postsynaptic density (PSD) is involved in the regulation of LTP (long-term potentiation) and

LTD (long-term depression), two major forms of synaptic plasticity. Trafficking of PSD-95 out of dendritic spines is regulated by phosphorylation of serine 73 (S73) via αCaMKII. Here we investigated PSD-95 trafficking after chemically-induced LTP and LTD in vitro in the stratum radiatum of CA1 hippocampal neurons with immunofluorescence. The level of total PSD-95 decreased 30 min after LTD but not after LTP. Furthermore, using AAV transfection approach we have found that unphosphorylable PSD-95 S73A overexpression prevented elimination of PSD-95 after LTD and had no effect on protein level after LTP. These results indicate that activity-dependent removal of PSD-95 is a mechanism occurring during long-term depression but not potentiation and it depends on its phosphorylation at serine 73 via αCaMKII. Next we injected the AAV viruses into the CA1 of the hippocampus of C57/BL6 mice and performed contextual fear conditioning with an extinction session. Mice overexpressing PSD-95 S73A didn't extinct fear memory. These results prove the crucial role of PSD-95 removal and its regulation by αCaMKII phosphorylation in memory remodeling. Overall this study contributes novel finding towards better understanding of molecular mechanisms of memory.

Myosin VI in nucleolar structure and function

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Myosin VI (MVI) is a unique actin-based motor protein moving towards the minus end of actin filaments, in the opposite direction than other known myosins. This unique molecular motor is involved in a range of cellular processes including cell migration and adhesion, endocytosis, intracellular trafficking, cytokinesis and, as it was quite recently shown, in gene expression. We found that in neurosecretory PC12 cells MVI upon their stimulation translocates into the nucleus where it localizes to several nuclear compartments and interacts with numerous proteins involved in nuclear functions. Among the identified putative partners was nucleolin, a major nucleolar protein which is implicated in rDNA transcription, rRNA maturation, ribosome assembly and nucleo-cytoplasmic transport. Additionally, we showed that other nucleolar proteins required for ribosome biogenesis, namely UBF, RNA polymerase I and B23 also interacted with MVI. As revealed by the immunogold technique, MVI is present within all nucleolar compartments, also at the nucleoplasm-nucleoloplasm border, indicating its involvement in ribosome biogenesis and in shuttling between these nuclear compartments. We also showed that depletion of MVI caused disorganization of the nucleolus and endoplasmic reticulum (ER), however, it did not significantly affect nucleolar transcription. In light of these data, we postulate that MVI is important for the nucleolar and ER maintenance but its effect on Pol 1-related transcription seems to be negligible.

Correlation between the motility of glioma cell lines and morphological properties of the cell nuclei - possible role of nuclear Rac1

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Uncontrolled migration is a characteristic feature of malignant cancer cells, including glioblastomas – highly metastatic, deadly brain tumors. Cell migration depends on an interplay between cytoskeleton dynamics and cell adhesion. Among many mechanisms controlling cell motility the role of cell nucleus as a physical cell component recently emerged. The nucleus is the biggest organelle of the cell, much stiffer that the cytoplasm, thus its mechanical properties can largely influence the cell's ability to migrate in 2D (culture dish) and 3D (culture scaffold/tissue) environments. It is already known that rigid nuclei with highly compacted chromatin is preferred for optimal cell migration as it efficiently transfers the forces needed to move the whole cell. Also, the nucleus size along with the composition and structure of the nuclear lamina determine the ability of the cell to deform and squeeze through confined environments without damaging the genetic material. One of the regulators of the nuclear architecture is the nuclear pool of Rac1 protein (Ras-related C3 botulinum toxin substrate 1). Rac1 belongs to RhoA GTP-ases family. The protein acts at the plasma membrane where it regulates actin polymerization and cell adhesion. Interestingly, nuclear Rac1 accumulation was found in some cancer tissues and cells. Recent data confirmed that Rac1 may undergo translocation to the cell nucleus. Moreover, its forced nuclear accumulation results in fragmentation of nuclear lamina and irregular cell shape and both processes depend on actin polymerization. Here we show how nuclear morphology differs in glioblastoma multiforme cell lines with various migratory characteristics. Using time-lapse microscopy combined with cell tracking algorithms we characterized spontaneous 2D motility of the studied cell lines (path, displacement, persistence etc.). Next, with image analysis techniques we determined the morphological properties of the cell nuclei (size, shape, Haralick's texture features). The preliminary data also revealed low Rac1 nuclear : total ratio in highly motile cells. On this basis we speculate that the efficient migration of glioblastoma cells correlates with elongated shape, smaller size and uniform chromatin staining pattern of their nuclei and that these aspects of nuclear architecture might be influenced by the nuclear pool of Rac1 protein.

The impact of self-esteem on preferential processing of self-related information

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Preferential processing of self-related information is a well-documented phenomenon on both the behavioral and neural levels. However, the impact of self-esteem on this self-preference has not been studied in a systematic way. Here, the electrophysiological correlates of explicit self-reflection were investigated in individuals with low (LSE) and high self-esteem (HSE). Participants evaluated trait adjectives in reference to the self or to an "other" person (close-other, famous) while EEG was recorded. The analysis of event-related potentials focused on the late positive component (LPC), which exhibits a fronto-central distribution and latency over 500 ms. In both LSE and HSE groups, the amplitudes of LPC were enhanced in the self condition when compared to control conditions (both close-other and famous). Crucially, LPC amplitudes in the HSE group were significantly higher than in the LSE group. Moreover, the self-preference effect, defined as the difference between amplitudes of LPC associated with the evaluation of words in relation to oneself vs. other people, was significantly higher in the

HSE group than in the LSE group. Overall, our findings indicate that people with high self-esteem tend to engage in self-referential processing to a higher extent.

Brain under pressure – effects of speed-accuracy trade-off

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Performance depends on the time spent on stimulus processing. Higher accuracy is obtained at the cost of longer response times - the phenomenon known as speed-accuracy trade-off (SAT). The neural basis of SAT as well as the way in which it influences other cognitive functions is poorly understood. In our experiment we compared performance and neuronal activity in two groups of individuals: „fast" – subjected to SAT (imaging by EEG; n = 42 and fMRI; n = 31) and „slow" – with response postponed after stimulus offset, thus devoided of SAT (EEG n = 13; fMRI n = 9). In both groups alpha band power decreased more in trials requiring anticipatory attention than in the control ones. However, spatio-temporal characteristics of this effect were different in these two groups. In the fast group attention related decrease of alpha power was present virtually over all recording EEG sites and persisted over entire anticipation period. In the slow group, it was localized over parietal and occipital cortex and present only in early stage of anticipation (0.5-2.5 s). In both groups the difference diminished over time, gradually causing both types of trials more alike. Percent decrease of alpha power during anticipation compared to fixation period was related to performance in the forthcoming task exclusively in the fast group. The amplitude of P300 evoked by the target matrix was bigger in the slow group and only in this group it was positively related to performance. In both control and attention trials SAT was related to lower alpha power over frontal cortex. fMRI data confirmed activation of dorsal attention network (bilaterally FEF and IPS) during anticipation period. ROI analysis revealed that IPS was more activated in the slow than in the fast group during anticipation of attention demanding task (the difference was bigger for right hemisphere). Whole brain analysis showed that SAT was related to bigger activity in ACC and motor cortex during cue of the control task. The same regions (plus anterior insula) were more active in the slow group during control task execution in regard to withholding the response until target matrix offset. To conclude, SAT resulted in bigger activation of frontal control regions preceding task execution suggesting its involvement in the decision stage of stimulus processing. It was related to lower amplitude of P300 and lower activation of IPS during anticipation – showing its restraining effects on top-down attention.

Investigation on novel probe binding to glycogen in living cells.

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Glycogen is noted to be widely available reservoir of energy in living cells. Concerted action of several enzymes, consecutively, glycogenin (GYG1, 39 kDa), glycogen synthase (GYS1, 83.7kDa) and glycogen branching enzyme (GBE1, 80 kDa), is responsible for the multibranched structure of the granule. Our previous data based on Fluorescence Correlation Spectroscopy shown differences in the diffusion rates between those enzymes involved into the synthesis of glycogen. Moreover, GYS1 seems to be attached to something bigger and for longer time than GBE1, likely glycogen granule. What is more, mentioned proteins have different expression pattern after transfection which can be observed under the microscope. Question arises whether this expression pattern depends on the distribution of glycogen

particles? Thus a probe to visualize glycogen particles is needed. Unfortunately there are no specific solutions to visualize this glucose polymer in living cells. For this purpose, we established a probe lacking enzymatic activity per se which may be used to bind to glycogen granules in living cells. Established probe contains carbohydrate binding membrane 20 (CBM20), found in human starch-binding domain containing protein 1 (STBD1) tagged to fluorescent protein mNeptune. Investigations were performed on several living cell lines, including eHap and its genetically modified (CrispR/Cas9 method) derivatives: knockouts of GYS1 (KO GYS1) and knockouts of GBE1 (KO GBE1), patient-derived fibroblasts with glycogenosis IV and healthy, age-matched control fibroblasts and finally U-2OS cell line with stable expression of the glycogen probe. Presented data indicates that p-mNeptune-C1-CBM20 probe may be used to visualize glycogen particles. However, the glycogen granules seem to be concentrated in several single spots in the cell rather than distributed as many smaller particles throughout the cells Acknowledgments: This work was supported from the source of the National Science Centre according to the decision number DEC-2013/08/W/NZ1/00687

Identification of AXL interacting partners by proximity-dependent biotin identification (BioID)

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AXL is a receptor tyrosine kinase (RTK) which, together with TYRO3 and MER, belongs to the TAM receptor family. A ligand for AXL is growth arrest specific 6 (GAS6), which is able to bind all three TAMs with the highest affinity for AXL. Upon GAS6 binding, AXL is activated and phosphorylates its downstream effectors, such as ERK or AKT. AXL is overexpressed in a variety of different cancers, which correlates with poor prognosis, metastasis and drug resistance. However, the AXL biology is still not fully understood and data concerning proteins that interact with the receptor are almost completely lacking. In the present study, we used proximity-dependent biotin identification (BioID) to find proteins that interact with AXL. This method allows identification of weak and transient interactions but also proteins which are located in close proximity of the tested protein. We constructed cell lines stably expressing a mutated biotin ligase BirA-HA or an AXL-BirA-HA fusion protein, which were then used for the BioID assay. Experiments were performed using HEK293 cells, which do not express AXL and human glioblastoma cell line LN229, which has high endogenous level of this receptor. Using mass spectrometry analysis of the biotinylated proteins obtained from three independent experiments, we identified in total 88 and 124 proteins that interact with AXL in HEK293 and LN229 cells, respectively. Among them 26 were unique for GAS6-treated HEK293 and 40 for GAS6-stimulated LN229 cells. Majority of the identified proteins are implicated in intracellular trafficking, signal transduction, extracellular transport and are associated with the plasma membrane as well as cytoskeleton. The latter suggests that AXL plays a role in actin remodeling, which is consistent with our unpublished observation that GAS6-activated AXL triggers formation of circular dorsal ruffles, actin-rich structures involved in mesenchymal migration. Interestingly, more profound analysis of proteins implicated in intracellular trafficking indicates that AXL might be internalized via clathrin-mediated endocytosis and recycled back to the plasma membrane in a sorting nexin (SNX)- or RAB11-dependent manner. This study provides for the first time comprehensive data about proteins interacting with AXL and reveals novel determinants of AXL signaling and endosomal transport. Thus, the obtained results will help to understand the biology of the AXL receptor.

SPHINGOMYELIN AFFECTS TLR4 LEVEL AND LPS-INDUCED CYTOKINE PRODUCTION IN MACROPHAGES

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Objectives Sphingomyelin (SM) is a component of plasma membrane rafts, where activation of TLR4 by lipopolysaccharide (LPS) takes place, triggering MyD88- and TRIF-dependent pathways. SM is produced from ceramide by sphingomyelin synthase (SMS). Two SMS isoforms are identified: SMS1 resides in Golgi, SMS2 in the plasma membrane. The aim was to investigate role of sphingomyelin in TLR4 signaling. Methods To affect sphingomyelin level, cells were exposed to D609 (SMS inhibitor) and Sgms genes expression was silenced in J774 cells. TLR4 total and cell surface levels were analyzed by immunoblotting and FACS, respectively. NFκB and IRF-3 activity was assessed by immublolotting, TNF and RANTES production by ELISA tests. Results TLR4 cellular and cell surface levels underwent LPS-induced depletion. D609 reduced both total and cell surface levels of TLR4 in resting cells in a dose-dependent manner. The drug subsequently accelerated the disappearance of TLR4 in LPS-stimulated cells. This reduction was accompanied by inhibition of MyD88- and TRIF-dependent cytokine production, correlating with inhibited transcriptional activity of NFκB and IRF-3. D609 treatment strongly decreased SMS2 activity and moderately reduced total sphingomyelin level. This effect was augmented after prolonged incubation of cells with the drug. As no parallel accumulation of ceramide, sphingomyelin precursor, was observed the data suggests that sphingomyelin level affects TLR4 amounts. To verify this, Sgms1 or Sgms2 expression was silenced. Unexpectedly Sgms2 depletion up-regulated TLR4 total and cell surface levels. In these conditions, enhancement of IRF3 activity and TRIF-dependent RANTES production was detected. In accordance, accumulation of SM, but not ceramide, was detected in LPS-stimulated SMS2-depleted cells. To resolve these results, expression of Sgms1 and Sgms2 was analyzed. It was found that silencing of Sgms2 is compensated by doubling expression of Sgms1, which could account for SM increase. Conclusions Obtained data suggests that SM metabolism affects TLR4 level of LPS-stimulated cells.

Optogenetic control of feeding

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Optogenetics, based on genetically encoded light-responsive proteins is a powerful method in neuroscience that enables study function of intact neuronal circuits. Opsins, are able to control neuronal activity and display a specific response, when excited with light of specified wavelengths. Channelrhodopsin-2 (ChR2) is a seven-transmembrane cation channel from Chlamydomonas reihardtii activated with blue light. To observe changes in feeding behaviour upon optogenetic stimulation, neurons in arcuate nucleus (Arc) of the hypothalamus are optogenetically modified. Arc nucleus consists of neurons controlling fed and fasted state of the animal, stimulating feeding and satiety, respectively. Agouti-related protein (AgRP) neurons are well defined hunger neurons which are activated during fasting and drive hunger and food seeking behaviour. There is also opposite mechanism within hypothalamus containing fast acting satiety neurons – vesicular glutamate transporter-2 (V-Glut2) neurons.

We have obtained ChR2 specific expression in AgRP and V-Glut2 neurons using Cre-loxP system by crossing respective Cre lines with Cre-responsive-ChR2 mouse line or using viral vector injection. Delivery of blue light at 470 nm into Arc nucleus is achieved by specially designed optoelectronic module consisting light emitting diode (LED) implanted in the Arc that allows wireless induction of light emission. Activation/inhibition of food intake will be analyzed in satiated/hungry mice upon photostimulation.

Intrinsic signal optical imaging as a tool to determine the plastic changes in the mouse primary visual cortex after associative pairing of visual stimulation and tail shock

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Aim: In this study we verify the hypothesis that intrinsic signal optical imaging (ISOI) can be used to determine plastic changes in the mouse primary visual cortex after classical conditioning with associative pairing of visual stimulation and electric tail shock. Methods: Two groups of 6-week old C57BJ6 mice were used in the experiment: naive mice that served as a control and trained mice which underwent classical conditioning. During training, monocularly presented visual stimulus (square-wave black-and-white gratings of 45° orientation, spatial frequency of 0.05 c/deg, temporal frequency of 1Hz) was coupled with electric shock applied to the tail simultaneously with visual stimulation. Twenty four hours after the training the ISOI was performed on mice under isoflurane anaesthesia. Similar imaging was performed on controls. Intrinsic signals were recorded using CCD camera set above the visual cortex and focused under cortex's surface on 400 μm. Imaging was performed under the control of Imager 3001 system (Optical Imaging Inc.). Visual stimuli were presented on a CRT monitor with the following protocol: square-wave black-and-white gratings of spatial frequency: 0.05 c/deg, temporal frequency: 1 Hz, four orientations: 0°, 45°, 90°, 135°, drifting in two directions, back and forth, randomly presented with uniform grey images in 16 trials. Collection of the data started 1 s before stimulus onset, lasted 7 s during visual stimulation and 8 s following stimulus offset. Results: Using the described protocol of visual stimulation and data collection we could successfully map cortical responses to visual stimuli of different orientations for both groups, naive and trained. Collected images showed the strongest responses for horizontally and vertically oriented gratings, for both groups, naive and trained and recorded ISOI signal from trained mice showed noticeable increase in absorption level. Conclusion: Our results support the hypothesis that ISOI can be used to determine the plastic changes in the mouse primary visual cortex after classical conditioning.
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CD44 as a novel regulator of synaptic plasticity

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CD44 adhesion molecule is a transmembrane receptor for hyaluronic acid, the major component of brain extracellular matrix (ECM). The role of CD44 in the adult nervous system, especially in neurons is unexplored. Recently, it was revealed that CD44 is a key molecule that regulates dendritic tree arborization (Skupien et al.,2014). Here, we show for the first time that CD44 influence small membranous protrusions that extend from neuronal

dendrites and bear excitatory synapses, that are dendritic spines. Immunoelectron microscopy revealed the ultrastructural localization of CD44 protein at the pre- and postsynaptic sites of the mature synapses. Moreover, expression level of CD44 affects dendritic spine structure in primary hippocampal neurons. Electrophysiological studies demonstrated that changes in CD44 expression level affect excitatory synaptic transmission, and this effect corresponds with a change in the number of Bassoon-positive presynaptic puncta together with PSD-95 level at the dendritic spines. We also discovered that CD44 influences the activity of small Rho-GTPases in neurons. The obtained results indicate that CD44 is a novel synaptic cell adhesion molecule that contributes to proper acquisition of dendritic spine structure and function by modifying the activity of actin cytoskeleton regulators Cdc42, RhoA and Rac1

Activity of Cofilin in dentate gyrus affects alcohol seeking

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Alcohol addiction is characterized by psychological and biochemical dependence on ethyl alcohol. It involves continuous and compulsive drinking despite of adverse effects on drinker's organism and his environment. This disease causes changes not only in behavior, but also in brain physiology. Cofilin modifies structure of synapses through depolimerization of F-actin. Its increased activity was liked with shrinkage of dendritic spines and therefore weakening of synapses. These changes might be connected with pathological behavior during alcoholism, however the role of cofilin and actin cytoskeleton in the regulation of alcohol addiction is poorly understood. Here we wanted to verify if the activity of cofilin and remodeling of actin cytoskeleton affect mouse behavior during alcohol training. To this end we analyzed cofilin expression and the levels of F-actin in the hippocampus and amygdala of the mice trained to drink alcohol. Our data show the level of active cofilin is decreased in the area CA1 and dentate gyrus (DG) of the hippocampus in mice drinking alcohol and during withdrawal F-actin level is increased. To further test the role of cofilin we overexpressed adeno-associated viruses (AAV2/9) coding cofilin in DG of the C57BL6/cmdb mice. We injected: AAV9-CaMKIIα0,4-CofilinS3A-HA (constitutively active cofilin), AAV9-CaMKIIα0,4-CoffilinS3D-HA (nonactive cofilin), AAV9-CaMKIIα0, 4-eGFP-HA (EGFP expression control), AAV9-CaMKIIα0,4-CofilinWT-HA (cofilin control). Next, the mice were trained to drink alcohol in IntelliCage system. Our data indicated that mice with high activity of cofilin (S3A) in DG increased alcohol seeking during withdrawal. Altogether, our data indicates the role of cofilin in DG in the regulation of alcohol addiction.

Identification of potassium channels in the mitochondria of human bronchial epithelial cells.

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Mitochondria have been recognized for their multifunctional roles in energy transduction, ion transport, signaling and cell death. It has been observed that potassium flux through the inner mitochondrial membrane regulates synthesis of the reactive oxygen species, affects the mitochondrial volume and changes both the mitochondrial membrane potential and the

transport of calcium ions into the mitochondria. Additionally, it has been shown that activation of mitochondrial potassium channels protects against cell death during myocardial infarction or cerebral hypoxia. Our studies using the patch-clamp technique proves the presence of two different potassium channels in the inner mitochondrial membrane of human bronchial epithelial cell line (16HBE14o-). We identified the activity of week rectifying potassium channel and large-conductance Ca2+-regulated potassium channel (mitoBKCa channel). Using reverse transcriptase-PCR, we detect mRNA transcript for KCNJI (ROMK), KCNJ3 (GIRK1) and KCNJ6 (GIRK2) pore-forming channel as a typed molecular component of the mitoKATP channel. Moreover, the protein of ROMK, GIRK1 and GIRK4 was also observed by Western blot analysis. Additionally, it has been confirmed the presence of α-subunit and modulatory β-subunits of BK channel proteins (Western Blot analysis) and genes expression (reverse transcriptase-PCR analysis). We believe that our findings of the mitochondria potassium channels of human bronchial epithelial cells, it will help us better understand of their role in global protective mechanisms. This study was supported by a grant 2016/21/B/NZ1/02769 from the National Science Centre, Poland and partially by the Nencki Institute of Experimental Biology. Work implemented as a part of Operational Project Knowledge Education Development 2014-2020 cofinanced by European Social Fund; Project number POWER.03.02.00-00-I007/16-00 (to Aleksandra Sęk).

Visualizing Retinotopy In Mouse Visual Cortex

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Understanding the neural basis of visual perception is one of the key focus areas of neuroscience. Using mouse model to study visual system is considered advantageous as neurons in the mouse visual cortex display many similar response properties as have been described for higher mammals. Intrinsic signal optical imaging (ISOI) is a rapid and noninvasive method for observing brain activity in vivo over a large area of the cortex. This technique is used in the present study to map the retinotopy in adult mouse primary visual cortex. The retinotopic map in the mouse visual cortex makes it possible to study mechanisms involved in cortical map formation and neuronal activity. Here we used drifting sine-wave gratings to activate selective population of responsive neurons in mouse visual cortex. Stimuli were presented at nominal 100% contrast for 5 sec with an interval of 7 sec. Retinotopic maps were computed by first-frame corrected images averaged over 16-30 stimulus repetitions. Results obtained from this study showed that small square shaped stimuli presented at different positions in the visual field activated corresponding retinotopic regions in the mouse primary visual cortex. The retinotopic map is arranged such that the upper visual field is represented in the posterior portion of the primary visual cortex, while lower visual field is represented in the anterior portion of the primary visual cortex. Also temporal stimuli were encoded by medial cortical areas and those appearing medially by lateral cortex. In conclusion, mouse primary visual cortex contains a complete and continuous map of the visual field and the intrinsic signal optical imaging successfully maps the retinotopic organization of the mouse primary visual cortex. Findings from the present study will be useful in our future studies where focal retinal lesion-induced reorganization of the retinotopic map in the initially silenced region of the visual cortex will be under investigation.

Liver mitochondrial alterations associated to an early stage of Non-Alcoholic Fatty Liver Disease

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NAFLD (Non-Alcoholic Fatty Liver Disease) is characterized by the accumulation of fat in the liver, a reversible stage that can progress to cirrhosis, hepatocellular carcinoma and ultimately ends with liver failure. The prevalence of NAFLD has been continuously rising, affecting nowadays up to 24% of the worldwide population. Western diets enriched in fat and sugar additionally accompanied by a sedentary lifestyle are considered as the main causes for the development of NAFLD. The molecular mechanisms underlying the progression of the disease are still debated. Several studies have demonstrated structural and functional mitochondrial alterations in a more progressive stage of the disease. Therefore, one suggested major mechanism implies mitochondrial reactive oxygen species (ROS) production that initiates vicious cycles of mitochondrial dysfunction and hepatocyte damage. Using a diet mimicking the Western society habits, we tested the effects of normal (C), high-fat (HF), high-sucrose (HS) or a combined high-fat and high-sucrose (HFHS) diets to address possible mitochondria-related alterations in NAFLD. C57BL/6 mice were fed with the above described diets for 16 weeks. As expected, HF and HFHS fed groups showed increased both body and liver weight comparing to the C group. Importantly, HF and HFHS diets induced a significant triglycerides accumulation in the form of macrosteatosis, while microsteatosis was mostly visible in the group fed with HS diet. The histological investigation showed that only very mild symptoms of liver damage and liver inflammation were apparent and practically no markers of fibrosis have been detected. Isolated mice liver mitochondria from the groups fed with HF, HS and HFHS diets showed a decreased oxygen consumption along with a decreased capability to generate a mitochondrial membrane potential. Those alterations were accompanied by a significant increase of H2O2 levels in mice fed with the HFHS diet. Moreover, our results suggest that increased ROS levels may be a cause or accompanying a pro-oxidative state revealed as increased lipid peroxidation level. This work showed that a combined HFHS diet is the most effective to induce NAFLD with the manifestation of the oxidative stress in mice. This diet appears to be associated not only with the enhanced ROS production but also with effects on mitochondrial bioenergetics.

New aspects of inhibition mechanism of thymidylate synthase catalyzed-reaction by dUMP analogues.

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Thymidylate synthase (TS), catalyzing dUMP methylation at the pyrimidine C5, with concomitant transformation of 5.10-methylenetetrahydrofolate (meTHF) to dihydrofolate, plays a crucial role in the early stages of DNA biosynthesis. In animal cells it is the only de novo pathway of deoxythymidyne monophosphate (dTMP) biosynthesis. As the enzyme is known to be expressed in cycling cells, for over 50 years it has been a target in anticancer chemotherapy, with 5-fluorouracil, designed in 1957, being still the most widely used anti-

cancer drug aimed at the enzyme. N4-hydroxy-dCMP (N4-OH-dCMP) is an TS inhibitor found to be competitive with respect to dUMP, time- and meTHF-dependent, and presumably accompanied by formation of a ternary complex, N4-OH-dCMP-meTHF-enzyme, with apparently differing interactions between the low molecular weight components and each of the two enzyme subunits. Not being a substrate, the analogue caused slow-binding inhibition, inactivated the enzyme in a way that was meTHF-dependent, hence mechanism-based, and blocked abstraction of the C(5) hydrogen. However, crystallographic results disproved the assumption that the analogue would form a ternary complex with meTHF and TS, indicating that in the presence of N4-OH-dCMP the meTHF methylene group is not transferred to C(5) of N4-OH -dCMP but reacts with an unknown group. At the same time tetrahydrofolate (THF) becomes oxidized to dihydrofolate, with concomitant reduction of N4-OH-dCMP pyrimidine C(5) carbon, leading to a covalent E-I complex, stable under denaturing conditions. Thus, interaction with N4-OH-dCMP appears to uncouple the two TS-catalyzed reactions, i.e. methylene group transfer and THF-promoted reduction. The latter posed a question whether THF alone is capable to promote the TS-catalyzed pyrimidine ring reduction in nucleotides, such as N4-OH-dCMP or FdUMP, resulting in formation of a covalent enzyme protein-inhibitor complex and TS inactivation. Evidence will be presented that THF promotes binding of each FdUMP and N4-OH-dCMP to TS, resulting in a TCA-resistant, thus covalent complex. The binding depends not anly on concentrations of FdUMP/N4-OH-dCMP and THF, but also on the reaction time and TS molecular activity, indicating TS-catalyzed reaction to be responsible for it. Furthermore, the binding is associated with tymidylate synthase inactivation, pointing to an unexpected and particularly interesting possibility of THF-dependent enzyme inactivation by FdUMP.

Expression of Sema3c during formation of interhemispheric connections in the opossum brain

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Mammals form few major pathways to transfer information between the two hemispheres of the brain: the anterior commissure (AC), hippocampal commissure (HC) and the corpus callosum (CC). In Metatheria and monotremes the main route of axons projecting to the neocortex of the opposite hemisphere is the AC, whereas Eutheria uses a combination of the AC and the CC, the pathway which is characteristic only for methaterian mammals. The aim of the current research was to study expression of axon guidance genes that are involved in directing and elongation of the interhemispheric main neocortical axons in the developing neocortex of the mouse, representative of eutherians and Monodelphis opossum, representative of marsupials. We focused on class 3 of semaphorins (Sema) which modulate development of the nervous system acting as repellents (Sema 3A) or attractants (Sema 3C) for growing axons and dendrites. We also examined neuropilins (Nrp) that act as co-receptors for semaphorines. In the opossum at P17 and P19, Sema3a was highly expressed in the upper neocortical layers, particularly in the cingulate cortex. In the mouse Sema3a gene was strongly expressed in the subventricular and the intermediate zone (IMZ) cells of germinal layers in the neocortex. mRNA for Sema3c was observed in the upper cortical layer at P14, P17 and P19 opossums. The expression was high in the cingulate cortex. In the mouse Sema3c was highly expressed exclusively in the IMZ of the neocortex. The receptor Nrp1 was expressed mainly in the upper layer cortical neurons of both investigated animals. Unlike

mice, neurons of upper neocortical layers in opossums weakly expressed Nrp1. The expression pattern of investigated genes such as Sema3a, Sema3c and their receptor Nrp1 was different in the mouse and opossum neocortex. Based on these differences we suggest that these genes are involved in regulation of different axon guidance signaling pathways in the marsupials and eutherians. This work was supported by grant no 2016/22/M/NZ4/00670 from the National Science Center Poland.

Expression of MBD3 (methyl-CpG binding domain protein 3) in cortical neurons cell culture

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The MBD3 protein belongs to the MBD protein family that contains a conversed methyl-CpG binding domain. This protein is a component of nucleosome remodeling and histone deacetylase (NuRD) complex. MBD3 appears to influence gene expression as a component of the NuRD complex, and it is indispensable for the assembly of this complex. The aim of this project was to evaluate the expression of MBD3 in cortical neurons cell culture. Primary cultures of cortical neurons were prepared from 18D rat embryos. Cells were transfected with a plasmid that expresses MBD3 (N4) on the 9th day in vitro. Level of MBD3 was determined using Western Blot and fluorescence. We observed that cells transfected with the plasmid that expressed MBD3 show high expression of MBD3 in nuclei of neurons. Western Blot analysis showed no significant difference in expression of MBD3 between cells transfected with the plasmid that expressed MBD3, and control plasmid, what may be caused by low transfection efficiency, which should be optimized. This work was supported by Polish Ministry of Science and Education grant G1401.

Myosin VI – a molecular motor protein engaged in myoblasts differentiation and function

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Myosin VI (MVI) is a protein which belongs to the group of unconventional myosins. This unique protein (due to the fact that it is the only myosin walking towards the minus end of microfilaments) plays a role in many cellular processes such as endocytosis, cell migration, adhesion, maintenance of the Golgi apparatus, autophagy, and in gene transcription. It was shown that in skeletal muscle MVI is present in the neuromuscular junction, sarcoplasmic reticulum and myofiber nuclei thus suggesting its important roles in proper functioning of the muscle (Karolczak et al. 2013, 2014). Moreover, we have also postulated that MVI could be involved in myoblast differentiation and myofiber maturation (Karolczak et al. 2015). In order to elucidate the role of MVI in myoblast differentiation, we derived myoblasts from hind limb muscles of Snell's waltzer (SV) mice. These mice have spontaneous mutation within MYO6 gene which prevents from MVI synthesis and therefore are considered as natural MVI knockout animals. We demonstrated that SV myoblasts differentiate in a different way than the cells from control littermates. Furthermore, we observed changes in cell adhesion and the level of proteins engaged in protein synthesis. What is more, we observed a reduction in mitochondrial activity and a decrease in Ca2+ concentration in SV myoblasts. These observations suggest impact of MVI on energy production and calcium homeostasis, the processes essential for myoblast differentiation and muscle contraction. Our data indicate that

MVI plays important roles in process of myoblast differentiation and function. Karolczak et al. (2013) Histochem Cell Biol 139:873-885 Karolczak et al. (2014) Anat Rec 297(9):1706-13 Karolczak et al. (2015) Histochem Cell Biol 144:21-38 Myosin VI, skeletal muscle, myotubes, myogenesis

Studying memory in real-life conditions: measuring stimulus-locked brain dynamics with fMRI

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While listening to a story, people tend to reconstruct the original events in their minds. How does one's memory operate as a filter as the story is being communicated to others? To answer this question, we asked people to tell stories as they would do in everyday life. Compared to artificial, highly controlled stimulus material traditionally used in experiments, complex stimulation (e.g. movies, stories) is more likely to elicit brain responses similar to those occurring naturally. It was shown that during such complex stimulation changes in brain response patterns tend to be similar across individuals, while being tightly related to the stimulus content. Moreover, it was demonstrated that such a high degree of similarity occurs not only during the presentation of a movie or story, but also when subjects later recall their content, in the absence of any sensory stimulation. In the present experiment we used publicly available brain data of subjects (story tellers) who watched a movie (50 min) and later recalled its content (10.8 – 43.9 min; 21.7 min on average) in an MRI scanner. Additionally, we plan to collect data from another group of subjects (story receivers), who will read or listen to the recorded recalls and will then undergo comprehension and memory tests. With the use of pattern similarity analysis we will attempt to predict properties of the story receivers' knowledge from brain activity of the story tellers, both during movie watching and during recall. Our preliminary results confirm previous findings, which demonstrated that brain response patterns in different subjects tend to become similar during complex stimulation, presumably due to similar underlying mental processes. Further analysis will be needed to verify if brain response patterns during movie watching and during recall can be used to predict how effectively the story is communicated to others.

Transfer of social information in mice

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Information about reward and danger spreads between individuals in the herd and plays a key role in adaptation to changes in the environment. Understanding the mechanism which regulates the transfer of emotions could help in developing the treatment for behavioral problems related to social deficits characteristic of autism spectrum disorders. Unfortunately, we know very little about emotional contagion, also there are only few established experimental protocols allowing the study of this phenomenon in laboratory conditions. Using Eco-HAB system, fully automated and ecologically relevant assay for behavioral experiments, and C57 males as a subject, we showed that aversive and appetitive emotions experienced by one mice can affect the behavior of the rest of the group. Mice were more

interested in bedding from a familiar mouse exposed to reward or stress conditions, compared to control (a non-stimulated mouse). Also, we observed changes in interactions and social structure specific to the type of odor used. The mice spent more time with stressed but not with rewarded or control mouse. Our results proved that laboratory mice are a suitable model for studying group empathic behavior which could help us understand the neuronal basis of emotions' regulation.

Short-term Western diet feeding induces metabolic distrubances and obesity resulting in enhanced astrogleiosis in early stage of Alzheimer's disease development

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Dietary pattern enriched in fat, cholesterol and sugar characteristic for Western society, called Western diet (WD) induces such symptoms as systemic inflammation, obesity and metabolic syndrome, which are the great cause of morbidity and mortality in the western world. Moreover, it was shown that obesity and systemic inflammation impact Alzheimer's disease (AD) progression, but the impact of WD consumption on AD development remains not fully explored. Our study addresses the question whether described WD can be consider as a civilizing risk factor of AD progression. We aim to verify the hypothesis that WD feeding by inducing blood biochemical disturbances and systemic inflammation may alter the neuroinflammation of the brain at the early stage of AD development. The analysis is performed in the blood and brain tissue from transgenic mice carrying the human mutant gene for the amyloid precursor protein (APPswe), a model of the familiar form of AD. In order to induce metabolic syndrome, systemic inflammation and neuroinflammation in the brain, APPswe mice were fed with WD, or/and intraperitoneally injected with Lipopolissacharide as a control. To determine the impact of applied factors on the metabolic phenotype and sequence of brain neuroinflammation events, the analysis was carried out in three age groups (4-,8-,12-month old). We show that WD results in hypercholesterolemia, hyperglycaemia, obesity and enhanced liver weight, what indicates that WD feeding induces systemic inflammation indirectly by triggering obesity and metabolic disorder. Moreover, WD-related systemic inflammation induced the response mediated by glial cells (astroglia) in the young (4 month old) APPswe mice hippocampus. Obtained results indicate that consuming of WD accelerates and intensifies the neuropathological changes observed during AD development, and suggest that WD can be considered as a common civilizing risk factor of AD progression.

Role of stearoyl-CoA desaturase 4 in regulation of cardiac lipid metabolism

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Stearoyl-CoA desaturase (SCD) is a rate-limiting enzyme involved in biosynthesis of monounsaturated fatty acids (MUFA) and important factor in the regulation of substrate utilization in the heart. Cardiomyocytes in normal conditions utilize up to 70% of energy from fatty acids (FA) oxidation. Abnormal lipid metabolism in the heart is associated with heart failure, cardiac fibrosis, reduced contractility and apoptosis. So far, four isoforms of SCD have been characterized in mouse tissues. The most abundant isoform is SCD1, while SCD4 is expressed exclusively in heart. It is known, that cardiomyocytes of SCD1 deficient mice

(SCD1-/-) are characterized by lower FA uptake and oxidation and increased glucose transport and oxidation. Moreover it was shown, that SCD1 inhibition leads to decrease in lipogenic proteins levels and elevation of lipolysis in cardiomyocytes. Still the role of SCD4 in these processes is unknown. The aim of the presented study was to determine the role of SCD4 in control of lipolysis in the heart. We used wild type (WT) and SCD4-/- mice fed high fat (HF) diet for 8 or 16 weeks. Performed analyses indicate that SCD1 deficiency leads to increased phosphorylation of hormone-sensitive lipase (HSL) at Ser563 (that is associated with higher HSL activity) and decreased HSL phosphorylation at Ser565 (that is known to inhibit HSL activity) in mouse heart. Interestingly, lack of SCD4 leads to decreased phosphorylation of HSL at Ser565 and AMP-activated protein kinase (AMPK) at Thr172, whereas HF feedings result in up-regulated phosphorylation of HSL at Ser565. Taken together, obtained data emphasize the important role of SCD in control of lipolysis in the heart and suggest that both isoforms of SCD play a key role in regulation of lipolysis in the heart.

siRNA ATG5 - evoked dysfunction of autophagy initiation attenuates C10-induced senescence in MCF-7 cells.

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Introduction: Autophagy is an evolutionally conserved cytoplasmic degradation system of intracellular components. It was demonstrated that autophagy is whirled with senescence and both processes play an important role in cancer initiation and progression. Thus, their regulation became new target in anticancer therapy. Presently, more attention is paid to autophagy or senescence regulators that are able to enhance efficacy of anticancer therapy. Recently, we have demonstrated that tacrine-melatonin heterodimer (C10) has anticancer properties due to simultaneous induction and blockade of autophagy. Moreover, we have found that 24-hour treatment with cytostatic IC50 dose of C10 followed by culture in drug free medium for few days led to cellular senescence of 20% of cells. Aim: To verify whether there is interconnection between autophagy and senescence evoked by C10 in MCF-7 cells. Methods: We inhibited autophagy genetically at initiation stage, using siRNA against ATG5. Cell viability was analyzed using MTT assay. Several markers of autophagy and senescence were analyzed by western blotting, immunostaining or flow cytometry. Results: After treatment with C10 MCF-7 cells transfected with siRNA ATG5 preserved enhanced proliferation capacity in comparison with the control cells. Moreover, cells with decreased level of ATG5 secreted lower amount of IL-6 and IL-8, both characteristic for senescence-associated secretory phenotype (SASP). Surprisingly no significant changes in SA-β-gal activity was observed. Conclusion: C10-evoked autophagy disorder is essential for strength of senescence phenotype that appears after drug removal. However, cells with faulty autophagy initiation (transfected with siRNA ATG5) are not able to develop full set of senescence features.

Cortical vasoactive intestinal polypeptide (VIP) interneurons in learning-induced plasticity.

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VIP interneurons constituting about twelve per cent of all cortical inhibitory interneurons, are an enigmatic group of cells and their role in brain circuitry and cognitive functioning is highly unexplored. A few studies have shown that VIP interneuron group is a key part in disinhibitory circuit that regulates adult visual cortical plasticity or assosciative learning in the auditory cortex. Here, using a chemogenetic approach we aimed to study a role of VIP interneurons in plasticity induced by fear learning in primary somatosensory cortex of mice. With immunofluorescence labeling we validated selectivity of inhibitory DREADDs expression in VIP interneurons. Next, under optical imaging recording we transduced cortical representation of row B whiskers with viral vectors expressing designer receptors. During fear learning, in which stimulation of vibrissae paired with tail shock results in plastic modification of the barrel cortex activation, we blocked the activity of VIP interneurons by activation of inhibitory designer receptors with CNO (1 mg/kg). After the conditioning [14C]-2-deoxyglucose brain mapping was performed. On autoradiograms of brain sections functional representation of the conditioned row (B) of whiskers and contralateral row on the other side of the snout were compared. Our preliminary results suggest that blocking of VIP interneurons can enhance plasticity in fear learning and the effect can be mediated by increased activity of somatostatin interneurons.

Evolutionarily conserved proteins,Tt990p and Tt070p, locate in close proximity to nexin-dynein regulatory complex (N-DRC) and regulate Tetrahymena cilia beating

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Cilia are highly conserved microtubule-based protrusions built of several hundred proteins. Proper beating of motile cilia powers a sperm motility, and in multicellular organisms, enables a shift of fluids or particles along the surface of the ciliated cells lining the Fallopian tubes, brain ventricles or respiratory tracts. In human dysfunction of motile cilia or asynchronous beating of motile cilia cause primary ciliary dyskinesia, genetic disorder that affects 1:10 000 individuals. The molecular mechanisms that regulate cilia beating is unclear partly because the knowledge of cilia protein composition is incomplete. To understand the molecular mechanisms that control cilia beating I analyze as-yet uncharacterized ciliary proteins that are indispensable for proper cilia beating. I focus on highly evolutionarily conserved, coiled-coils-containing protein encoded by a single gene in mammals and by two orthologous genes, TT990 and TT070 in a ciliate Tetrahymena thermophila. In Tetrahymena cells lack of TT990p or TT070p impairs cells motility and alters the swimming paths. The biochemical and genetic studies suggest that TT990 and Tt070 are positioned in close proximity and that the ciliary localization of TT990 and TT070 is interdependent. The TT990p was not targeted to cilia in TT070-KO cells and reverse, TT070p was absent in cilia isolated from TT990-KO cell. Interestingly, based on BioID data, TT990 and TT070 proteins are positioned in close proximity to main ciliary regulator, N-DRC (nexin-dynein regulatory complex). Thus, it is possible that TT990 and TT070 form a heterodimer that interacts with N-DRC complex. Supported by National Science Centre, Poland (Harmonia 6, 2014/14/M/NZ3/00511)

Increased plasma neurofilament light chain concentration and neuronal damage in mice with frontotemporal dementia-type tauopathy but not in mice with tauopathy of Alzheimer-type.

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Both of the two common neurodegenerative disorders, namely Alzheimer's disease (AD) and frontotemporal dementia (FTD), have multiple evidence that tau pathology is implicated in their pathogenesis. Abnormal forms of tau trigger neurodegeneration. More recently, neurofilament light (NfL) concentrations in body fluids has been reported to be elevated in neurodegenerative conditions such as AD and FTD. NfL level in CSF and serum reflects symptoms of cerebral proteopathies also in mouse models. Here we studied NfL changes in plasma of two mouse models resembling tauopathies of Alzheimer-type (line L1) and frontotemporal dementia-type (line L66) using ELISA immunoassay. Post mortem, mice brains were sectioned for evaluation of neurodegeneration using Fluoro-Jade C (FJC) and Nissl staining. Adjacent sections were stained immunohistochemically with antibodies selective against aggregated tau. We found plasma NfL concentrations to be significantly increased over control wild type animals (WT-NMRI) in L66-FTD-mice but not in L1-AD tauopathic mice. There was increased labeling of FJC in the cortex, hippocampus and dentate gyrus in L66 transgenic mice of FTD when compare with WT-NMRI. No FJC positive degenerating neurons were found in L1-AD tauopathic mice. Consistently, examination of Nissl stained section also revealed numerous damaged neurons in L66-FTD mice and only limited neurodegeneration in L1-AD mice in comparison to controls. These findings demonstrate that tauopathy exaggerate neuronal damage and NfL concentration in plasma in mouse analogue of FTD but not in AD model. Our results indicate that data regarding the potential use of plasma NfL as a biomarker for neurodegeneration should be interpreted with caution.

Effects of non-invasive transorbital pulse current stimulation (tPCS) on visual evoked potentials in healthy humans

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Aim: The main purpose of this study was to explore how single current pulses applied transorbitally before presentation of checkerboard pattern reversals affects visual evoked potentials resulting from the latter in healthy humans. Methods: We studied 24 healthy (11 females) volunteers in age of 20-40 years (26.2 ± 3.6) divided into two groups: SHAM (7 females, 5 males, age 27.0 ± 3.9) and EXP (4 females, 8 males, age 25.4 ± 3.3). Participants watched checkerboard pattern reversals presented on the computer screen in a darkened room. Visual stimulation was performed alone (gr. SHAM) or preceded with tPCS of different amplitude and duration (gr. EXP). Current stimulation was generated by DC-Stimulator MC (neuroConn, Germany) and applied via four 15x20 mm electrodes located below and above the participants' eyes. During the current and visual stimulations, 128-channel EEG signal was recorded and then analysed with the use of Python programming language. We measured amplitudes and latencies of visual evoked potential's main components, i.e. N1 (N75), P1 (P100) and N2 (N145). Results: tPCS caused noticeable decrease in P1-N1 and P1-N2 amplitudes and latencies in all considered scalp regions, i.e. occipital, parieto-occipital and parietal. Conclusions: Our findings suggests the potential capabilities of tPCS in at least short-

term modulation of visual information processing in healthy humans. Research was supported by National Science Centre Poland Grant 2016/23/N/HS6/02346.

In vivo attenuation of hub nodes in fear memory extinction network.

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A traumatic memory formed by acquiring a strong, aversive stimuli can become longlasting and in serious cases could even lead to a Post Traumatic Stress Disorder (PTSD). Psychiatry uses a treatment based on presenting the previously acquired aversive stimuli in safe conditions to attenuate the fear response. This is called extinction treatment. Since traumatic memories are harder to manipulate thus harder to attenuate the fear response to the aversive stimuli causing extinction therapy less effective. Moreover little is known about the mechanisms and neuronal networks behind REMOTE (ie. 30-day-old) fear memory extinction. Here we wanted to find and understand brain regions responsible for remote fear extinction memory in WT mice. By mapping brain-wide expression of the activity-regulated gene *c-fos*, we identified a networks of brain regions co-activated by RECENT (1-day-old) and REMOTE (30-day-old) fear memory. Next we attenuate activity of their main components using DREADD (Designer Receptor Exclusively Activated by Designed Drug) tool, to verify its role on fear memory extinction processing over time. Recent fear memory extinction induced cFos in primary visual (V1), infralimbic (IL) cortices, central medial (CM) and paraventricular (PV) thalamus nuclei and central division of central amygdala nucleus (CeM). Remote fear memory extinction additionally hyperactivates V1, PV and lateral amygdala (LA). Moreover this study presents the largest yet functional network of fear memory extinction in two time-points. Surprisingly *in vivo* attenuation of activity in hub nodes showed no significant results in consolidation of fear memory extinction but revealed the time-dependent modality of nucleus reuniens (RE). Together, this study supports a "multiple trace theory" of remote memory processing and suggests a new targets for therapeutic approaches against traumatic memories with special emphasis on RE.